Efficient Repair of DNA Damage Induced by Heavy Ion Particles in Meiotic Prophase I Nuclei of *Caenorhabditis elegans*

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The effects of heavy ion particle irradiation on meiosis and reproductive development in the nematode *Caenorhabditis elegans* were studied. Meiotic pachytene nuclei are significantly resistant to particle irradiation by the heavy ions carbon and argon, as well as to X-rays, but not UV, whereas diplotene to diakinesis stage oocytes and early embryonic cells are not. Chromosomal abnormalities appear in mitotic cells and in maturing oocytes irradiated with heavy ion particles during the diplotene to the early diakinesis stages, but not in oocytes irradiated during the pachytene stage. The pachytene nuclei of *ced-3* mutants, which are defective in apoptosis, are similarly resistant to ionizing radiation, but pachytene nuclei depleted for *Ce-atl-1* (ataxia-telangiectasia like 1) or *Ce-rdh-1/rad-51* are more sensitive. Pachytene nuclei thus appear to effectively repair heavy ion-induced DNA damage by the meiotic homologous recombination system.

INTRODUCTION

Meiosis is essential for generating haploid gametes during sexual reproduction. An important feature of meiotic prophase I is homologous recombination, which is accompanied by dynamic chromosomal changes, including pairing and synapsis. Meiotic recombination is initiated by programmed DNA double-strand breaks (DSBs) catalyzed by the widely conserved eukaryotic Spo11 protein.1–3] In all cells, DSBs caused by ionizing radiation and some chemical agents can induce great damage to genetic material. Heavy ion particle irradiation, which is more effective than gamma-irradiation in inducing tumors in animals,4–6] has been developed as a cancer therapy for killing tumor cells.7] Recently, Masumura *et al.*8] reported that carbon particles induce deletions, generally greater than 1,000 base pairs in size, whereas gamma-rays induce base substitutions and deletions of less than 100 base pairs.

We previously demonstrated that meiotic pachytene nuclei of the nematode *Caenorhabditis elegans* are hyper-resistant to ionizing radiation, whereas early (post-fertilization) embryonic cells are not.9] The high level of expression of enzymes involved in meiotic homologous recombination accounts for these differences in resistance. We also reported that *Ce-atl-1*, which is related to *ATM* (ataxia-telangiectasia mutated) and *ATR* (*rad3*+ related), is important for early embryogenesis and chromosome segregation in *C. elegans*.10] Fungal members of the *ATM* family, including *Saccharomyces cerevisiae* MEC1/ESR1 and *Schizosaccharomyces pombe* rad3*, are involved in cell-cycle checkpoint control induced by DNA damage caused by UV light, X-rays, alkylating agents, and hydroxyurea.11–16] Mammalian AT cells also exhibit hypersensitivity to ionizing radiation and are defective in DSB repair after irradiation.17–20] In this study, we evaluated whether DNA lesions induced by heavy ion particle irradiation in *C. elegans* could be effectively repaired by the meiotic homologous recombination system. In addition, we studied the effects of depletion of *Ce-atl-1* on the hyper-resistance of meiotic pachytene nuclei to ionizing radiation.

MATERIALS AND METHODS

*C. elegans* manipulations

Wild-type N2 and *ced-3* (n717) MT1522 mutant21] *C. elegans* hermaphrodites were generously supplied by the *C. elegans* Genetic Center. General methods used for *C. elegans* culture have been described.22] *Ce-rdh-1/rad-51* and *Ce-atl-1* RNA interference (RNAi) procedures were carried out as described previously.9,10,23] All nematode experiments were performed at 20°C.
Effects of X-rays, carbon and argon ion particles, and UV light on egg hatching frequency and chromosome structure

For each experiment, eight young gravid *C. elegans* hermaphrodites were irradiated with heavy ion particles (carbon and argon), X-rays, or UV light. Accelerated carbon mono peak beams of a carbon ion particle (LET 23 keV/µm, specific energy 135 MeV/u) and accelerated argon mono peak beams of an argon ion particle (LET 311 keV/µm, specific energy 95 MeV/u) were generated at the Ring Cyclotron in RIKEN (Saitama, Japan). 150 kVp X-ray irradiation was delivered at a dose rate of 1.6 Gy per min (Hitachi Co., Ltd., model 1520R, filtering with 0.5 mm Al and 0.1 mm Cu). UV irradiation (254 nm) was carried out with a UV transilluminator and measured with a UVX digital radiometer (UVP, Inc.). Following irradiation, the animals were immediately transferred to new culture plates, and the egg-hatching frequency was measured for half of the irradiated animals (F1 generation) after an additional 24 h of incubation at 20°C. The gonadal nuclei of the remaining animals were stained with 4′,6- diamidino-2-phenylindole (DAPI). For RNAi experiments, young gravid hermaphrodites were X-irradiated (20 Gy) 24 h after injection with dsRNA specific to *Ce-rdh-1*/rad-51 or *Ce-atl-1*, and the hatching frequency of eggs laid from 8 to 24 h after X-irradiation was measured.

