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Repair of Extensive Ionizing-Radiation DNA Damage at 95°C in the Hyperthermophilic Archaeon *Pyrococcus furiosus*†

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We investigated the capacity of the hyperthermophile *Pyrococcus furiosus* for DNA repair by measuring survival at high levels of ^{60}Co γ-irradiation. The *P. furiosus* 2-Mb chromosome was fragmented into pieces ranging from 500 kb to shorter than 30 kb at a dose of 2,500 Gy and was fully restored upon incubation at 95°C. We suggest that recombination repair could be an extremely active repair mechanism in *P. furiosus* and that it might be an important determinant of survival of hyperthermophiles at high temperatures.

Pyrococcus furiosus is a hyperthermophile growing optimally at 100°C. It is a member of the *Archaea*, a group of prokaryotes which have many molecular features in common with modern eukaryotes, and which are thought by many to be ancestral life forms (25). The exceptional degree of tolerance and resistance of hyperthermophiles to high temperatures must include adaptations that affect all levels of the cellular machinery, including the enzymes that are involved in maintaining the integrity and stability of genomic DNA.

Kopylov et al. (14) reported that a closely related member of the *Archaea*, *Thermococcus stetteri*, is 12 times more resistant to γ-irradiation than *Escherichia coli* but 2 times more sensitive than the bacterium *Deinococcus radiodurans*. In addition, Peak et al. (20) have shown that at 100°C, the DNA of *P. furiosus* is 20 times more resistant to thermal breakage in vivo than the DNA from the mesophile *E. coli*. Thermophilic members of the *Archaea* have histone proteins (24) known to give partial protection of plasmid DNA to fast neutrons and γ-photons (12) and to prevent thermal denaturation of DNA (5). However, the protective effect of DNA-binding proteins cannot account for the extreme resistance of these microorganisms to heat and γ-irradiation (12, 14, 20), suggesting the presence of very active mechanisms for DNA repair.

Among the lesions induced by ionizing radiation in cellular DNA, double-strand breaks (DSBs) are the least efficiently repaired, and their frequency is correlated with cell death (9). Indeed, *E. coli* and most other organisms cannot survive if more than two or three DSBs are introduced per chromosome, independently of their physiological state (15, 21). DSBs are noninformative lesions that affect the DNA double helix at the same site, eliminating intact template for repair and precluding any excision repair processes. However, *D. radiodurans* has been found to be extremely resistant to ionizing radiation (19). This organism can repair more than 100 DSBs per chromosome, induced by ionizing radiation, without loss of viability (19). Its extreme resistance to γ-irradiation is attributed to possible adaptation to desiccation (16). RecA-dependent recombinational repair and single-strand annealing are the two mechanisms proposed to account for repair of ionizing-radiation damage in *D. radiodurans* (16, 18).

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In contrast, there is currently very little information about DNA repair systems in hyperthermophiles, and what information there is consists mainly of the characterization of DNA repair-like genes and the observation of extreme resistance of *P. furiosus* to heat-induced DNA breakage (3, 4, 23). We describe here the effect of ionizing-radiation exposure on the survival of *P. furiosus* and its ability to repair its chromosome at 95°C after fragmentation by γ-irradiation.

Radioresistance of *P. furiosus*. *P. furiosus* cultures were grown anaerobically in 100-ml serum bottles for 3 h or to early stationary phase at 95°C (to approximately 3.5×10^5 and 3×10^7 cells/ml, respectively) (22) and irradiated on ice without a change of broth. Irradiation was performed with a ^{60}Co γ-ray source (at the National Institute of Standards and Technology) from 1,000 to 4,500 Gy at a rate of 45.2 Gy/min. Following irradiation, cultures were incubated at 95°C and sampled at regular intervals for determination of total cell and viable cell counts. Total cell counts were performed by the acridine orange direct count (AODC) method (11), and viable cell counts were carried out by the most probable number (MPN) technique (1). MPN cultures were incubated for 24 h at 95°C before scoring. Cells irradiated in the early stationary phase showed no loss of viability up to 2,000 Gy and 75% survival when the culture was exposed to a dose of 2,500 Gy (Fig. 1). Compared to *D. radiodurans*, which can sustain up to 15,000 Gy without loss of viability, *P. furiosus* cells are 7 times less resistant to ionizing radiation, but they are 10 times more resistant than *E. coli* cells (17). At higher doses, the survival rate decreased very rapidly as reported for other microorganisms (Fig. 2) (14, 17).

