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Extreme resistance of bdelloid rotifers to ionizing radiation

Eugene Gladyshev* and Matthew Meselson*†‡

*Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138; and †Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, MA 02543

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Rotifers of class Bdelloidea are common invertebrate animals with highly unusual characteristics, including apparently obligate asexuality, the ability to resume reproduction after desiccation at any life stage, and a paucity of transposable genetic elements of types not prone to horizontal transmission. We find that bdelloids are also extraordinarily resistant to ionizing radiation (IR). Reproduction of the bdelloids Adineta vaga and Philodina roseola is much more resistant to IR than that of Euchlanis dilatata, a rotifer belonging to the desiccation-intolerant and facultatively sexual class Monogononta, and all other animals for which we have found relevant data. By analogy with the desiccation- and radiation-resistant bacterium Deinococcus radiodurans, we suggest that the extraordinary radiation resistance of bdelloid rotifers is a consequence of their evolutionary adaptation to survive episodes of desiccation encountered in their characteristic habitats and that the damage incurred in such episodes includes DNA breakage that is repaired upon rehydration. Such breakage and repair may have maintained bdelloid chromosomes as colinear pairs and kept the load of transposable genetic elements low and may also have contributed to the success of bdelloid rotifers in avoiding the early extinction suffered by most asexuals.

Results

Reproduction. The reproduction of Adineta vaga and Philodina roseola was much more resistant to IR than that of Euchlanis dilatata. The relative parental fecundity of E. dilatata was reduced 10-fold by a dose of ∼200 Gy, whereas a corresponding reduction in bdelloid fecundity required a dose five times greater. A similar difference between the monogonont and the bdelloids was seen in relative parental fertility. The reproductive effects of IR extended to the daughters of irradiated parents, with dose-dependent reductions in fecundity and fertility almost as great as seen for the parents. Even at the highest doses tested, irradiated rotifers remained active for the 1–2 weeks during which they were observed.

Table 1 presents data on parental and F₁ reproduction of rotifers exposed to doses up to 280 Gy for the monogonont and 1,120 Gy for the bdelloids. For each dose group of 96 animals, including nonirradiated controls, Table 1 gives the number of animals that deposited eggs, the number that produced at least one daughter, the proportion of animals giving daughters normalized to the corresponding proportion for nonirradiated rotifers (relative parental fertility), and, for E. dilatata and P. roseola, the number of hatched eggs per daughter normalized to the number produced per daughter by daughters of nonirradiated rotifers (relative F₁ fecundity). For E. dilatata, the number of hatched eggs was counted directly, whereas for the bdelloids it was taken as the number of progeny, as described in Materials and Methods.

The proportion of parental rotifers not depositing eggs averaged ∼5% for bdelloids and 12% for E. dilatata, independent of dose. Such animals were apparently infertile or had reached the end of their reproductive lifespan, ∼5 days for E. dilatata and 2 weeks for the bdelloids under our conditions. The total number of eggs deposited per individual averaged 12 and 19 for A. vaga and P. roseola, respectively, whereas the average for E. dilatata was ∼3 in the first two experiments and 6 in the third, a variation possibly reflecting differences in the age of the parents.

Relative parental and F₁ fecundity and fertility are plotted as a function of dose in Fig. 1. The two bdelloid species are similar in their dose–response relations, with an indication that reproduction of P. roseola may be a little more radiosensitive than that of A. vaga. Parental fecundity, which measures the total number of daughters produced by irradiated animals, is only moderately more radiosensitive than parental fertility, which requires only the production of at least one daughter. Bdelloid parental and F₁ fertility and fecundity display an extended shoulder in their dose–response relations, which they are able to repair.

Author contributions: E.G. and M.M. designed research; E.G. performed research; E.G. and M.M. analyzed data; and M.M. wrote the paper.

The authors declare no conflict of interest.

