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Role of DNA Repair in *Bacillus subtilis* Spore Resistance

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Wet-heat or hydrogen peroxide treatment of wild-type *Bacillus subtilis* spores did not result in induction of *lacZ* fusions to three DNA repair-related genes (*dinR*, *recA*, and *uvrC*) during spore outgrowth. However, these genes were induced during outgrowth of wild-type spores treated with dry heat or UV. Wet-heat, desiccation, dry-heat, or UV treatment of spores lacking major DNA-binding proteins (termed $\alpha^-\beta^-$ spores) also resulted in induction of the three DNA repair genes during spore outgrowth. Hydrogen peroxide treatment of $\alpha^-\beta^-$ spores did not result in induction of *dinR*- and *recA-lacZ* but did cause induction of *uvrC-lacZ* during spore outgrowth. Spores of a *recA* mutant were approximately twofold more UV sensitive and approximately ninefold more sensitive to dry heat than were wild-type spores but were no more sensitive to wet heat and hydrogen peroxide. In contrast, $\alpha^-\beta^-$ *recA* spores were significantly more sensitive than were $\alpha^-\beta^-$ spores to all four treatments, as well as to desiccation. Surprisingly, RecA levels were quite low in dormant spores, but RecA was synthesized during spore outgrowth. Taken together, these data (i) are consistent with previous suggestions that some treatments (dry heat and UV with wild-type spores; desiccation, dry and wet heat, hydrogen peroxide, and UV with $\alpha^-\beta^-$ spores) that kill spores do so in large part by causing DNA damage and (ii) indicate that repair of DNA damage during spore outgrowth is an important component of spore resistance to a number of treatments, as has been shown previously for UV.

Dormant spores of various gram-positive organisms are much more resistant than their growing-cell counterparts to a variety of treatments, including wet and dry heat, desiccation, UV, and oxidizing agents such as hydrogen peroxide (9, 10, 12, 33, 40, 44). There are a number of factors involved in spore resistance, including the desiccation and mineralization of the spore core (11) and decreased spore permeability to damaging chemicals (1, 13, 15). Studies with *Bacillus subtilis* spores have shown that another factor in spore resistance is the protection of spore DNA from damage through its saturation with a group of DNA-binding proteins termed α/β-type small, acid-soluble spore proteins (SASP) (44, 45). These proteins are synthesized only during sporulation in the developing spore and are degraded early in spore germination (43, 45). The saturation of spore DNA with α/β-type SASP provides protection against DNA base loss due to wet heat (i.e., depurination) and DNA single-strand breakage caused by desiccation and hydrogen peroxide (9, 10, 29, 39, 45). As a consequence of this DNA protection, death of wild-type spores in water at temperatures from 10 to 90°C or upon treatment with hydrogen peroxide is apparently not due to DNA damage (9, 39). Although binding of α/β-type SASP also provides significant protection against spore DNA damage due to dry heat, killing of wild-type spores by this treatment is due at least in part to DNA damage; this damage results in DNA single-strand breaks, possibly as a consequence of DNA depurination (40). As might be expected, spores lacking most α/β-type SASP (termed $\alpha^-\beta^-$ spores) are much more sensitive to desiccation, dry and wet heat, and hydrogen peroxide than are wild-type spores; all of these treatments kill $\alpha^-\beta^-$ spores in large part by damaging DNA (45).

Binding of α/β-type SASP also plays a major role in the resistance of wild-type spores to UV damage by altering the spore DNA photochemistry (42, 44, 45). The major deleterious DNA photoproduct generated by UV irradiation of growing cells is a cyclobutane-type dimer between adjacent thymine residues (termed TT), but TT is not formed in spores. Rather, the major UV photoproduct formed in spore DNA is a thyminyl-thymine adduct termed the spore photoproduct (SP), which is also formed between adjacent thymine residues. $\alpha^-\beta^-$ spores are ~30 times more UV sensitive than are wild-type spores, and UV generates a large amount of TT in $\alpha^-\beta^-$ spores (37). Generation of TT is the reason for the decreased UV resistance of $\alpha^-\beta^-$ spores. Several studies have shown that the binding of α/β-type SASP is the major, if not the only, reason for the altered UV photochemistry of spore DNA (29, 39, 45).