RESULTS

Meiotic pachytene nuclei are resistant to heavy ion particle irradiation

The sequential phases of oogenesis can be observed in the gonads of a single young gravid hermaphrodite. Each such animal has about 15 fertilized eggs in the uterus, and the two gonads contain about 15 oocytes at the diakinesis stage, about 10 oocytes at the diplotene stage, and more than 200 pachytene nuclei. Eggs are usually retained in the uterus until about the 32-blastomere stage (Fig. 1). The survival of eggs, as measured by the hatching frequency, laid by young gravid hermaphrodites after irradiation or a treatment with DNA-damaging agents can be easily measured. In this study, we measured the hatching frequency of eggs from four young gravid *C. elegans* hermaphrodites after irradiation with X-rays, carbon ion and argon ion particles, and UV light, as shown in Fig. 2. The HD50 (50% hatching rate) values for eggs laid 0–8 h and 8–22 h after irradiation were calculated from Fig. 2, and are summarized in Table 1. These results indicate that, compared with eggs laid 0–8 h after irradiation, eggs laid 8–22 h thereafter were less sensitive to X-rays, carbon ion particles, argon ion particles, and UV, by factors of 2.9, 3.0, 2.2 and 1.6, respectively. The average number of eggs laid by each young gravid hermaphrodite 0–8 h and 8–22 h after irradiation was 37.2 ± 5.9 and 65.1 ± 6.9, respectively. Extrapolating backwards in time, eggs laid between 0 and 8 h had been irradiated at the 32-blastomere stage and at the diakinesis to diplotene stages of oogenesis, while eggs laid between 8 and 22 h had been irradiated at the pachytene stage. These results indicate that meiotic pachytene oocytes are less sensitive to X-rays, carbon ion particles and argon ion particles than are diplotene- to diakinesis-stage oocytes and fertilized eggs.

Chromosomal aberrations observed after X-ray and argon ion particle irradiation

Gonadal chromosome structures were studied at 4 and 24 h following irradiation with X-ray or argon ion particles, as shown in Fig. 3. Oocytes observed at 4 h and 24 h after irradiation had been exposed during the diplotene to early diakinesis stages and during the pachytene stage, respectively. In the unirradiated control, round mitotic nuclei were observed in the distal tip of the gonad, and six condensed diakinesis-stage bivalents were observed in maturing oocytes at the point of entry into the spermatheca (Figs. 1 and 3A, 0 Gy control). Four hours after X-irradiation (more than 50 Gy), the chromosomes of maturing oocytes at the same position appeared to be abnormally narrow and kinked, and were often fragmented, but the chromosomes of mitotic nuclei in the gonad tip did not show such abnormalities. In contrast, 24 h after X-irradiation (less than 100 Gy), the chro-
mosomes of maturing oocytes appeared normal and formed six condensed bivalents, but abnormally enlarged and broken nuclei could be seen in the mitotic region of the gonad tip (Fig. 3A). Similar phenomena were observed after argon ion particle irradiation (Fig. 3B). The presence of chromosomal abnormalities in maturing oocytes (prior to their entrance into the spermatheca) is consistent with the observed hatching rate of the eggs.

Table 1. Doses of X-rays, carbon ion particles, argon ion particles and UV resulting in an egg hatching rate of 50% (HD50).

|                      | Dose producing HD50 for eggs laid |
|----------------------|-----------------------------------|
|                      | 0–8 h after irradiation | 8–22 h after irradiation |
| X rays               | 55 Gy                      | 160 Gy                     |
| Carbon ion particles | 40 Gy                      | 120 Gy                     |
| Argon ion particles  | 30 Gy                      | 65 Gy                      |
| UV (254 nm)          | 65 Jm⁻²                    | 100 Jm⁻²                   |

Fig. 2. Hatching rate of eggs laid by wild-type *C. elegans* hermaphrodites following irradiation with X-rays, heavy ion particles (carbon and argon), and UV light. For each treatment, four young gravid hermaphrodites (N2 wild-type) were irradiated with 10–200 Gy X-rays (A), 20–200 Gy carbon particles (B), 10–100 Gy argon particles (C), and 50–100 Jm⁻² UV (254 nm) (D). The eggs laid from 0 h to 8 h following irradiation and from 8 h to 22 h following irradiation were collected and their hatching rates were scored. For oocytes irradiated at early embryogenesis and diakinesis to diplotene, 37.2 ± 5.9 eggs were laid per animal; indicated as closed circles. For oocytes irradiated at pachytene, 65.1 ± 6.9 eggs were laid per animal; indicated as open circles. Independent duplicate experiments were carried out and the averages of scores were plotted.