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Table 1. Reproductive performance of bdelloid and monogont rotifers exposed to IR

<table>
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<th>Species</th>
<th>Wells with eggs, n</th>
<th>Wells with F1, n</th>
<th>Total eggs, n</th>
<th>Total eggs hatched, n</th>
<th>Relative parental fertility</th>
<th>Relative parental fecundity</th>
<th>Wells with eggs, n</th>
<th>Wells with F1, n</th>
<th>Total eggs, n</th>
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</table>

Rotifers were irradiated on ice for various times with a 137Cs source delivering 140 Gy per h. Parental and F1 relative fecundity and relative fertility are defined in Materials and Methods. The number of daughters transferred to new wells was in each case equal to the number of parental wells in which daughters were produced. For E. dilatata the number of hatched eggs was counted directly. For bdelloids it was taken as the number of progeny, as described in Materials and Methods.

Discussion

Reproduction of Irradiated Rotifers. We find that reproduction of the bdelloid rotifers A. vaga and P. roseola is much more resistant to 137Cs γ-radiation than that of the monogonont rotifer E. dilatata. A dose of ~200 Gy reduced the number of daughters produced by the monogonont by ~90%, whereas a corresponding reduction in bdelloid fecundity required a dose five times higher. The principal difference between the bdelloid and monogonont dose–response relations lies in the extended shoulder over which bdelloid fertility and fecundity remain high before falling steeply, whereas only a much less extended shoulder is found for the monogonont. The sterilizing effect of IR extends to the daughters of irradiated parents, with dose–response relations shifted to somewhat higher doses. Taking the size of the A. vaga genome as ~180 Mbp (R. Gregory, personal communication), the average molecular size of 350 kbp observed after a dose of 560 Gy, causing a reduction in fecundity of only 20%, corresponds to ~500 DSBs per genome.

The reproduction of A. vaga and P. roseola is more resistant to IR than that of any other metazoan for which we have found relevant data. Such resistance to IR is likely to be characteristic of the Bdelloidea generally, as A. vaga and P. roseola represent families that diverged millions of years ago (14) and because of the probable association, discussed below, of bdelloid radiation resistance with anhydrobiosis. Doses of IR or high-energy electrons required to prevent reproduction have been measured for many arthropod species at various life stages, mainly for purposes of pest control. In a tabulation of such sterilizing doses for male arthropods, which are generally more radiosensitive than females, including 285 species representing 61 families of insects and other arthropods, irradiated mainly as pupae or nymphs and generally achieving at least 90% parental or F1 sterility, the average dose is ~110 Gy (ref. 15 and www.ididas.iaea.org/ididas/). For the most radio-resistant species in the tabulation, the lepidopteran Spilosoma (Diaceira) obliqua, a dose of 200 Gy to pupal or adult males, the most resistant sex, resulted in >99% F1 sterilization.8 Among other invertebrates, sensitivity to IR has been measured for X-irradiation of Caenorhabditis elegans eggs in buffer, for which ~30 Gy reduced the number developing into adults by 90%.

References

studied in the tardigrades anhydrobiosis at any life stage, resistance to IR has been observed in animal species known to be capable, like bdelloid rotifers, of surviving after exposure to 70 Gy (16, 17). Among the very few species with extraordinary resistance, two tardigrade species, E. roseola and P. roseola, which produced only sterile eggs after exposure to 70 Gy (24, 25) yet is essentially unaffected even at the highest doses tested, suggesting either that egg production can proceed without maturation divisions or that the sterilizing effect of IR is manifested only later in development.

The observation that rotifers rendered sterile by IR nevertheless remain active is consistent with the fact that somatic cell division is already complete at the time of hatching (20–23) and with the general observation that metabolic processes are less radiosensitive than cell division. Egg production, however, involves two maturation divisions starting from primary oocytes present in the hatched animal (24, 25) and the snail Zonitoides arboreus, which produced only sterile eggs after exposure to 70 Gy (16, 17). Among the very few animal species known to be capable, like bdelloid rotifers, resistance to IR has been studied in the tardigrades Richtersius coronifer and Milnesium tardigradum, which produced only sterile eggs after exposure to the lowest doses tested, 500 and 1,000 Gy, respectively (18, 19).

