Mutational effects of γ-rays and carbon ion beams on Arabidopsis seedlings

Ryouhei YOSHIHARA1, Shigeki NOZAWA2, Yoshihiro HASE2, Issay NARUMI3, Jun HIDEMA4 and Ayako N. SAKAMOTO2,*

1Research Center for Environmental Genomics, Kobe University, 1-1 Rokkodai, Nada, Kobe 657-8501, Japan
2Ion Beam Mutagenesis Research Group, Medical and Biotechnological Application Division, Quantum Beam Science Directorate, Japan Atomic Energy Agency, 1233 Watanuki, Takasaki, 370-1292, Japan
3Department of Life Sciences, Faculty of Life Sciences, Toyo University, 1-1-1 Izumino, Itakura, 374-0193, Japan
4Graduate School of Life Sciences, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan
*Corresponding author. Ion Beam Mutagenesis Research Group, Medical and Biotechnological Application Division, Quantum Beam Science Directorate, Japan Atomic Energy Agency, 1233 Watanuki, Takasaki, 370-1292, Japan. Tel: +81-27-346-9537; Fax: +81-27-346-9688; Email: sakamoto.ayako@jaea.go.jp

(Received 27 February 2013; revised 23 April 2013; accepted 25 April 2013)

To assess the mutational effects of radiation on vigorously proliferating plant tissue, the mutation spectrum was analyzed with Arabidopsis seedlings using the plasmid-rescue method. Transgenic plants containing the Escherichia coli rpsL gene were irradiated with γ-rays and carbon ion beams (320-MeV 12C6+), and mutations in the rpsL gene were analyzed. Mutant frequency increased significantly following irradiation by γ-rays, but not by 320-MeV 12C6+. Mutation spectra showed that both radiations increased the frequency of frameshifts and other mutations, including deletions and insertions, but only γ-rays increased the frequency of total base substitutions. These results suggest that the type of DNA lesions which cause base substitutions were less often induced by 320-MeV 12C6+ than by γ-rays in Arabidopsis seedlings. Furthermore, γ-rays never increased the frequencies of G:C to T:A or A:T to C:G transversions, which are caused by oxidized guanine; 320-MeV 12C6+, however, produced a slight increase in both transversions. Instead, γ-rays produced a significant increase in the frequency of G:C to A:T transitions. These results suggest that 8-oxoguanine has little effect on mutagenesis in Arabidopsis cells.

Keywords: mutation spectrum; γ-rays; carbon ion beams; Arabidopsis; rpsL gene

INTRODUCTION

The biological effect of ionizing radiation has been studied in bacteria and animals over many decades. Ion beams and γ-rays are utilized as models of high-linear energy transfer (LET) and low-LET radiation, respectively, to study the biological effects from various radiations. It is known that high-LET radiations tend to confer greater biological effects (such as cell-killing and mutagenesis) than low-LET radiations [1–3]. It is thought that the greater cell-killing effects of high LET radiation are due to the formation of double-strand breaks (DSBs) and clustered DNA damage, which are deleterious for organisms [4, 5]. Mutations induced by high- and low-LET radiations are also of different types. It has been reported for mammalian cells that ion beams induce large-sized deletions and insertions, and chromosomal rearrangements, whereas γ-rays induce shorter deletions and insertions, and more frequent base substitutions [3].

Several studies have reported that high-LET radiations confer greater and more distinctive biological effects on plant cells than low-LET radiations. For instance, high-LET radiations produce a more lethal effect on Arabidopsis and tobacco cells [6–9]. As for the types of mutation, irradiation of Arabidopsis dry seeds by carbon ion beams induces point-like mutations (base substitutions and several-base deletions/insertions) and chromosomal rearrangements with similar frequencies, whereas electron beams, one of the low-LET forms of radiation, predominantly induce point-like mutations [10, 11]. Furthermore, genomic in situ hybridization in wheat has shown that ion beams induce chromosomal...
rearrangements more frequently than X-rays [12]. Recently, Hase et al. (2012) showed that high-LET ion beams induce DSBs that are seldom repaired by the non-homologous end-joining (NHEJ) pathway in Arabidopsis dry seed [13]. These results suggest that the significant biological effects of high-LET radiation on plants seem to be due to differences in DNA damage, as reported in other organisms.