SP is a potentially lethal DNA lesion and is formed in spore DNA with about the same efficiency as a function of UV fluence as is TT in cell DNA (38). Consequently, SP must be repaired in order for spores to exhibit high UV resistance. This repair cannot take place in the dormant spore because of its lack of ATP and the inactivity of enzymes in the spore core (44). SP is, however, repaired efficiently in the first minutes of spore germination by two independent systems (24, 25, 45). One is the excision repair system that removes DNA lesions, including TT, in cells. Expression of the genes involved in the excision repair system, including *uvrC*, is induced by DNA damage, and these genes are controlled at least in part by the *recA* and *dinR* genes, as is the case in *Escherichia coli*. The *uvrC*, *recA*, and *dinR* genes of *B. subtilis* are homologs of the *uvrC*, *recA*, and *lexA* genes, respectively, of *E. coli* and are themselves controlled by RecA and DinR (5, 6, 11, 16, 31, 32, 48). The second repair system is SP specific and utilizes an enzyme termed Spl which is synthesized only in the forespore; *spl* expression is very likely not under RecA-DinR control and does not appear to be inducible by DNA damage (27). Unlike excision repair, which cleaves the DNA backbone and can generate a short region of single-stranded DNA, Spl converts SP to two thymine residues without DNA backbone cleavage (23). Mutations in either the excision repair system or *spl* reduce spore UV resistance 2- to 7-fold, while spores of double mutants in both systems are 30- to 50-fold more UV sensitive than are wild-type spores (42).

Since DNA damage contributes significantly to the killing of

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TABLE 1. *B. subtilis* strains used

Strain	Genotype or phenotype	Source or reference
PS356	$\alpha^-\beta^-$ (parent is PS832)	19
PS832	Wild-type 168 derivative	Laboratory stock
PS2318	*recA260* Cm^r Ery^r	YB3000→PS832
PS2319	$\alpha^-\beta^-$ *recA260* Cm^r Ery^r	YB3000→PS356
PS2271	*recA-lacZ*::*amyE* Cm^r	YB3001→PS832
PS2272	$\alpha^-\beta^-$ *recA-lacZ*::*amyE* Cm^r	YB3001→PS356
PS2302	YB886A with *uvrC-lacZ*::*amyE* Cm^r	Ron Yasbin (see Materials and Methods)
PS2320	*uvrC-lacZ*::*amyE* Cm^r	PS2302→PS832
PS2321	$\alpha^-\beta^-$ *uvrC-lacZ*::*amyE* Cm^r	PS2302→PS356
PS2331	YB886A with *dinR-lacZ*::*amyE* Cm^r	Kevin Winterling (see Materials and Methods)
PS2332	*dinR-lacZ*::*amyE* Cm^r	PS2331→PS832
PS2333	$\alpha^-\beta^-$ *dinR-lacZ*::*amyE* Cm^r	PS2331→PS356
YB3000	YB886 *recA260* Cm^r Ery^r	Rich McVeigh (7)
YB3001	YB886A *recA-lacZ*::*amyE* Cm^r	Ron Yasbin (7)

wild-type spores by dry heat and to the killing of $\alpha^-\beta^-$ spores by all treatments tested (19, 40, 45), DNA repair could make an important contribution to spore resistance to these treatments as well. However, there are no detailed studies of this possibility, with only one report indicating a minimal effect, if any, of a *recA* mutation on the wet-heat resistance of wild-type spores (14). Consequently, in this study we have examined the expression of genes involved in DNA repair during germination and outgrowth of both wild-type and $\alpha^-\beta^-$ spores which have been exposed to a variety of treatments. We have also examined the effect of a *recA* mutation on the resistance of wild-type and $\alpha^-\beta^-$ spores to these treatments. These studies indicate that DNA repair in spore outgrowth is an important factor in spore resistance to treatments which kill spores at least in part by DNA damage.