However, the effect of radiation in *P. furiosus* depends strongly on physiological states, such as age of the culture, with increased resistance of cells in stationary phase (results not shown). This effect has been previously reported for several bacteria, along with the influence of other factors, such as growth medium composition, irradiation medium, and temperature (13, 19).

Repair of the *P. furiosus* chromosome at 95°C after γ-irradiation. *P. furiosus* cultures were irradiated at 2,500 Gy at a rate of 45.2 Gy/min followed by incubation at 95°C for 0, 4, and 24 h. For each incubation time point, chromosomal DNA was analyzed by pulsed-field gel electrophoresis (PFGE) as described by Borges et al. (2). The genome size of *P. furiosus* has been estimated to be 2 Mb (2), and the genome was fragmented by irradiation of the cells at 2,500 Gy (Fig. 3, lane 0h).

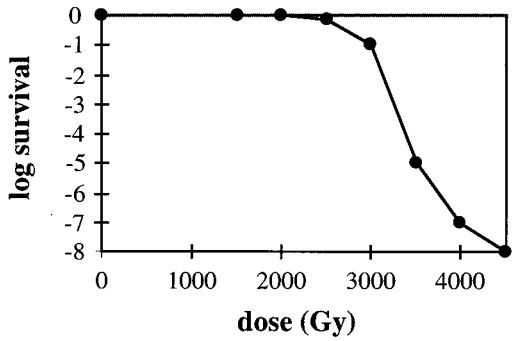

FIG. 1. Survival of *P. furiosus* determined by the MPN method after ^{60}Co γ-irradiation at 45.2 Gy/min. Prior to irradiation, cells were grown to early stationary phase. Following irradiation, appropriate dilutions were made in fresh medium and cultures were incubated at 95°C for 24 h. Values are the means of two experiments with duplicate cultures per experiment.

No intact chromosomal band could be detected, and the fragment sizes ranged from 500 kb to less than 30 kb, indicating a large number of DSBs per chromosome. Untreated cultures did not show evidence of DNA DSBs (Fig. 3, lane C). As shown in Fig. 3, at the 4-h postincubation time point, there was a considerable accumulation of intact chromosomes. After 20 h of incubation at 95°C, the chromosome of *P. furiosus* was fully restored to its 2-Mb original size. In Fig. 3, the band corresponding to 30 kb in lanes C 0h, 4h, and 20h is the result of chromosome degradation during preparation of the chromosomal DNA for PFGE. Treatment with RNases did not affect the intensity of the band (20a), and the band disappeared when the DNA was treated with restriction enzymes (2).

No significant variation in cell number, determined by AODC, was observed during the recovery period (results not shown), and although the survival rate at 2,500 Gy was 75%, the results in Fig. 3 clearly show that chromosomes from all of the cells were fragmented. Moreover, irradiation at lower doses, 1,000 and 2,000 Gy, which did not cause any loss of viability, displayed chromosome fragmentation (results not shown). In addition, the DSB repair process in *P. furiosus* did not require the dilution of the cells to a suitable density for growth, as in *D. radiodurans* (7, 18).

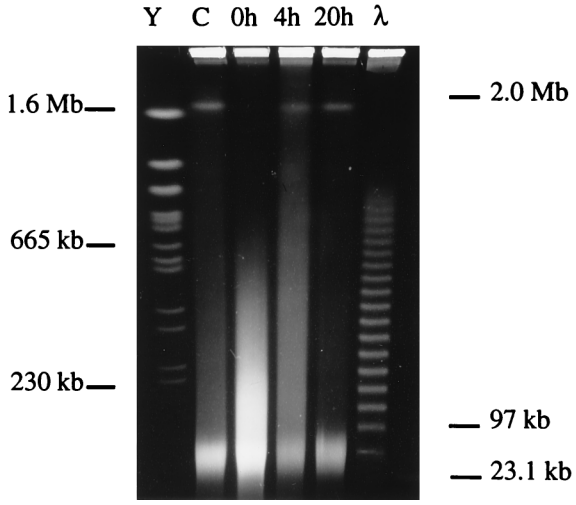

FIG. 3. PFGE of *P. furiosus* chromosomal DNA following ^{60}Co γ-irradiation of 2,500 Gy. Each lane contains DNA from 5×10^7 cells and was visualized by ethidium bromide staining. C, control not irradiated; 0h, 4h, and 20h, hours of incubation at 95°C after irradiation; Y, *S. cerevisiae* SFY 526 chromosomes; λ, lambda concatemers. Electrophoresis conditions were 40 to 60 s at 200 V and 30 h at 14°C.