In general, ionizing radiation predominantly induces the formation of radicals by water radiolysis, and these then attack DNA and produce oxidative damage. The formation of 8-oxoguanine is one of the major forms of radiation-induced oxidative damage in animal and bacterial cells, and it is believed to induce major base substitutions involving G:C to T:A and A:T to C:G transversions [14]. The oxidative DNA damage causes mispairings during DNA replication [15].

In a prior study, we irradiated Arabidopsis dry seeds with carbon ion beams and γ-rays to analyze radiation-induced mutation in Arabidopsis somatic cells. Our results showed no significant increase of G:C to T:A or A:T to C:G transversions with either type of radiation, suggesting the low effect of 8-oxoguanine in Arabidopsis dry seeds [16]. This may be because the low water content and/or low cell proliferation activity in dry seed help to avoid the effect of 8-oxoguanine and other oxidative damage. In this study, we analyzed the mutations induced by carbon ion beams (320-MeV 12C6+) and γ-rays in Arabidopsis seedlings. This is the first report detecting radiation-induced mutations in vigorous plant cells with high water content and high cell proliferation activity. Our results provide an important insight for evaluation of the mutational effects of ionizing radiation on plant systems.

**Materials and Methods**

**Plant and bacterial strains**

For mutation spectrum analysis, we used transgenic Arabidopsis containing the E. coli rpsL gene integrated into the chromosomal DNA [17]. Mutations occurring in the rpsL gene were detected by means of the plasmid-rescue technique. E. coli strain DH10B [F mcrA Δ(mrr-hsdRMS-mcrBC) 680d lacZΔM15 ΔlacX74 deoR recA1 endA1 araD139 Δ(ara leu)7697 galU galK rpsL supF λ-] carrying the supF-containing plasmid pFSE101 (DH10B/pFSE101) was used for the screening of rpsL mutant clones [18].

**Ionizing radiation**

Transgenic Arabidopsis seedlings were irradiated with carbon ion (12C6+) beams generated using an azimuthally varying field (AVF) cyclotron and 60Co γ-rays (Japan Atomic Energy Agency, Takasaki, Gunma, Japan). The energy of a carbon ion beam was 320-MeV (26.7 MeV/μ) and the average LET was 86.2 keV/μm, which was calculated using the ELOSSM code, a program used to calculate the energy loss of heavy ions under practical irradiation conditions [19].

**Plant cultivation and irradiation**

The rpsL-transgenic plants were sown on 1/2 B5 medium (1/2 × Gamborg’s B5 salts, 1/1000 × hyponex, 1% sucrose, 0.7% agar) aseptically. After a three-day 4°C treatment, plants were grown at 23°C under a 16-h light and 8-h dark cycle for 7 days. For mutation spectrum analysis, 7-day-old plants were irradiated with carbon ions or γ-rays. Irradiated plants were grown under the same conditions for another 7 days and used for mutation analysis.

**Sensitivity to ionizing radiation**

Sensitivity to ionizing radiation was evaluated by measuring the area of the fifth leaf of irradiated 14-day-old plants. The leaves were mounted on a paper and the leaf area was measured using Adobe Photoshop Elements 4.0 software (Adobe systems Inc., San Jose, CA, USA). Radiation sensitivity was determined as the relative leaf size of irradiated leaves to that of non-irradiated control leaves. Sensitivity curves fitted using a least-square method were defined by the following equation:

\[
\text{Sensitivity rate} = 1 - \left(1 - e^{-D/D_0}\right)^m,
\]

where \(D, D_0\), and \(m\) indicate the dose, the dose conferring a 37% sensitivity rate (mean lethal dose), and the extrapolated number (the number of targets), respectively.