Fig. 3. Chromosomal structures in the mitotic nuclei of the gonad tip and in maturing oocytes just before entrance into the spermatheca 4 and 24 h after X-irradiation (A) and argon particle (B) irradiation, as observed by staining with DAPI. White arrowheads and white arrows indicate chromosomal fragmentations and abnormally enlarged nuclei, respectively. Scale bars represent 10 µm.

Genes involved in the hyper-resistance of meiotic pachytene nuclei to radiation

Our previous report indicates that silencing of the *Ce-rdh-1/ rad-51* gene by RNAi causes meiotic pachytene cells to become more sensitive than early embryonic cells to X-irradiation, and suggests that the ionizing radiation hyper-resistance of meiotic pachytene cells is due to the strong expression of one or more enzymes involved in meiotic homologous recombination. To verify whether the checkpoint control that activates the DNA repair system and/or apoptosis also regulates the radiation hyper-resistance of meiotic pachytene nuclei, we measured the radiation sensitivity of meiotic cells that were depleted for *Ce-atl-1* by RNAi, or that bore the *ced-3* (n717) mutation. *Ce-atl-1* is a homolog of the mammalian ataxia-telangiectasia mutated gene.
Table 2. Relative hatching rate of X-irradiated pachytene oocytes of Ce-atl-1 and Ce-rdh-1/rad-51 by RNAi.

|                         | Relative hatching rate of eggs laid 8 to 24 h after 20 Gy X-irradiation |
|-------------------------|-------------------------------------------------------------------------|
| Wild type               | 0.98 (0.97±0.99)                                                        |
| ced-3 (n717)            | 0.99 (0.98±0.99)                                                        |
| Ce-atl-1 RNAi           | 0.60 (0.39±0.65)                                                        |
| Ce-rdh-1/rad-51 RNAi    | 0.12 (0.083±0.69)                                                        |

(hatching rates of X-irradiated eggs/unirradiated control) In each experiment, four young gravid hermaphrodites were exposed to 20 Gy X-rays, and the hatching rates of eggs laid from 8 h to 24 h after irradiation were scored. Independent duplicate experiments were carried out and score averages were calculated.

DISCUSSION

In the adult C. elegans hermaphrodite, germline nuclei in the tip of the gonad arm divide mitotically and thereafter enter meiotic prophase I, progressing from the leptotene to the zygote stages in the proximal region (Fig. 1). This linear array of the developmental phases of oogenesis makes the adult hermaphrodite a convenient model system to study the effects of DNA damaging agents on both meiotic and mitotic cells. In this study, we found that meiotic pachytene oocytes are less sensitive to heavy ion particle irradiation, as well as to X-irradiation, than are diploptene to diakinesis stage oocytes and fertilized eggs (Fig. 2).

Heavy ion particle irradiation induces DNA lesions with larger deletions than does low-LET ionizing radiation. Masumura et al.80 reported that carbon-particle irradiation significantly increases the frequency of mutations in the liver, spleen, and kidney of gpt delta transgenic mice. They also revealed that carbon particles induce DNA deletions greater than 1,000 base pairs in extent. The incidence of DNA lesions is known to increase in a LET-dependent manner.24 In addition, isochromatic breaks are frequently introduced by high LET ionizing radiation, including heavy ion particle irradiation.25,26

Gartner et al.27 reported that a checkpoint mediates DNA damage-induced apoptosis during C. elegans meiosis. This activity, however, is relatively weak, since defective oocytes with DNA damage are not completely removed by apoptosis, and dead eggs are commonly produced by C. elegans. This may explain why the radiation hyper-resistance of meiotic pachytene cells was not affected by the ced-3 mutation (Table 2).

The homologous recombination that takes place between the zygote to pachytene stages of meiotic prophase I makes use of a specialized version of the DNA DSB repair pathway28–33 and is initiated by DSBs catalyzed by the widely conserved Spo11 protein.1–3 DSB ends are processed to form single-stranded tails by an exonuclease activity associated with the Rad50, Xrs2 and Mre11 protein complex (termed the Mre11 complex).11–35 These single-stranded tails invade a homologous DNA duplex with the aid of a protein complex that includes the reca-like proteins Rad51 and Dmc1.36–41 The Mre11 complex and Rad51 are also required for the recombinational repair of DNA DSBs induced by several genotoxic agents.30,35,39 Phosphorylation of Mre11 and Nbs1 (a mammalian Xrs2 homolog), which is dependent on the Atm protein function, activates an S-phase checkpoint system.30–41 In addition, Atm-deficient mice are sterile due to the arrest of gametogenesis in the early prophase of meiosis I, similar to what is seen for Dmc1-deficient mice.43,46 These findings suggest that Ce-atl-1 probably functions as an activator for one or more enzymes involved in meiotic homologous recombination, and in its absence meiotic cells are more radiation sensitive (Table 2).

Taken together, these observations suggest that meiotic pachytene oocytes can effectively repair DNA damage induced by heavy ion particle irradiation and that the widely conserved meiotic recombination system is likely to be responsible for this repair.

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