Roles of the Major, Small, Acid-Soluble Spore Proteins and Spore-Specific and Universal DNA Repair Mechanisms in Resistance of *Bacillus subtilis* Spores to Ionizing Radiation from X Rays and High-Energy Charged-Particle Bombardment

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The role of DNA repair by nonhomologous end joining (NHEJ), homologous recombination, spore photoproduct lyase, and DNA polymerase I and genome protection via α/β-type small, acid-soluble spore proteins (SASP) in *Bacillus subtilis* spore resistance to accelerated heavy ions (high-energy charged [HZE] particles) and X rays has been studied. Spores deficient in NHEJ and α/β-type SASP were significantly more sensitive to HZE particle bombardment and X-ray irradiation than were the *recA*, *polA*, and *splB* mutant and wild-type spores, indicating that NHEJ provides an efficient DNA double-strand break repair pathway during spore germination and that the loss of the α/β-type SASP leads to a significant radiosensitivity to ionizing radiation, suggesting the essential function of these spore proteins as protectants of spore DNA against ionizing radiation.

Endospores of the gram-positive bacterium *Bacillus subtilis* are highly resistant to inactivation by environmental stresses, such as biocidal agents and toxic chemicals, desiccation, pressure and temperature extremes, and high fluences of UV radiation (reviewed in references 44, 45, and 61) and are a powerful biodosimetric system for terrestrial environmental monitoring and astrobiological studies (44). On Earth, understanding extreme spore resistance to ionizing radiation is important in the areas of food preservation, medical sterilization, and decontamination from bioterror attack (4, 17, 47; reviewed in references 42 and 43). Off Earth, spore radiation resistance is important both in space flight and in ground-based simulations, in order to obtain information on the biological damage produced by exposure to space conditions (23, 25, 26, 44). Onboard several spacecraft (Apollo 16, Spacelab 1, LDEF, D2, and FOTON), spores of *Bacillus subtilis* were exposed to selected parameters of space, such as space vacuum and different spectral ranges of solar UV radiation and cosmic rays, applied separately or in combination (5, 9, 19–21, 23, 24, 26). Especially, the radiation environment on Earth, on Mars, in low-Earth orbit, and in deep space is typified by a wide variety of primary particles covering an extended range of energies. Galactic cosmic rays (GCR) are charged particles that originate from sources beyond our solar system. The distribution of GCR is believed to be isotropic throughout interstellar space. The spectrum of the GCR consists of 98% protons and heavier ions (baryon component) and 2% electrons and positrons (lepton component). The baryon component is composed of 87% protons, 12% helium ions (alpha particles), and the remaining 1% heavy ions of charge 3 from lithium to 92 from uranium. Due to their high abundance, iron ions are highly penetrating, giving them a large potential for radiobiological damage (3, 21).

While the UV photochemistry of spore DNA and repair of UV damage to DNA during germination are well characterized (34, 44, 59–61), there has been relatively little work on the nature of DNA damage in spores caused by ionizing radiation, the protective role of α/β-type small, acid-soluble spore proteins (SASP), or the occurrence of a specific DNA repair system(s) for repair of this damage, as ionizing radiation is potentially lethal and mutagenic to all organisms. The cellular response to ionizing radiation is complex due to the variety of targets in a cell (29, 52). Ionizing radiation can damage cellular components though direct deposition of radiation energy into biomolecules and also indirectly by generating reactive oxygen species (ROS). Hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals (HO·) are major oxidizing species produced by the radiolysis of water, and superoxide ions (O$_2^-·$) are formed in the presence of dissolved oxygen (54). Generally, the cytotoxic and mutagenic effects induced by ionizing radiation are thought to be the result of DNA damage caused during the course of irradiation, which includes single-strand breaks (SSB), double-strand breaks (DSB), base modification, abasic sites, and sugar modification (14, 27, 32, 33). The amounts of DNA damage caused by given doses of ionizing radiation for different bacteria are very similar, although the range of ionizing-radiation resistance levels is wide.