**Mutation analysis**

We used ~270 plants per experimental plot. The experiment was repeated at least five times. Chromosomal DNA was isolated by the CTAB method [20]. Approximately 80 μg of DNA was digested with 20 units of BamHI (Takara Bio, Otsu, Shiga, Japan) in a 400-μl reaction mixture. Digested DNA was purified by phenol/chloroform extraction and ethanol precipitation. The DNA was self-ligated in an 800-μl reaction mixture containing 11 Weiss units of T4 DNA ligase (Takara Bio). Reconstructed plasmid purified by phenol/chloroform extraction and ethanol precipitation was electroporated into E. coli DH10B/pFSE101. To select clones carrying a mutated rpsL gene, this E. coli was screened on agar medium containing 100 μg/ml of kanamycin (Km) and 60 μg/ml of streptomycin (Sm). The number of total clones was determined by plating a portion of electroporated cells on the medium containing 100 μg/ml of Km. Mutant frequency was calculated as the ratio of mutant clone to total clone. Sequence analysis was performed using a GenomeLab GeXP Genetic Analysis System (Beckman Coulter Inc., Brea, CA, USA).

We analyzed a total of 3.1–20 × 10^4 clones in each rescue experiment and repeated experiments at least five times.
Plural mutations in one clone, i.e. multiple base changes, a base change combined with a frameshift, or a deletion accompanied with an insertion, were scored as a ‘complex mutation’. All mutations from independent clones were counted as independent mutations.

RESULTS

Sensitivity of Arabidopsis seedlings to γ-rays and 320-MeV $^{12}$C$^{6+}$

To compare the effects of γ-rays and 320-MeV $^{12}$C$^{6+}$ ion beams on plant tissues, the growth inhibition on young plant tissues was quantified. Seven-day-old seedlings, on which 1–2 true leaves have emerged, were irradiated with γ-rays and 320-MeV $^{12}$C$^{6+}$ ion beams. The plants were grown for another 7 days, by which time the plants had developed 4–6 true leaves. We cut the fifth leaf from each plant and measured the area of the leaf using graphics software. Results showed that 320-MeV $^{12}$C$^{6+}$ more severely inhibited the growth of plant leaves than the same dose of γ-rays (Fig. 1). This is due to the deleterious effect of 320-MeV $^{12}$C$^{6+}$ on cell proliferation. To standardize the effect of both radiations, we adopted the Multi-Target Single-Hit model (Fig. 1) that has been shown to work well in plants [7, 9, 16]. Based on these sensitivity curves, 40–100 Gy of γ-rays and 5–35 Gy of 320-MeV $^{12}$C$^{6+}$ reduced the leaf size dose-dependently. We expected that these ranges of radiations might induce mutations effectively in the plant. Based on this prediction, we chose the dose of 64 Gy and 20 Gy for γ-rays and 320-MeV $^{12}$C$^{6+}$, respectively, which reduced the leaf size to ~50%, and then conducted mutation spectrum analysis.

Mutagenicity of γ-rays and 320-MeV $^{12}$C$^{6+}$ ion beams

To investigate the mutational effect of γ-rays and 320-MeV $^{12}$C$^{6+}$ ion beams on Arabidopsis seedlings, the mutation frequencies were determined using the plasmid-rescue method (Table 1). The mutant frequency of unirradiated seedlings was 3.4 x 10$^{-5}$, as found in our previous report [16]. By irradiating with 64 Gy of γ-rays, the mutant frequency increased significantly (4.1-fold, $P < 0.01$). In contrast, 20 Gy of carbon ions increased the mutant frequency by 2.9-fold, but this increase was not statistically significant. Therefore, these results suggest that a 320-MeV $^{12}$C$^{6+}$ ion beam is less effective than γ-rays in inducing detectable mutations in this assay system.