As we find for bdelloid and monogonont rotifers, the effect of IR on other animals generally extends beyond the irradiated parents to their progeny. F1 sterility induced by parental irradiation, widely made use of in insect control programs, is associated with chromosome aberrations, mutations, and possibly deleterious epigenetic effects expressed as impaired reproduction in generations beyond that which is irradiated.

The observation that rotifers rendered sterile by IR nevertheless remain active is consistent with the fact that somatic cell division is already complete at the time of hatching (20–23) and with the general observation that metabolic processes are less radiosensitive than cell division. Egg production, however, involves two maturation divisions starting from primary oocytes present in the hatched animal (24, 25) yet is essentially unaffected even at the highest doses tested, suggesting either that egg production can proceed without maturation divisions or that the sterilizing effect of IR is manifested only later in development.

**Basis of Radiation Resistance.** The extraordinary resistance of bdelloid reproduction to IR cannot be attributed to an unusually small genome size, as the genome sizes of A. vaga and P. roseola are comparable to or larger than those of C. elegans, Drosophila melanogaster and many other animals that display no comparable radiation resistance (15, 21, 26, 27). Nor can bdelloid radiation resistance be explained by genetic redundancy or the multitudes of repair options available to homologous repair. Belloid primary oocytes are in G1 (21, 27) and, as described in ref. 28, bdelloids appear to be degenerate tetraploids, with only two copies of most genes.

The possibility that bdelloid radiation resistance reflects a novel chromosome alignment that keeps homologous regions together, facilitating access of broken ends to homologous templates, has been proposed for D. radiodurans where it appears unlikely for a number of reasons (29). Moreover, neither chromosome alignment nor the novel variant of synthesis-dependent strand annealing proposed to facilitate DSB repair at high levels of DNA breakage (30) provide an explanation for the extended shoulder in bdelloid and D. radiodurans dose–response relations that distinguishes them from radiosensitive organisms.

It might be thought that the measured efficiency of IR breakage rather that the germ line DNA is somehow protected. We think this unlikely as no such resistance is seen in D. radiodurans or in other radiation-resistant prokaryotes, where the number of breaks made per Mbp per Gy, ∼0.004 (31, 32), is not significantly different from that for radiosensitive bacteria, fungi, and animals. More likely, the ability of bdelloids to remain fertile after extensive DNA breakage and other damage inflicted by IR derives from attributes of the systems that repair such damage or those that protect the repair systems, or both.

The finding that IR killing of D. radiodurans is paralleled by oxidative damage to its proteins has led to the proposal that the extraordinary radio resistance of D. radiodurans results from unusually effective protection of its proteins against toxic products of IR (33), a conclusion consistent with the ability of extracts of D. radiodurans to protect E. coli from radiation killing (34) and with the much higher scavenging ability for reactive oxygen species in extracts of D. radiodurans than in similarly prepared extracts of E. coli (35). Although protein damage in highly radiosensitive species may cause lethality even before significant DNA damage occurs (36, 37), this is clearly not the case for bdelloid rotifers, where hundreds of DSBs are made by a dose of IR that causes but little reduction in fecundity.
The picture that emerges from the above considerations is that a major defense against radiation damage in *D. radiodurans* and bdelloid rotifers, accounting for the distinctive shoulders in their dose–response relations, is an enhanced capacity for scavenging the reactive molecular species generated by IR and that the proteins and other cellular components thereby protected include those essential for the repair of broken DNA. This, however, leaves the question of why DNA, in both *D. radiodurans* and bdelloid rotifers, is not similarly protected but instead accumulates DSBs in direct proportion to dose and to the same extent per Gy per Mbp as in radio-sensitive organisms (Fig. 1). A possible answer lies in evidence that IR-induced DSBs result from closely spaced scission events on opposite chains of the DNA duplex that are caused by clusters of hydroxyl radicals or other reactive species generated in close association with DNA duplex that are caused by clusters of hydroxyl radicals or other reactive species generated in close association with DNA that are relatively immune or inaccessible to elimination by scavengers (38, 39).