In addition to their high UV resistance, bacterial spores are often more resistant than growing cells to ionizing radiation.
formed by water radiolysis (27, 41). It is thought that DNA acid seem to have only minor influence on spore resistance to (28). However, sulfur-rich spore coat proteins and dipicolinic acid protect by sulfur-rich coat proteins (18) and dipicolinic acid impact on spore resistance to ionizing radiation, in particular directly. Several further factors have been investigated for their impact on spore resistance to ionizing radiation, mainly acting as ROS scavengers of radicals formed by water radiolysis (27, 41). It is thought that DNA DSB are the most critical damage caused by ionizing radiation (67).

Recently, we reported that B. subtilis spores defective in the nonhomologous end joining (NHEJ) DSB repair system were significantly more sensitive to DNA SSB and DSB generated by treatments such as ultrahigh vacuum, mono- and polychromatic UV, and X rays, thus establishing a major role for NHEJ in spore resistance to these extreme environmental parameters (38). In this communication, we have examined the protective role of α/β-type SASP, as well as the roles of both spore-specific DNA repair pathways (spore photoprotein [SP] lyase and NHEJ), the universal homologous recombination (recA) DNA repair pathway, and the repair DNA polymerase I (polA), which participates in nucleotide excision repair and base excision repair pathways, in spore resistance to ionizing treatments such as X-ray irradiation and bombardment by heavy ions (the so-called high-energy charged [HZE] particles [according to reference 21]).

Endospores of eight different Bacillus subtilis 168-derived strains were used in this work, all of which either differ in their capacity for DNA damage repair by homologous recombination (recA), SP lyase (splB), DNA polymerase I (polA), or NHEJ (ykoU, ykv, and ykoV) or lack the two major DNA protective α/β-type SASP, SASP-α and -β (encoded by sspA and sspB, respectively) (Table 1). All strains were used to the wild-type strain 168, with the exception of strain HA101(59)F (Table 1).

Spores were obtained by cultivation under vigorous aeration in double-strength liquid Schaeffer sporulation medium (56), under identical conditions for each strain, and the spores were purified and stored as described previously (35–38). Spore preparations consisted of single spores with no detectable clumps and were free (>99%) of growing cells, germinating spores, and cell debris, as seen in the phase-contrast microscope.

For studying the impact of heavy ion exposure and X-ray irradiation-induced DNA damage on spore survival, air-dried monolayers of 2 × 10⁷ spores on 7-mm quartz discs (38, 51) were irradiated at room temperature with X rays (150 keV/19 mA) generated by an X-ray tube (Mueller Type MG 150, Germany; as described previously by Moeller et al. [38]) and three heavy ion species (linear energy transfer [LET] range from 2 to 200 keV/μm) up to a final dose of 0.5 kGy. Heavy ion irradiations were performed at the Heavy Ion Medical Accelerator (HIMAC) at the National Institute for Radiological Sciences in Chiba, Japan, under the aegis of HIMAC research project 17B463 [Gene Activation of Heavy Ion-Treated Bacillus subtilis 168 (DSM 402) Endospores during Germination-Involved DNA Repair] in the years 2005 to 2007. The following three ion species were applied (energies [in MeV/nucleon (n)] and LET values [in keV/μm] are in parentheses): helium (He; 150 MeV/n; 2.2 keV/μm), argon (Ar; 500 MeV/n; 90 keV/μm), and iron (Fe; 500 MeV/n; 200 keV/μm). These three ion species were chosen because they are abundant components of space radiation and span a range of LET values (3, 21). All irradiations were carried out in the Biological Irradiation Room, where a maximum beam diameter of 10 cm is obtained by using a pair of wobbler magnets and a scatterer. Reference doses were retrieved from a high-precision Farmer-type ionization chamber with a water-equivalent thickness of 1 mm installed upstream of the target, which was also used to check the uniformity of the beam in one dimension, yielding maximum fluctuations of <5% over the circular beam area for the employed ion species. Further details on the irradiation geometry of the HIMAC facility, beam monitoring, dosimetry, and dose calculations have been given by Okayasu et al. (48).