Mutation induced by γ-rays and a 320-MeV $^{12}$C$^{6+}$ ion beam in Arabidopsis seedlings

We analyzed mutation spectra induced by γ-rays and a 320-MeV $^{12}$C$^{6+}$ ion beam, as well as unirradiated (‘background’) seedlings. All mutations were classified into three groups: base substitutions, frameshifts, and ‘other’ mutations (Fig. 2). In comparison with unirradiated plants, both radiations increased the frequency of frameshifts and ‘other’ mutations, but only γ-rays resulted in a significant increase in the frequency of total base substitutions.

We analyzed the type of each mutation (Table 2). Regarding the types of base substitutions, γ-rays resulted in a significant increase in the frequency of G:C to A:T transitions ($P < 0.01$). Furthermore, γ-rays increased the frequencies of −1 and −2 frameshifts, deletions/insertions, and complex mutations ($P < 0.01$). In contrast, 320-MeV $^{12}$C$^{6+}$ increased G:C to T:A and A:T to C:G transversions, −1 frameshifts and complex mutations ($P < 0.01$). There was no increase in both transversions associated with γ-rays, which was consistent with our previous mutation analysis in Arabidopsis dry seeds [16].

Table 1. Mutant frequencies induced by γ-rays and 320-MeV $^{12}$C$^{6+}$ ion beams

| Mutant clone | Total clone | Mutant frequency |
|--------------|-------------|-----------------|
| 33 | 9.2 | 3.4 ± 0.8 |
| 44 | 3.6 | 14 ± 7* |
| 27 | 3.3 | 10 ± 12 |

*aSum of 5–10 independent experiments. bAverage of 5–10 independent experiments with SD. *Statistically significant compared to unirradiated (‘background’) plants ($P < 0.01$).
DISCUSSION

In this study, we irradiated Arabidopsis seedlings with 64 Gy of γ-rays and 20 Gy of 320-MeV $^{12}$C$^{6+}$ ion beams, which reduced leaf size to ~50%, then analyzed the mutation spectrum. In the previous study, we irradiated Arabidopsis dry seeds with 740 Gy of γ-rays and 140 Gy of 208-MeV $^{12}$C$^{5+}$, each of which correspond to 0.8 Dq of survivals and provide similar mutation frequencies (Yoshihara et al., 2010). Here, we used the reduction of leaf size as an index and expected the doses causing a similar degree of reduction to also cause a similar induction of mutation frequency. However, results revealed that the mutant frequency increased following irradiation by γ-rays (64 Gy), but did not increase significantly following irradiation by 320-MeV $^{12}$C$^{6+}$ (20 Gy), although both conditions reduced leaf size to ~50%. Since the growth of leaves depends on multiple factors, such as cell death, cell-cycle arrest, or cell-expansion, it did not simply correlate with the DNA damage and/or mutations. Namely, the reason that the 320-MeV $^{12}$C$^{6+}$ ion beam did not elevate mutant frequency could be because the dose was too low to induce mutations. It would be necessary to analyze the dose response of mutations in order to elucidate all the characteristics of these radiations. Nevertheless, though limited, the data obtained in this work shed light on the understanding of mutations by γ-rays and a 320-MeV $^{12}$C$^{6+}$ ion beam, as discussed below.

The mutation spectrum analysis showed an increase in the total number of base substitutions after γ-ray irradiation, but not after radiation from the 320-MeV $^{12}$C$^{6+}$ ion beam (Fig. 2). Oxidative damage to DNA, which is mainly caused by radicals from water radiolysis as an indirect effect of ionizing radiation, is often involved in the induction of base substitutions [21]. Therefore, it is possible that more radicals were induced in plant cells by γ-rays than by the 320-MeV $^{12}$C$^{6+}$ ion beam in these assay conditions. This difference might have caused the difference in mutant frequencies.