Figure 4 shows a 4-h inhibition of growth in the cultures inoculated immediately following exposure to γ-irradiation. During that time, many of the DSBs are eliminated and some intact chromosomes are present (Fig. 3, lane 4h). The cultures inoculated after 4 and 20 h of postincubation resumed exponential growth at the same rate as the unirradiated culture (Fig. 4).

These results suggest that recombination repair could be a mechanism by which DSBs are repaired in *P. furiosus*. In *D. radiodurans*, interchromosomal recombination has been suggested as the major repair pathway for chromosomal mending as well as a single-strand annealing repair mechanism immediately following irradiation (6, 8). In this organism, the onset of recombination and growth are separated by about 7 h, suggesting that extensive recombination repair occurs before DNA replication (8). We showed that in *P. furiosus*, a maximum of 4 h was required for extensive repair before continu-

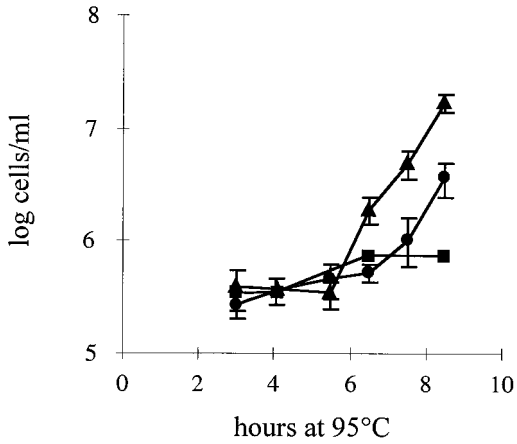

FIG. 2. Growth of *P. furiosus* determined by AODC after ^{60}Co γ-irradiation at 45.2 Gy/min. Prior to irradiation, cells were grown for 3 h at 95°C to approximately 3.5×10^5 cells/ml. ▲, 0 Gy; ●, 1,000 Gy; ■, 4,000 Gy.

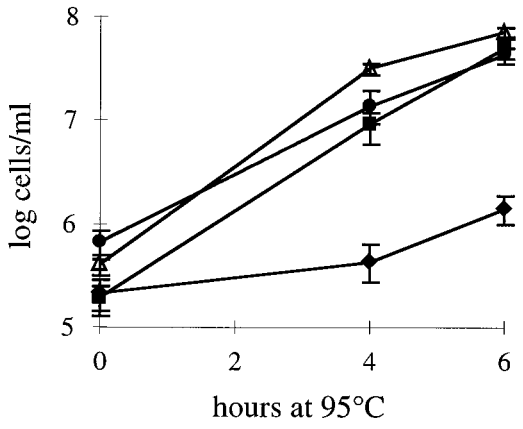

FIG. 4. Recovery of growth of *P. furiosus* determined by AODC after ^{60}Co γ-irradiation at 2,500 Gy. Fresh medium was inoculated at low density (2% inoculum) with cultures incubated at 95°C for 0 (◆), 4 (■), and 20 (●) h after irradiation. △, unirradiated control.

ation of growth, indicating relatively rapid chromosomal restoration. In hyperthermophilic members of the *Archaea*, interchromosomal recombination has only been reported for *Sulfolobus acidocaldarius* in the formation of stable genetic recombinants selectable by auxotrophic markers (10).

Given the extensive DNA damage imposed by 2.5 kGy of irradiation on the chromosome of *P. furiosus*, hydrolytic damage affecting the membrane and protein components of the cells is expected. However, the anaerobic irradiation and growth conditions may diminish the oxygen effect and secondary oxidative damages induced by ionizing irradiation (9).

Conclusion. We have shown here that *P. furiosus* can repair its chromosome at 95°C after dramatic fragmentation by ionizing radiation, suggesting that recombination repair could be one of the mechanisms involved in the mending process of the chromosome. Moreover, in *P. furiosus*, these repair processes occur at 95°C and could represent an adaptation to life at high temperatures in the same way that the radioresistance of *D. radiodurans* has been described as an adaptation to desiccation. In addition, we have found in *P. furiosus* two *recA*-like genes highly homologous to the *radA* genes described for other members of the domain *Archaea* and to eukaryotic *RAD 51* genes (8a, 9, 23).

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