To recover the spores from the quartz discs after irradiation, spore monolayers were covered by a 10% aqueous polystyrene alcohol solution, and after drying the spore polystyrene alcohol layer was stripped off as described previously (22, 26) and was resuspended in 1 ml sterile distilled water, resulting in >95% recovery of the spores. This procedure does not affect spore viability (26). Spore survival was determined from appropriate dilutions in distilled water for Bacillus subtilis spores as colony-forming ability after incubation overnight at 37°C on nutrient broth agar plates (Difco, Detroit, MI) as described previously (35–38).

The surviving fraction of B. subtilis spores was determined from the quotient N/N₀ with N being the number of CFU of the irradiated sample and N₀ being that of the nonirradiated controls. Spore survival was plotted as a function of X rays and heavy ion irradiation dose. Data are reported as Dₓ values, the dose lethal for 63% of the initial spore population, determined from the linearity of the semilogarithmic curve (reciprocal of the spore inactivation constant kᵢ [according to references 36 and 50]). The ratio of the Dₓ values of the X-ray- to the heavy ion-irradiated spores was used for data normalization to determine the sporicidal effectiveness of the applied HZE particle. The best-fit curves were used to calculate Dₓ values for statistical comparison. This latter value reflects the biological

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype or phenotype</th>
<th>Source and/or reference</th>
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</thead>
<tbody>
<tr>
<td>168</td>
<td>trpC2</td>
<td>DSM 402, DSMZ (64)</td>
</tr>
<tr>
<td>WN463</td>
<td>trpC2 recA::ermC MLS</td>
<td>R. E. Yashin (6)</td>
</tr>
<tr>
<td>HA101(59)F</td>
<td>trpC2 his leu metB polA59'</td>
<td>G. Hornbeck (13)</td>
</tr>
<tr>
<td>TKJ6324</td>
<td>trpC2 splB1'</td>
<td>N. Munakata (10)</td>
</tr>
<tr>
<td>PSS56</td>
<td>trpC2 ΔsspA ΔsspB Cm'</td>
<td>P. Setlow (8)</td>
</tr>
<tr>
<td>BFS1845</td>
<td>trpC2 ykoU::pMUTIN4 Cm'</td>
<td>A. J. Doherty (67)</td>
</tr>
<tr>
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<td>trpC2 ykoV::pMUTIN4 Cm'</td>
<td>A. J. Doherty (67)</td>
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<tr>
<td>BFS1846</td>
<td>trpC2 ykoV::pMUTIN4 Cm'</td>
<td>A. J. Doherty (67)</td>
</tr>
</tbody>
</table>

* Strain was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) GmbH, Braunschweig, Germany.
  † MLS', resistant to lincomycin (25 μg/ml) and erythromycin (1 μg/ml).
  ‡ polA59 is a DNA polymerase I mutation (13, 16).
  § splB1 indicates that the strain carries two point mutations causing changes in amino acids G168R and G242D in SP lyase (10).
  ¶ Deletions of the sspA and sspB genes (8, 31).
  ‡ Cm', resistant to chloramphenicol (3 or 5 μg/ml).
impact of the LET on spore survival. Each experiment was repeated at least three times, and the data shown are expressed as averages ± standard deviations. The treated spores were compared statistically using Student’s t test. Values were analyzed in multigroup pairwise combinations, and differences with P values of ≤0.05 were considered statistically significant (35–38).

In order to assess the possible importance of the major SASP and selected DNA repair mechanisms in B. subtilis spore resistance to HZE particle and X-ray irradiation, wild-type and ykoU- and ykoV-type SASP-deficient spores, as well as spores from DNA repair-deficient strains, were subjected to X rays and HZE particle bombardment. Typical inactivation curves for air-dried spores of all strains in response to X rays and the heavy ions He, Ar, and Fe, in an LET range of 2 to 200 keV/μm, are depicted in Fig. 1. After exposure to X rays or HZE particles, spores of all strains exhibited strict exponential inactivation kinetics with no shoulder apparent, as indicated by n (ordinate of the extrapolated semilog straight line) approximating 1 (Fig. 1). Spore resistance was expressed as the D_{37} value (Table 2).