In contrast, both types of radiations increased the frequency of deletions/insertions and complex mutations compared with that observed in unirradiated plants (Fig. 2). It is known that these mutations occur during the process of DSB repair by non-homologous end-joining (NHEJ) [22]. It is reasonable to claim that both radiations induced DSBs in Arabidopsis plants. In our previous work, Arabidopsis plants with a disrupted DNA Ligase IV gene, a key component of the NHEJ pathway, showed a markedly higher sensitivity than the wild-type to ion beams [13]. These data also support the idea that DSBs formed by the radiation exposure were mostly repaired by the NHEJ pathway in Arabidopsis plants.

Mutational effects of radiation on seedlings

Fig. 2. Frequency of mutations classified into three categories. The frequency of mutations was calculated from the ratio of mutant clone numbers to predicted total analyzed clone numbers. Frameshifts include one base insertion, one base deletion, and deletion of two contiguous bases. Others are a deletion or insertion of ≥3 bases, plural base substitutions, base substitution accompanied by a frameshift, and deletion accompanied by an insertion. *Statistically significant compared with unirradiated (‘background’) plants according to the Poisson test ($P < 0.01$).

Table 2. Mutation spectra of Arabidopsis seedlings following ionizing radiation

|                | Background | γ-rays | 320-MeV $^{12}$C$^{6+}$ |
|----------------|------------|--------|------------------------|
|                | NM (×10$^{-5}$) | MF | NM (×10$^{-5}$) | MF | NM (×10$^{-5}$) | MF |
| Transition     |             |       |                        |       |       |   |
| G → A          | 9 (1.0)     | 11 (3.1)* | 2 (0.6)                |
| A → G          | 1 (0.1)     | 0 (<0.3) | 0 (<0.3)               |
| Transversion   |             |       |                        |       |       |   |
| G → T          | 1 (0.1)     | 2 (0.6)  | 3 (0.9)*               |
| G → C          | 3 (0.3)     | 2 (0.6)  | 2 (0.6)                |
| A → T          | 3 (0.3)     | 4 (1.1)** | 0 (<0.3)              |
| A → C          | 0 (<0.1)    | 1 (0.3)  | 2 (0.6)*               |
| Frameshifts    |             |       |                        |       |       |   |
| +1             | 0 (<0.1)    | 1 (0.3)  | 1 (0.3)                |
| –1             | 8 (0.9)     | 9 (2.5)* | 8 (2.4)*               |
| –2             | 0 (<0.1)    | 2 (0.6)* | 0 (<0.3)               |
| Deletion       | 8 (0.9)     | 9 (2.5)* | 6 (1.8)**              |
| Complex        | 0 (<0.1)    | 3 (0.8)* | 3 (0.9)*               |
| Total          | 33 (3.6)    | 44 (12.4)| 27 (8.2)               |

Mutant frequency is derived from the ratio of mutant clone numbers to predicted total analyzed clone numbers. NM = number of mutants, MF = mutant frequency. The statistical significance of differences between the irradiated group and the unirradiated (‘background’) group was examined using the Poisson test ($^*P < 0.01$, $$P < 0.05$).
seedlings. The simplest interpretation is that the γ-rays and 320-MeV $^{12}$C$^{6+}$ carbon beam induced a comparable number of DSBs, which led to deletions/insertions or complex mutations with similar frequencies (3.4 and 2.7 × 10$^{-5}$, respectively) (Fig. 2).