**IR and Desiccation.** Naturally occurring bacterial isolates that are highly resistant to IR are also resistant to desiccation, and mutations that diminish radiation resistance in *D. radiodurans* also reduce its resistance to desiccation. These observations and the low levels of IR in the natural habitats of *D. radiodurans* and other radioresistant bacteria indicate that their radiation resistance is a consequence of evolutionary adaptation to survive desiccation (40). Similarly, the extraordinary radiation resistance of bdelloid rotifers is almost certainly an adaptation to survive in their characteristic ephemeral aquatic habitats.

The association of resistance to IR with anhydrobiosis and the observation that desiccation, like exposure to IR, is accompanied by the production of reactive oxygen species (41) and protein oxidation in diverse biological systems and that proteins of desiccation-resistant bacteria are protected against such damage (33, 42) indicates that at least part of the damage caused by desiccation is the same as that caused by radiation. In *D. radiodurans* this includes DNA breakage, which increases with desiccation time and can reach a high level before there is appreciable killing (40). Although DNA breakage has not been investigated in desiccated bdelloid rotifers, their ability to resume reproduction as a function of desiccation time also exhibits an extended shoulder (2), and it seems likely that, as in *D. radiodurans*, desiccation is accompanied by extensive DNA breakage, implying that the evolution of bdelloid genomes has been accompanied by unusually frequent and extensive DNA breakage and repair.

**Implications for Bdelloid Genome Structure and Evolution.** Accurate repair of a DSB requires the presence of a homologous template. Although bdelloid primary oocytes are in G1 and therefore lack sister chromatids, the requirement for homologous templates would be satisfied if bdelloid primary chromosomes were present as colinear pairs. In fact, this appears to be the case, as described in ref. 28, showing that bdelloids are probably degenerate tetraploids. Homology of colinear chromosomes sufficient for efficient repair could be maintained by selection against clones in which such homology becomes inadequate to support DSB repair by template-dependent repair processes. Local relaxation of the requirement for homology might occur, however, if subor neo-functionality becomes established at particular sites and where the decreased efficiency of repair is more than offset by selection against clones in which such sites have been homogenized.

There are several ways in which the anhydrobiotic lifestyle of bdelloid rotifers may have contributed to their success in avoiding the early extinction that is the usual fate of asexuals. Desiccation can be of benefit to surviving bdelloids by facilitating their dispersal and freeing them from desiccation-sensitive competitors, parasites, pathogens, and predators. In addition to such ecological factors, bdelloid anhydrobiosis may have genetic benefits. In degenerate tetraploids, homogenization associated with the repair of desiccation-damaged DNA may significantly accelerate the appearance of clones homozygous for recessive beneficial mutations in a few days (43).

Finally, frequent DSB repair may act to keep the deleterious load of transposable elements low and account for the remarkable paucity in bdelloid genomes of transposable elements (TEs) of types that are not prone to horizontal transfer, namely long interspersed element LINE-like and other nonviral retrotransposons (44–46). TEs could be deleted or truncated by single-strand annealing mediated by microhomologies and, if not homozygous, by removal of nonhomologous 3’ ends in the course of synthesis-dependent strand annealing (47). Synergistic selection against TEs could occur, for example, if in the course of DSB repair an extended invading strand dissociates after copying a donor TE. Reversion and continued replication may then occur either at a eutopic site, leading to faithful repair, or at an ectopic TE, giving a lethal or semilethal nonreciprocal translocation (48). As the probability of a DSB being near a TE and the probability of ectopic reintegration are both proportional to the density of homologous TEs, such template switching would cause synergistic selection against them. Nonreciprocal translocations, probably mediated by such template switching, accompany the formation of novel TEs in some species of *D. radiodurans* and *D. viridis* (49). As the probability of a DSB being near a TEs is not zero, the presence of substantial numbers of IR-induced TEs in the genome of a species may account for frequent nonreciprocal translocations, and for the extraordinary paucity of TEs in bdelloid genomes.