MATERIALS AND METHODS

Bacterial strains used and production of spores and cells. All strains used in this work are derivatives of *B. subtilis* 168 and are listed in Table 1. The transcriptional *lacZ* fusions to *dinR* and *uvrC* were constructed by Leendert Hamoen and Bert Jan Haijema of the University of Groningen, Groningen, The Netherlands, with PCR being used to generate fragments from bp 95 to 398 of *dinR* (32) and from bp 236 to 530 of *uvrC* (5), respectively. These fragments were cloned in plasmid pDH32 (28), which when linearized will integrate into the *B. subtilis amyE* locus by a double-crossover event. Various transcriptional *lacZ* fusions were introduced into strain PS832 or PS356 by transformation to chloramphenicol (3 μg/ml) resistance (Cm^r) with chromosomal DNA (~1 μg) from strains carrying the original *lacZ* fusions, as described previously (47). The integration of these *lacZ* fusions at the *amyE* locus was verified as described previously (47). Generation of *recA* derivatives of PS832 and PS356 was by transformation to Cm^r and erythromycin (3 μg/ml) resistance (Ery^r) with chromosomal DNA (~1 μg) from strain YB3000. The latter strain contains a plasmid carrying Cm^r and Ery^r genes integrated in *recA*, rendering this gene inactive (7). The *recA* phenotype of Cm^r Ery^r transformants was confirmed by their sensitivity to mitomycin C (5 μg/ml) on LB plates (per liter: tryptone, 10 g; yeast extract, 5 g; NaCl, 10 g; agar, 15 g; 1 M NaOH, 1 ml) as described previously (49).

Spores of various strains were produced at 37°C in 2× SG medium (26); sporulation was performed in the dark for *recA* strains. Spores were purified as described previously (26), a procedure which generally took about 2 weeks, and were stored at 10°C in water. All spores used in this work were free (>95%) of sporulating cells and germinated spores.

Growth of cells for analysis of expression of DNA repair genes or RecA levels was at 37°C in liquid LB medium; cells were harvested at an optical density at 600 nm (OD_{600}) of ~1.0. For induction of DNA repair genes, cells in LB medium at an OD_{600} of 0.25 were treated with 0.3 μg of mitomycin C per ml (49); cells were harvested after 90 min of incubation, when the OD_{600} was ~1.0.

Spore killing. Dormant spores in water (OD_{600}, 1 to 30) were treated with wet heat, hydrogen peroxide, or UV at 254 nm as described previously (9, 19, 37, 39). After hydrogen peroxide treatment, catalase was used to inactivate the hydrogen peroxide (39). Treatment of spores with dry heat or multiple cycles of freeze-thawing or freeze-drying and rehydration was also as described (10, 40). Spore killing was assessed by plating at least three different dilutions on LB plates with appropriate antibiotics and counting survivors after 16 to 36 h. For assessment of spore resistance, at least two different times points for each treatment were analyzed; for most treatments, these measurements were carried out at least twice, always with similar (±15%) results.