In this study, plural base substitutions, base substitution accompanied by a frameshift, and a deletion combined with an insertion, were categorized as ‘complex mutations’. These mutations are known to be induced by repair or replication of damaged DNA. For example, in the NHEJ repair process, 1–2 bp of microhomology near the DNA end is often utilized to rejoin the DSB. After annealing of the homologous base(s), the flanking mismatched base(s) is sometimes deleted or inserted by fill-in activity [23, 24]. Some DNA polymerase can fill the strand gap even when the template has a gap, a damaged base, or a mismatched base [25–27]. Such error-prone repair may lead to a deletion combined with an insertion, or a base substitution accompanied by a frameshift, which is likely to occur when the DSB end is not complementary. It is known that a template involving a damaged base causes primer–template misalignment, which may induce plural base substitutions or a base substitution accompanied by a frameshift [28, 29]. Such error-prone replications are likely to occur when DNA damage is not completely removed before replication. The mutation spectra showed that both γ-rays and 320-MeV $^{12}$C$^{6+}$ ion beams significantly induced complex mutations (Fig. 2). We therefore speculate that some DSBs, or other base damage induced by both radiations, are difficult to repair, and that the plant cells are subject to error-prone repair or replication.

Both γ-rays and 320-MeV $^{12}$C$^{6+}$ ion beams increased the frequency of −1 frameshift mutations (Table 2). It is thought that +1 and −1 frameshifts are mainly caused by primer–template misalignment at or near the damaged base during DNA replication [30–32]. In addition, it has been shown that some +1 and −1 frameshifts may be induced in the process of DSB repair by NHEJ [33, 34]. In yeast, it was estimated that ~50% of +1 and −1 frameshifts are induced by the NHEJ pathway [34]. Considering the few base substitutions induced by the 320-MeV $^{12}$C$^{6+}$ ion beams (Fig. 2), it is possible that the formation of base lesions was not elevated by 320-MeV $^{12}$C$^{6+}$ ion beams compared with that of unirradiated plants. In contrast, the frameshifting were significantly higher in 320-MeV $^{12}$C$^{6+}$ ion beam-irradiated plants than in unirradiated plants (Fig. 2). Therefore, it is likely that the increase in −1 frameshifts in irradiated seedlings was due to failed DSB repair by the NHEJ pathway, at least in part.

The G:C to T:A and A:T to C:G transversions are mainly accompanied by a frameshift, and a deletion combined with damaged base, or a mismatched base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29]. Such error-prone replications induce plural base substitutions or a base substitution accompanied by a frameshift, or a base substitution accompanied by a frameshift, and a deletion combined with damaged base [28, 29].
CONCLUSION
In conclusion, our mutation spectrum analysis suggested that plants might have a unique mechanism for maintaining genomic stability against radiation-induced oxidative damage. Further studies, including dose–response analysis of mutagenesis for both radiations, are needed to elucidate the details of the DNA repair pathways and radiation mutagenesis in higher plants.

ACKNOWLEDGEMENTS
We are grateful to Y. Nakatsu for providing plasmid containing the rpsL gene and E. coli strain DH10B/pFSE101. We thank S. Toki, M. Teranishi and H. Saika for valuable discussions, C. Suzuki for technical assistance, and K. Takimoto and A. Tanaka for the critical reading of this manuscript. We also thank all members of the Ion beam Mutagenesis Research Group for helpful support. Part of the results were presented at the EMBO Workshop ‘Genetic Stability and Change’, May 2012, as ‘Mutation Spectrum Analysis in Higher Plants’, by R.Y. et al.

FUNDING
This work was supported by the Atomic Energy Basic Infrastructure Research Initiative (210105) from the Japan Science and Technology Agency (JST), as well as Grants-in-Aid for Scientific Research (A) (24241028) and Scientific Research (C) (22570055) from the Japan Society for the Promotion of Science (JSPS).