**Irradiation and Scoring Reproductive Performance.** Approximately 1,000 rotifers were transferred by pipette to a 5-cm plastic Petri dish containing 5 ml of half-strength MBL medium and kept 6–10 h at room temperature to clear the animals of food organisms. As rapid cooling was found to be lethal, the dish was wrapped in a cloth towel, and kept for 6 h in the cold room before being taken, on ice, to the irradiation facility. The procedure followed for both monogononts and bdelloids was the same as in *D. radiodurans* described above, except that *D. radiodurans* was used as a control in each case. After irradiation all dishes were refrigerated and used within 10 days. Irradiated animals were examined daily for the presence of eggs and, if any were present, the dish was removed from the cold room and reincubated in a 60° C incubator for 2 days to allow egg hatching (50). Animals were removed from the Petri dishes and counted in a hemacytometer (50). The irradiated rotifer population was then transferred to fresh medium in 5-cm Petri dishes containing fresh medium every week or so. For determination of reproductive performance, the dish was removed after a few days, and the animals were scored for the presence of fertilized eggs and nauplii. The rotifer population was maintained in a rotating 1,000 ml jar containing fresh medium every few days for 4 to 6 weeks after irradiation. Irradiated rotifer populations were maintained for 4 to 6 weeks after irradiation in 1,000 ml jars containing fresh medium every few days. The irradiated rotifer population was then transferred to fresh medium in 5-cm Petri dishes containing 5 ml of half-strength MBL medium and kept 6–10 h at room temperature to clear the animals of food organisms. As rapid cooling was found to be lethal, the dish was wrapped in a cloth towel, and kept for 6 h in the cold room before being taken, on ice, to the irradiation facility. The procedure followed for both monogononts and bdelloids. After addition of 0.5 ml of freshly prepared 2 mM-L-cysteine and 0.5 ml of freshly prepared 2 mM-kit, the irradiation facility, a procedure followed for both monogononts and bdelloids. After addition of 0.5 ml of freshly prepared 2 mM-L-cysteine and 0.5 ml of freshly prepared 2 mM-kit, the irradiation facility, a procedure followed for both monogononts and bdelloids. After addition of 0.5 ml of freshly prepared 2 mM-L-cysteine and 0.5 ml of freshly prepared 2 mM-kit, the irradiation facility, a procedure followed for both monogononts and bdelloids. After addition of 0.5 ml of freshly prepared 2 mM-L-cysteine and 0.5 ml of freshly prepared 2 mM-kit, the irradiation facility, a procedure followed for both monogononts and bdelloids. After addition of 0.5 ml of freshly prepared 2 mM-L-cysteine and 0.5 ml of freshly prepared 2 mM-kit, the irradiation facility, a procedure followed for both monogononts and bdelloids. After addition of 0.5 ml of freshly prepared 2 mM-L-cysteine and 0.5 ml of freshly prepared 2 mM-kit, the irradiation facility, a procedure followed for both monogononts and bdelloids.
from the irradiator at specific intervals, and individual animals were transferred by pipette to each well of a 96-well microtiter dish containing 0.15 ml of MBL medium well and a small amount of food. The dish was kept on ice during sampling, making it relatively easy to dislodge A. vaga by pipetting. Relative parental fertility was scored as the proportion of irradiated animals producing at least one active daughter, normalized to the corresponding proportion for nonirradiated animals. Each day or two until egg deposition ceased, ~1 week for E. dilatata and 2 weeks for the bdelloids, any daughters present, distinguished from the parent by their smaller size, were removed to avoid including the eggs of daughters in counts of eggs deposited by their mothers. A daughter from each fertile parental well was placed in a well of another microtiter dish containing food and 0.15 ml of MBL medium, and every few days any granddaughters present were removed. Relative F1 fertility was scored as the proportion of daughters producing at least one grand-daughter, normalized to the proportion produced by daughters of nonirradiated parents. For the reason explained below, bdelloid daughters were counted as they were removed from parental wells, as were bdelloid grand-daughters removed from daughter wells. Hatched and nonhatched eggs of E. dilatata and nonhatched bdelloid eggs were easily counted, but the transparency of hatched bdelloid eggs prevented their reliable identification. As inactive progeny were only rarely encountered, the number of hatched bdelloid eggs was therefore taken to be the same as the number of bdelloid progeny. At each dose, relative parental and F1 fecundity was taken as the total number of hatched eggs (monogononts) or progeny (bdelloids), normalized to the corresponding number from the nonirradiated animals. Three such experiments were performed with E. dilatata and one each with A. vaga and P. roseola.