Spores of all mutant strains tested were significantly more sensitive to X rays than were wild-type spores, with the ykoU ykoV spores being the most sensitive. In comparison to wild-type spores, the order of X-ray sensitivity of spores of the various strains from most to least sensitive was ykoU ykoV spores > ykoV spores > ykoU spores > sspA sspB spores > recA spores > polA spores > splB spores > wild-type spores (Table 2). These observations are in good agreement with the results from previous experiments in which a subset of these mutant spores were exposed to high doses (>2 kGy) of X rays (38). The previous experiments used D_{10} values to determine the order of X-ray sensitivity as ykoU ykoV spores > ykoV spores > ykoU spores > recA spores > splB spores ≈ wild-type spores (38). Taken together, the results from previous (38) and

![FIG. 1. Survival curves of B. subtilis spores in response to X rays (A), helium ions (B), argon ions (C), and iron ions (D). Strains are 168 (wild type; filled circles), polA mutant (filled triangles), recA mutant (open circles), splB mutant (open triangles), sspA sspB mutant (filled inverted triangles), ykoU mutant (open inverted triangles), ykoV mutant (filled squares), and ykoU ykoV mutant (open squares). Data are expressed as averages and standard deviations (n = 3); error bars either are shown or are smaller than the symbols.](https://jb.asm.org/)

**TABLE 2.** D_{17} values (Gy) of the X-ray- and accelerated HZE particle-irradiated spores of B. subtilis

<table>
<thead>
<tr>
<th>Strain</th>
<th>X rays</th>
<th>He</th>
<th>Ar</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>587.9 ± 56.7*</td>
<td>344.3 ± 29.3</td>
<td>193.8 ± 18.1</td>
<td>125.8 ± 13.2</td>
</tr>
<tr>
<td>polA mutant</td>
<td>251.4 ± 83.2*</td>
<td>167.9 ± 24.7*</td>
<td>133.5 ± 14.6*</td>
<td>93.6 ± 8.6*</td>
</tr>
<tr>
<td>recA mutant</td>
<td>167.1 ± 26.1*</td>
<td>130.6 ± 17.4*</td>
<td>99.7 ± 12.3*</td>
<td>73.2 ± 6.9*</td>
</tr>
<tr>
<td>splB mutant</td>
<td>396.9 ± 31.3*</td>
<td>249.4 ± 22.6</td>
<td>158.7 ± 9.6</td>
<td>108.9 ± 10.8</td>
</tr>
<tr>
<td>sspA sspB mutant</td>
<td>123.3 ± 19.5*</td>
<td>111.5 ± 9.3</td>
<td>87.4 ± 10.2*</td>
<td>68.9 ± 5.4*</td>
</tr>
<tr>
<td>ykoU mutant</td>
<td>104.8 ± 16.4*</td>
<td>97.1 ± 10.2*</td>
<td>75.2 ± 7.1*</td>
<td>59.9 ± 5.9*</td>
</tr>
<tr>
<td>ykoV mutant</td>
<td>90.8 ± 14.8*</td>
<td>83.0 ± 8.7*</td>
<td>67.6 ± 8.1*</td>
<td>54.8 ± 4.3*</td>
</tr>
<tr>
<td>ykoU ykoV mutant</td>
<td>73.6 ± 9.6*</td>
<td>69.2 ± 7.6*</td>
<td>58.3 ± 6.2*</td>
<td>52.6 ± 3.9*</td>
</tr>
</tbody>
</table>

* Data are averages and standard deviations (n = 3). Asterisks indicate survival values that were significantly different (P values of ≤0.05) from the survival value for wild-type spores of B. subtilis 168.

* Value obtained by extrapolation.
current experiments (i) confirm that the DSB repair pathway encoded by the ykoWVU operon is most important in the resistance of spores to X-ray damage; (ii) indicate that α/β-type SASP encoded by sspA and sspB are important protectants of DNA from damage by ionizing X-ray damage; (iii) indicate that homologous recombination and/or the SOS response mediated by recA and polA-encoded repair DNA polymerase I play lesser but still significant roles in repair of X-ray-induced damage; and (iv) indicate that SP lyase encoded by splB plays only a minor role, if any, in repair of X-ray-induced DNA damage in spores. In order to investigate spore resistance to HZE particle bombardment simulating a component of GCR, air-dried spore films were irradiated with three heavy ions as described previously. Strictly exponential survival curves were obtained for spores of all strains tested in response to HZE particle bombardment by He ions (Fig. 1B), Ar ions (Fig. 1C), and Fe ions (Fig. 1D). Interestingly, wild-type spores showed different sensitivities to X rays and HZE particle bombardment, which is in good agreement with previous reports by Horneck and Buecker (22) and Micke et al. (32, 33). Exposure of wild-type spores to He, Ar, and Fe resulted in 1.8-, 3.1-, and 4.8-fold-greater inactivation, respectively, than did X-ray exposure, based on comparison of D37 values (Table 2). These results can be explained in terms of the LET values of the X rays and HZE particles used.