REFERENCES
1. Blakely EA. Cell inactivation by heavy charged particles. Radiat Environ Biophys 1992;31:181–96.
2. Nikjoo H, Uehara S, Wilson WE et al. Track structure in radiation biology: theory and applications. Int J Radiat Biol 1998;73:355–64.
3. Yatagai F. Mutations induced by heavy charged particles. Biol Sci Space 2004;18:224–34.
4. Okayasu R, Okada M, Okabe A et al. Repair of DNA damage induced by accelerated heavy ions in mammalian cells proficient and deficient in the non-homologous end-joining pathway. Radiat Res 2006;165:59–67.
5. Asai-thamby A, Hu B, Chen DJ. Unrepaired clustered DNA lesions induce chromosome breakage in human cells. Proc Natl Acad Sci U S A 2011;108:8293–8.
6. Tanaka A, Shikazono N, Yokota Y et al. Effects of heavy ions on the germination and survival of Arabidopsis thaliana. Int J Radiat Biol 1997;72:121–7.
7. Shikazono N, Tanaka A, Kitayama S et al. LET dependence of lethality in Arabidopsis thaliana irradiated by heavy ions. Radiat Environ Biophys 2002;41:159–62.
8. Hase Y, Yamaguchi M, Inoue M et al. Reduction of survival and induction of chromosome aberrations in tobacco irradiated by carbon ions with different linear energy transfers. Int J Radiat Biol 2002;78:799–806.
9. Yokota Y, Hase Y, Shikazono N et al. LET dependence of lethality of carbon ion irradiation to single tobacco cells. Int J Radiat Biol 2003;79:681–5.
10. Shikazono N, Tanaka A, Watanabe H et al. Rearrangements of the DNA in carbon ion-induced mutants of Arabidopsis thaliana. Genetics 2001;157:379–87.
11. Shikazono N, Suzuki C, Kitamura S et al. Analysis of mutations induced by carbon ions in Arabidopsis thaliana. J Exp Bot 2005;56:587–96.
12. Kikuchi S, Saito Y, Ryuto H et al. Effects of heavy-ion beams on chromosomes of common wheat, Triticum aestivum. Mutat Res 2009;669:63–6.
13. Hase Y, Yoshihara R, Nozawa S et al. Mutagenic effects of carbon ions near the range end in plants. Mutat Res 2012;731:41–7.
14. Bjelland S, Seeberg E. Mutagenicity, toxicity and repair of DNA base damage induced by oxidation. Mutat Res 2003;531:37–80.
15. Nakabeppu Y, Sakumi K, Sakamoto K et al. Mutagenesis and carcinogenesis caused by the oxidation of nucleic acids. Biol Chem 2006;387:373–9.
16. Yoshihara R, Hase Y, Sato R et al. Mutational effects of different LET radiations in rpsL transgenic Arabidopsis. Int J Radiat Biol 2010;86:125–31.
17. Yoshihara R, Nakane C, Takimoto K. A new system for detecting mutations in Arabidopsis thaliana and the mutational spectra resulting from ethylmethanesulfonate treatment. J Radiat Res 2006;47:223–8.
18. Murai H, Takeuchi S, Nakatsu Y et al. Studies of in vivo mutations in rpsL transgene in UVB-irradiated epidermis of XPA-deficient mice. Mutat Res 2000;450:181–92.
19. Tanaka S, Fukuda M, Nishimura K et al. IRACM: a code system to calculate induced radioactivity produced by ions and neutrons. JAERI-Data/Code 97–019. (Tokai, Ibaraki: Japan Atomic Energy Research Institute) 1997 [in Japanese].
20. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 1980;8:4321–5.
21. Ward JF. DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparable. Prog Nucleic Acid Res Mol Biol 1988;35:95–122.
22. Wyman C, Kanaar R. DNA double-strand break repair: all’s well that ends well. Annu Rev Genet 2006;40:363–83.
23. Gorbunova V, Levy AA. How plants make ends meet: DNA reparability. Trends Plant Sci 1999;4:263–9.
24. Li P, Li J, Li M et al. Multiple end joining mechanisms repair a chromosomal DNA break in fission yeast. DNA Repair 2012;11:120–30.
25. Lee JW, Blanco L, Zhou T et al. Implication of DNA polymerase lambda in alignment-based gap filling for nonhomologous DNA end joining in human nuclear extracts. J Biol Chem 2004;279:805–11.
26. Zhou RZ, Blanco L, Garcia-Diaz M et al. Tolerance for 8-oxoguanine but not thymine glycol in alignment-based gap filling of partially complementary double-strand break ends by
DNA polymerase λ in human nuclear extracts. *Nucleic Acids Res* 2008;36:2895–905.

