Comparison of biological effect between low- and high-LET irradiation on DSB repair in the filamentous fungus *Neurospora crassa*†

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Ionizing radiations induce various cellular injuries, especially DNA double-strand breaks (DSBs). To obtain insights on the biological effects on several forms of ionizing radiations causing DSBs, we examined the cell killing effect, mutation frequency, and mutation type profile using the model filamentous fungus *Neurospora crassa*.[1)]

Two repair pathways, homologous recombination (HR) repair and non-homologous end-joining (NHEJ), recognizes and processes DSBs to maintain the integrity of genetic information. Asexual spores of wild-type *Neurospora crassa* and two DSB repair-deficient mutants (NHEJ-related *mus-52* knockout strain and HR-related *mei-3* nonsense mutant) were irradiated with X-ray (200 kVp, 2–5 keV/μm) as a low-linear energy transfer (LET) beam, and C- (135 MeV/nucleon, 30 keV/μm), Ar- (95 MeV/nucleon, 287 keV/μm), and Fe- (90 MeV/nucleon, 641 keV/μm) ion beams as high-LET beams. The survival rates were examined by a colony formation assay. Mutagenesis frequencies at *ad-3* loci were calculated as a ratio of visual purple colonies to total colonies. Mutation spectra at the *ad-3B* gene of collected mutants were examined by DNA sequencing. The experimental schema is shown in Fig. 1.

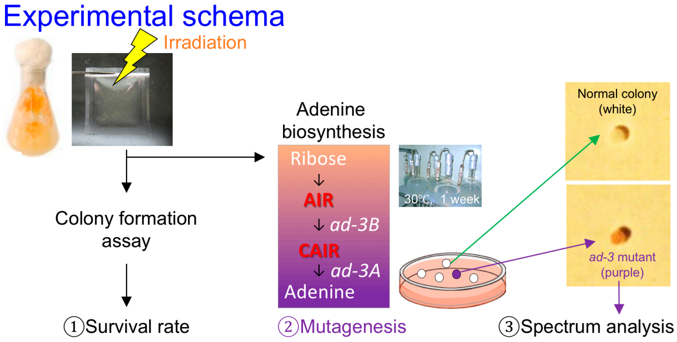

Fig. 1. Experimental schema of biological effect evaluation.

To determine the relative biological effectiveness (RBE) values on the killing effect, the doses with 50% survival in each ion-beam irradiation were compared with those obtained after X-ray irradiation as the reference radiation. RBEs of C-, Ar-, and Fe-ion beams were calculated to be 3.0, 7.4, and 5.8, respectively. Therefore, the Ar-ion displayed the greatest lethal effect on *Neurospora crassa*, which was found to be 2.5 times more effective than the C-ion. The rank order of RBE was Ar- > Fe- > C-ion > X-ray.

The killing effect profile of the X-ray irradiation was quite similar to the C-ion beam. The *mus-52* strain exhibited sensitivity to X-ray similar to that observed in the *mei-3* strain at 100 Gy. However, little additional cell death was observed at 200, 300, and 400 Gy. The survival rate in response to the high-LET ion-beam irradiation progressively decreased with the absorbed dose for all irradiated strains. In the case of C-ion beam irradiation, the sensitivity of the *mus-52* strain was higher than that of the wild-type and *mei-3* strains at low doses. In the case of Ar-ion beam irradiation, the *mei-3* strain exhibited the highest sensitivity at all doses. In the case of Fe-ion beam irradiation, the survival curves of three strains did not differ at lower doses, whereas obvious differences in sensitivity (*mei-3* > *mus-52* >WT) were observed at higher doses.

The mutation frequency in the *ad-3* loci indicated that *mei-3* > wild type > *mus-52* in all irradiated beams. The Ar-ion beam demonstrated a higher mutagenic capability than the Fe-ion beam in both the wild-type and *mei-3* strains. The mutation frequency of the wild-type strain was lower in response to Fe-ion beams than the X-rays. The deletion mutations were the most frequent but the deletion size increased with the increasing value of LET. The high-LET ion beams tended to induce larger deletions, with Fe-ion beams inducing deletions > 100 bp and Ar-ion beams causing deletions that ranged from 2 to 100 bp. Alternatively, low-LET X-ray induced deletions of 1 bp.

Our results indicated that high-LET ion beams exhibit higher cell killing and increased deletion size than low-LET X-ray in *Neurospora crassa*. These different LET-dependent phenomena may be due to the physical properties of each radiation and repair mechanism of the damage induced in *Neurospora crassa*. Our results may be useful in selecting the appropriate beam source to mutagenize fungi for further research and agricultural applications. For example, C-ion beams, which predominantly cause single-nucleotide deletions and base exchanges but few large deletions compared with higher-LET Ar- and Fe-ion beams, may be useful in generating novel fungal strains harboring mutation in essential genes without destroying the neighboring genes.

Reference
1) L. Ma *et al.*, Fungal Biol. **117**, 227–38 (2013).

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