Examination of the relative sensitivities of mutant spores in comparison to the wild type yielded an order of spore sensitivity to HZE particle bombardment identical to what had been observed with X-ray treatment (Table 2). Again, spores lacking NHEJ were most sensitive to heavy ion irradiation, followed by spores lacking major α/β-type SASP, strongly suggesting that α/β-type SASP and NHEJ are the major factors involved in protection against and repair of DNA damage by X rays and HZE particles. Spores carrying mutations in recA or polA were also significantly more sensitive to HZE particles than were wild-type spores but less sensitive than spores lacking NHEJ or α/β-type SASP. Spores lacking SP lyase were slightly more sensitive to HZE particles than were wild-type spores, but the difference was not statistically significant at the P < 0.05 level of confidence (Table 2).

Wild-type spores were more sensitive to HZE particles than to X-rays and were more sensitive to HZE particles with higher LET values (Table 2), in good agreement with previous observations (2, 66). To determine the sporicidal effectiveness of HZE particles relative to X rays for spores of all strains, the ratios of D37 values of the X-ray-irradiated strains to the respective D37 values with each heavy ion species were calculated (Table 2). A strict linear relationship was obtained when this value for all spores was plotted versus LET, with spores of each strain exhibiting their own characteristic slope (Fig. 2). Spores most resistant to X rays and HZE particles (wild-type and sspB, polA, and recA spores) gave the steepest slopes (Fig. 2A), indicating a strong dependence on LET. In contrast, spores least resistant to X rays and HZE particles by virtue of their lack of protective α/β-type SASP (sspA sspB) or DSB repair via NHEJ (ykoU, ykoV, and ykoU ykoV mutants) exhibited much shallower slopes (Fig. 2B), indicating that sensitivity of these strains to HZE particles was less dependent on LET.

Previously, we reported that spore survival upon treatments known to induce DSB and SSB (UV, high-dose X rays, and ultrahigh vacuum) was significantly impaired in mutants lacking NHEJ (42). The current results confirm that NHEJ is a major DNA repair system responsible for spore resistance to X rays (38), and we further extend this observation to DNA strand breaks caused by HZE particle bombardment. Among the ionizing components of radiation in space, the heavy primaries (HZE particles) are the most biologically effective component of the cosmic rays (reviewed in references 19 to 21 and 44).

Although ionizing radiation can damage a number of cellular molecules, DSB are one of the most lethal forms of DNA damage, as even a single unrepaired DSB is sufficient to kill a cell (reviewed in reference 30). Two major repair pathways have evolved to repair DNA DSB and ensure genomic stability: homologous recombination and NHEJ. In homologous recombination repair, an intact DNA duplex homologous to
the DSB site acts as a template for the resynthesis of the broken strands (reviewed in references 49 and 55). However, spores of *B. subtilis* are monogenic with the genome arranged in a toroidal shape (11, 53). Consequently, homologous recombination repair, which requires at least two homologous chromosomes, cannot operate during the early stages of spore germination prior to the first round of DNA replication (65).