Spore germination and outgrowth and measurement of β-galactosidase. Spores underwent germination and outgrowth at 37°C and an initial OD_{600} (Genesys 5 spectrophotometer) of 0.3 to 0.8 in 25 ml of Spizizen's minimal medium (46) with tryptophan (25 μg/ml) and Casamino Acids (0.1%) plus 4 mM L-alanine to stimulate spore germination; the dormant spores used were not heat shocked. In this medium, ≥90% of untreated spores had initiated germination within 30 min. In some experiments, [U-^{3}H]L-leucine (10 μCi; 50 mCi/μmol) was added to this medium to allow measurement of protein accumulation during germination and outgrowth. In other experiments, [*methyl*-^{3}H]thymidine (25 μCi; 10 μM) and deoxyadenosine (0.4 mM) were added to the medium to allow measurement of DNA synthesis during germination and outgrowth. At various times after addition of spores to the medium, aliquots (500 μl) were removed and added to 500 μl of cold (4°C) 10% trichloracetic acid for measurement of the incorporation of [^{3}H]leucine and [^{3}H]thymidine into acid-insoluble forms in order to quantitate protein and DNA synthesis, respectively (36). Samples (1 to 4 ml) were also harvested at various times by centrifugation, and the pellets were frozen for eventual assay of β-galactosidase. The outgrowing spores or cells in these samples were made permeable by lysozyme treatment, and β-galactosidase was assayed with either *o*-nitrophenyl-β-D-galactopyranoside (ONPG) or methylumbelliferyl-β-D-galactopyranoside (MUG) as the substrate (26). For experiments in which protein accumulation was measured, the specific activity of β-galactosidase in the assay mixture, as determined with ONPG, was expressed as the ratio of the change in OD_{420} per minute per milliliter of culture to the percentage of total leucine incorporated into protein; for the assay using MUG, the specific activity of β-galactosidase was expressed as the ratio of the picomoles of MUG hydrolyzed per minute per milliliter of culture to the percentage of total leucine incorporated into protein. For experiments in which protein accumulation was not measured, the specific activity of β-galactosidase, as determined with ONPG, was expressed in Miller units; with MUG as the substrate, β-galactosidase specific activity was expressed as the ratio of the picomoles of MUG hydrolyzed per minute per milliliter of culture to the OD_{600} of the culture. All β-galactosidase specific activities reported have been corrected for the extremely low specific activities found in outgrowing spores and cells lacking *lacZ* fusions. These values were always ≤10% of the values obtained for spores or cells carrying the various *lacZ* fusions.

Assays of β-galactosidase in cells growing in LB medium or in dormant spores were performed as described above, but dormant spores were first made permeable to lysozyme by treatment with urea-dithiothreitol-sodium dodecyl sulfate (SDS) as described previously (26). β-Galactosidase in growing cells and in dormant spores was assayed with ONPG, and specific activities were expressed in Miller units (21).

Western blot (immunoblot) analysis of RecA. Cell extracts from 10 ml of culture of various strains were prepared for analysis of RecA as described previously (17). Dormant-spore extracts were prepared by dry rupturing of spores (15 to 20 mg [dry weight]) for 8 min with glass beads (100 mg) as the abrasive (35). Disrupted spores were extracted with 1.5 ml of a solution containing 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride for 30 min on ice; this was followed by centrifugation in a microcentrifuge. Dormant spores of strain PS2272 ($\alpha^-\beta^-$ *recA-lacZ*), with and without UV irradiation, were germinated as described above in 250 ml of medium without [^{3}H]leucine. Spores were harvested by centrifugation after 140 min; pellets were frozen, lyophilized, disrupted, and extracted as described above for dormant spores. In parallel with the 250-ml germinating cultures, [^{3}H]leucine was added to a 10-ml aliquot, and at 140 min protein synthesis was measured, β-galactosidase was assayed with MUG, and β-galactosidase specific activity was expressed relative to protein accumulation. Samples of supernatant fluid from cell and spore extracts were subjected to SDS-polyacrylamide (10%) gel electrophoresis,

TABLE 2. Levels of β-galactosidase in cells and spores carrying *lacZ* fusions to DNA repair genes[a]

lacZ fusion to	β-Galactosidase specific activity (Miller units)			
	In cells[b]		In dormant spores	
	Wild type	$\alpha^-\beta^-$	Wild type	$\alpha^-\beta^-$
dinR	70	72	0.3	0.4
recA	5.9	5.6	<0.1	<0.1
uvrC	8.2	9.2	<0.1	<0.1
No fusion	0.1	0.1	<0.1	<0.1

[a] Cells and dormant spores of strains PS356, PS832, PS2271, PS2272, PS2320, PS2321, PS2332, and PS2333 were isolated, extracted, and analyzed for β-galactosidase with ONPG as described in Materials and Methods.