**Pulsed-Field Gel Electrophoresis.** For examination of DNA breakage ~1,000 starved, prechilled rotifers were irradiated as described above for 0, 1/6, 2, 4, 6, or 8 h, harvested by centrifugation in the cold at ~10,000 × g for 5 min, resuspended in 0.2 ml of cold 50 mM EDTA and 10 mM Tris (pH 8.0), and centrifuged again. After removing 0.16 ml of supernatant, tubes were placed in a 42°C water bath and supplemented with 40 µl of freshly melted 1% low melting point agarose (LMPA; NuSieve GTG) in 2× Tris-EDTA (200 mM EDTA, 100 mM Tris, pH 8.0) at 42°C, mixed well by pipetting, transferred to a plug mold, and left to solidify for 30 min in the cold room. Each plug and an additional plug containing S. cerevisiae chromosomes (New England BioLabs N0345S) was individually placed in 0.2 ml of digestion buffer (1× Tris-EDTA supplemented with 1% sarcosyl and 1 mg/ml freshly dissolved proteinase K) and kept for 1 h in the cold room and 18 h at 55°C. Plugs were then rinsed with 0.5× 40 mM Tris acetate, 1 mM EDTA, pH 8.5 (TAE), kept with gentle rocking for 3 h in 1 ml of 0.5× TAE at room temperature and embedded in a 5-mm deep slab of 0.7% LMPA in 0.5× TAE. Electrophoresis was done in a BioRad CHEF-DR III electrophoretic system at 14°C and 5.5 V/cm, with a switch angle of 20° and switch times of 50–250 s for 18 h. After electrophoresis, the gel was placed in SYBR Gold (Invitrogen) freshly diluted 1:1000 in water, gently rocked overnight, and scanned with a BioRad Molecular Imager FX with Quantity One quantitation software (SYBR Gold settings: 488-m/z excitation, 530-m/z band pass). Under the conditions used, signal intensity is a linear function of DNA concentration in the gel (53). After baseline subtraction, each photometric scan was divided into 33 equal intervals and the direction of migration (c) of the number average molecular size of DNA was calculated as [ intensity [(intensity)] by summation over intervals, taking molecular sizes from a plot of the migration distance of the yeast markers against their sizes.

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9. Gladyshev VP, Meselson M (2000) Evidence for the evolution of bdelloid rotifers from the irradiator at specific intervals, and individual animals were transferred by pipette to each well of a 96-well microtiter dish containing 0.15 ml of MBL medium well and a small amount of food. The dish was kept on ice during sampling, making it relatively easy to dislodge A. vaga by pipetting. Relative parental fertility was scored as the proportion of irradiated animals producing at least one active daughter, normalized to the corresponding proportion for nonirradiated animals. Each day or two until egg deposition ceased, ~1 week for E. dilatata and 2 weeks for the bdelloids, any daughters present, distinguished from the parent by their smaller size, were removed to avoid including the eggs of daughters in counts of eggs deposited by their mothers. A daughter from each fertile parental well was placed in a well of another microtiter dish containing food and 0.15 ml of MBL medium, and every few days any granddaughters present were removed. Relative F1 fertility was scored as the proportion of daughters producing at least one grand-daughter, normalized to the proportion produced by daughters of nonirradiated parents. For the reason explained below, bdelloid daughters were counted as they were removed from parental wells, as were bdelloid grand-daughters removed from daughter wells. Hatched and nonhatched eggs of E. dilatata and nonhatched bdelloid eggs were easily counted, but the transparency of hatched bdelloid eggs prevented their reliable identification. As inactive progeny were only rarely encountered, the number of hatched bdelloid eggs was therefore taken to be the same as the number of bdelloid progeny. At each dose, relative parental and F1 fecundity was taken as the total number of hatched eggs (monogononts) or progeny (bdelloids), normalized to the corresponding number from the nonirradiated animals. Three such experiments were performed with E. dilatata and one each with A. vaga and P. roseola.

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