In contrast to homologous recombination repair, NHEJ repair can rejoin two DNA ends directly without extensive homology between DNA strands. The first step in the NHEJ DNA repair mechanism involves the binding of the Ku complex to the two DNA ends. The next proteins to be recruited to the complex are those that lead to resection of the ends of a DSB. The final steps in NHEJ join the two ends by a specific DNA ligase, thus restoring the integrity of the DNA. The ligation of DNA strands is an energy-dependent process, and ATP is required for the formation of a phosphodiester bond at the site of an SS in a duplex DNA (68). Recently, Weller et al. (67) identified a Ku homolog (encoded by the ykoV gene) in *B. subtilis*, which has the biochemical characteristics of the eukaryotic Ku heterodimer. The bacterial Ku specifically recruits a DNA ligase (encoded by ykoU) to DNA ends and thereby stimulates DNA ligation. It is worthwhile to note that dormant spores do not contain detectable amounts of endogenous ATP; rather, high-energy phosphate is stored in the spore as a large depot of 3-phosphoglyceric acid. In the first minutes of spore germination, 3-phosphoglyceric acid rapidly generates ATP by the lower part of glycolysis (57, 62). Thus, the activity of the NHEJ pathway is dependent upon ATP generation during spore germination. In the NHEJ systems of bacterial species such as *Mycobacterium smegmatis* and *Pseudomonas aeruginosa*, the ATP-dependent DNA ligase is a multidomain protein composed of polymerase and nuclease domains in addition to the core ligase domain (49). However, in the *Bacillus subtilis* NHEJ ligase YkoU, the nuclease domain appears to be absent and the polymerase domain is fused with core ligase domain (reviewed in reference 49). Functional characterization of these nuclease domains and their activity to ensure genomic stability remain to be investigated. Also unclear at present is the potential role of the first cistron of the ykoWVYU operon, ykoW, which encodes a protein with similarities to putative sensory box proteins and/or diguanylate cyclase/phosphodiesterases (according to the SubtiList database, http://genolist.pasteur.fr/SubtiList [according to reference 39]). In this regard it is interesting that Mun et al. (40) reported that nonhomologous ends were removed by a DNA phosphodiesterase to eliminate radiolytic products (e.g., thymine glycol adducts) at broken DNA ends in *Deinococcus radiodurans*, suggesting the intriguing possibility that the YkoW product may perform an analogous function in *B. subtilis* NHEJ.

The α/β-type SASP have long been known to bind to and protect spore DNA, but their role in spore resistance to ionizing radiation has remained unclear (reviewed in references 44 and 61). Our results show that α/β-type SASP are major factors in spore resistance to X rays and HZE particles, second only to the NHEJ repair system itself. SASP-deficient spores irradiated with X rays and accelerated heavy ions in an LET range of 2 to 200 keV/μm were observed to be two to five times more sensitive to ionizing radiation than wild-type spores. Protection of DNA from ionizing-radiation damage by α/β-type SASP is likely due to a direct effect of their binding on and thereby stabilizing spore DNA integrity (46). In addition, α/β-type SASP may also serve as a potential scavenger for ionizing-radiation-induced ROS (7, 27). Taken together, NHEJ and α/β-type SASP appear to be the most important factors in the prevention and repair of ionizing-radiation-induced DNA damage. Recently, Wang et al. (65) reported on the role of NHEJ (alone and coupled with the influence of SASPs) in spore resistance to dry heat, which is a potential inducer of DNA strand breaks in spore DNA, perhaps by generating apurinic or apyrimidinic sites (58). Both NHEJ- and α/β-type SASP-deficient (and the respective double-mutant) spores were significantly more sensitive to dry heat than wild-type spores; these data are of value because of the simulation of the effect of thermal spikes or shock waves caused by high-LET HZE particles (see below). Our results after X-ray and HZE particle irradiation of air-dried spores complement data obtained by Hackett and Setlow (15) as a result of 60Co irradiation of *B. subtilis* spores lacking major SASP (SASP-α, -β, and -γ) in water. However, the impact of levels of spore core water on the spore resistance to ionizing radiation remains to be investigated.

Spores carrying mutations in the recA or polA gene were significantly more sensitive than wild-type spores to X rays and HZE particles, but the contribution of the RecA protein or the repair DNA polymerase I encoded by *polA* was less than that of the SASP or NHEJ proteins (Table 2). DNA polymerase I functions mainly in DNA repair processes requiring gap-filling activities, such as nucleotide excision repair or base excision repair (12), suggesting that some of the products of ionizing radiation may be intrastrand dimers, modified bases, or apurinic/apyrimidinic sites. These types of damage might be expected given the relatively broad spectrum of targets susceptible to ionization.