27. Pardo B, Ma E, Marcand S. Mismatch tolerance by DNA polymerase Pol4 in the course of nonhomologous end joining in *Saccharomyces cerevisiae*. *Genetics* 2006;172:2689–94.

28. Harfe BD, Jinks-Robertson S. DNA polymerase ζ introduces multiple mutations when bypassing spontaneous DNA damage in *Saccharomyces cerevisiae*. *Genetics* 2006;172:2689–94.

29. Zang H, Goodenough AK, Choi JY et al. DNA adduct bypass polymerization by *Sulfolobus solfataricus* DNA polymerase Dpo4: analysis and crystal structures of multiple base pair substitution and frameshift products with the adduct 1, N²-ethenoguanine. *J Biol Chem* 2005;280:29750–64.

30. Streisinger G, Okada Y, Emrich J et al. Frameshift mutations and the genetic code. *Cold Spring Harb Symp Quant Biol* 1966;31:77–84.

31. Bebenek K, Kunkel TA. Streisinger revisited: DNA synthesis errors mediated by substrate misalignment. *Cold Spring Harb Symp Quant Biol* 2000;65:81–91.

32. Bloom LB, Chen X, Fygenson DK et al. Fidelity of *Escherichia coli* DNA polymerase III holoenzyme. The effects of β, y complex processivity proteins and ε proofreading exonuclease on nucleotide misincorporation efficiencies. *J Biol Chem* 1997;272:27919–30.

33. Heidenreich E, Eisler H. Non-homologous end joining dependency of γ-irradiation-induced adaptive frameshift mutation formation in cell cycle-arrested yeast cells. *Mutat Res* 2004;556:201–8.

34. Lehner K, Mudrak SV, Minesinger BK et al. Frameshift mutagenesis: the roles of primer-template misalignment and the nonhomologous end-joining pathway in *Saccharomyces cerevisiae*. *Genetics* 2012;190:501–10.

35. Grollman AP, Moriya M. Mutagenesis by 8-oxoguanine: an enemy within. *Trends Genet* 1993;9:246–9.

36. Nakabeppu Y, Tsuchimoto D, Furuiichi M et al. The defense mechanisms in mammalian cells against oxidative damage in nucleic acids and their involvement in the suppression of mutagenesis and cell death. *Mutat Res* 2007;614:69–76.

37. Britt AB. Repair of damaged bases. The Arabidopsis Book 2002;1:e0005. http://www.bioone.org/doi/full/10.1199/tab.0005 (26 February 2013, date last accessed).

38. Murphy TM. What is base excision repair good for?: knockout mutants for FPG and OGG glycosylase genes in *Arabidopsis*. *Physiol Plant* 2005;123:227–32.

39. Yoshimura K, Ogawa T, Ueda Y et al. AtNUDX1, an 8-oxo-7,8-dihydro-2'-deoxyguanosine 5'-triphosphate pyrophosphohydrolase, is responsible for eliminating oxidized nucleotides in *Arabidopsis*. *Plant Cell Physiol* 2007;48:1438–49.

40. An Q, Robins P, Lindahl T et al. C◊T mutagenesis and γ-radiation sensitivity due to deficiency in the Smug1 and Ung DNA glycosylases. *EMBO J* 2005;24:2205–13.

41. Córdoba-Cañero D, Dubois E, Ariza RR et al. *Arabidopsis* uracil DNA glycosylase (UNG) is required for base excision repair of uracil and increases plant sensitivity to 5-fluorouracil. *J Biol Chem* 2010;285:7475–83.

42. Roldán-Arjona T, Ariza RR. Repair and tolerance of oxidative DNA damage in plants. *Mutat Res* 2009;681:169–79.