The role of RecA is twofold, the first being that of a direct participant in homologous recombination. As discussed above, the fact that dormant *B. subtilis* spores contain a single copy of the genome results in a lack of duplex substrate for RecA to act upon. However, even initiation of the first round of replication would result in production of at least a partial duplex chromosome on which RecA could function. Second, RecA is also an important signaling molecule in the SOS response, via its protease activity on the Lex repressor (1, 34). Previous results indicated that neither the *polA* gene nor the *ykoWV* operon was induced as part of the SOS regulon in either UV- or mitomycin C-treated vegetative cells (1) or germinating UV-treated spores (29). The fact that dormant *B. subtilis* spores contain a single copy of the genome results in a lack of duplex substrate for RecA to act upon. However, even initiation of the first round of replication would result in production of at least a partial duplex chromosome on which RecA could function. Second, RecA is also an important signaling molecule in the SOS response, via its protease activity on the Lex repressor (1, 34). Previous results indicated that neither the *polA* gene nor the *ykoWV* operon was induced as part of the SOS regulon in either UV- or mitomycin C-treated vegetative cells (1) or germinating UV-irradiated spores (R. Moeller and W. L. Nicholson, unpublished observations) of *B. subtilis*. However, included in the SOS regulon of genes induced by UV or mitomycin C were a number of operons encoding putative or unknown functions (1; Moeller and Nicholson, unpublished). Some of these currently unknown genes might encode proteins involved in repair of ionizing-radiation damage, and thus, inactivation of their SOS induction in the recA mutant may explain this strain’s increased sensitivity to X rays and HZE particles. These SOS-inducible genes of currently unknown function are promising targets for future exploration.

SP lyase encoded by the *splB* gene is an example of a protein dedicated to the repair of a specific type of DNA damage, the “spore photoproduct” SP, an intrastrand thymine dimer (63).
Therefore, an increased sensitivity of spbB mutant spores to ionizing-radiation damage presumably reflects the production of SP in spore DNA. Examination of the data indicated that SP lyase indeed contributed significantly to spore X-ray resistance (Table 2), strongly suggesting that the X rays produce a significant amount of SP in spore DNA. Although spbB mutant spores were less resistant to HZE particles than were wild-type spores, these differences were not statistically significant (Table 2), suggesting that HZE particles produce relatively fewer SP lesions in spore DNA than do X rays. In order to better understand the complete spectrum of DNA damage in spores exposed to X rays and various heavy ion species, it would be very interesting in future experiments to measure these lesions, as has previously been done with UV-irradiated spores (8, 35).

The data presented in this communication suggest that ionizing radiation, and in particular HZE particles, cause damage to cellular components other than DNA. For example, spores lacking various DNA repair functions were up to eightfold more X ray sensitive than wild-type spores but only up to 2.4-fold more sensitive to Fe particle bombardment (Table 2), indicating that a substantial fraction of the lethal damage caused in spores by HZE particles involved non-DNA targets. A similar observation was made previously concerning the environmental UV resistance of *B. subtilis* spores, where it was found that longer-wavelength UV-A and UV-B radiation caused non-DSN lethal damage to spores whereas short-wave-length UV-C radiation caused mainly lethal damage to DNA (69).

In conclusion, when faced with prolonged exposure to ionizing radiation, dormant bacterial spores must repair accumulated DSB to ensure their survival and genome integrity during germination (reviewed in reference 45). Dormant *B. subtilis* spores can survive irradiation with high doses of HZE particles, and the sporidical effectiveness is closely dependent on the LET of the heavy ion species (Fig. 2). However, future experiments testing HZE particle irradiation with particles carrying energies of >200 keV/μm are needed to analyze the possible LET survival dependency. Our results lend support to the hypothesis that NHEJ is a key strategy used during spore germination to repair DSB caused by key components of GCR encountered during exposure to space and/or high doses of terrestrial background radiation. Our results further demonstrate that major α/β-type SASP also play a major role in spore survival after X-ray or HZE particle exposure. However, further studies on the nature of the ionizing-radiation-induced damage (directly and indirectly caused) to the spore are needed to obtained detailed information on spore ionizing-radiation resistance.

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These results will be included in the research reports of the HIMAC project (17B463).

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