[b] Cells were harvested in late log phase of growth (OD_{600} = 1.0).

proteins on the gel were transferred to polyvinylidene difluoride paper, and RecA was detected by incubation with a 1:5,000 dilution of a polyclonal rabbit anti-*B. subtilis* RecA serum (18); this was followed by incubation with alkaline phosphatase-conjugated goat antirabbit serum and detection with Lumiphos (35).

RESULTS

Induction of DNA repair genes during spore outgrowth. Since DNA damage is a major cause of killing of $\alpha^-\beta^-$ spores by all treatments so far examined and of wild-type spores by dry heat and UV, DNA repair during spore germination and outgrowth might contribute significantly to some types of spore resistance, as is the case with spore UV resistance (45). We reasoned that if DNA repair is important in resistance of spores to a particular treatment, then expression of genes involved in DNA repair might be induced during germination and outgrowth of spores subjected to this treatment when they were dormant. Consequently, we prepared wild-type and $\alpha^-\beta^-$ strains carrying *lacZ* fusions to three genes (*dinR*, *recA*, and *uvrC*) known to have key roles in various aspects of DNA repair in *B. subtilis* (11, 22, 32, 48). The *lacZ* fusions were integrated at the *amyE* locus, so all strains tested contained a functional copy of the DNA repair gene, and resistance of spores carrying these *lacZ* fusions was identical to that of spores lacking the fusions (data not shown). For all three fusions, significant levels of β-galactosidase were present in growing cells, and we found no differences between the levels in growing and sporulating cells of wild-type strains as well as of $\alpha^-\beta^-$ strains (Table 2 and data not shown). However, for all three fusions, the level of spore β-galactosidase was 50- to 200-fold lower than that in growing cells containing the fusions.

After addition of spores to the germination medium, there was an initial fall in OD_{600} which is characteristic of the initiation of spore germination (Fig. 1). This initial decrease in OD_{600} was followed by a period when the OD_{600} remained relatively constant and then a period when it began to rise. The latter two periods, up to the completion of the first cell division, encompass spore outgrowth, which is followed by cell growth (41). With the wild-type strain, DNA replication in this medium began at 90 to 100 min (Fig. 1) as found previously (41); the same was true with the $\alpha^-\beta^-$ strain (data not shown). Assay of β-galactosidase from *uvrC-lacZ* in the wild-type strain showed that *uvrC* expression began soon after the initiation of spore germination, with β-galactosidase specific activity (expressed relative to the culture's OD_{600}) rising throughout outgrowth and into cell growth (Fig. 1). Similar results were obtained when expression of the *dinR*- and *recA-lacZ* fusions was measured during germination and outgrowth of wild-type spores (data not shown).

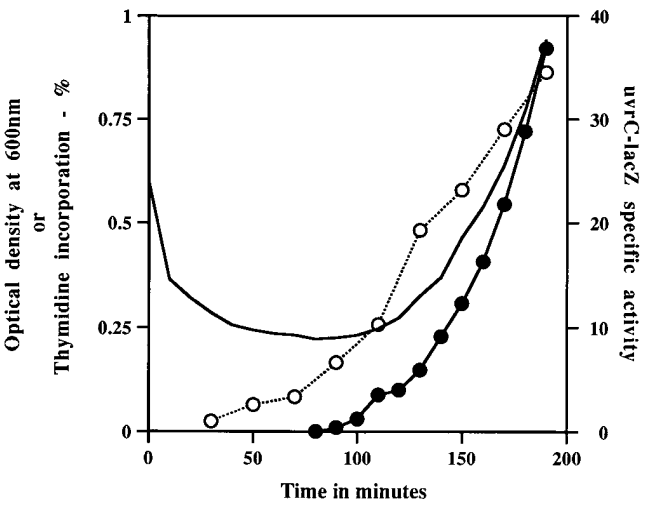

FIG. 1. Synthesis of β-galactosidase and DNA during germination and outgrowth of spores carrying *uvrC-lacZ* fusions. Spores of strain PS2320 (wild type) carrying *uvrC-lacZ* fusions were added to germination medium containing [^{3}H]thymidine. At various times, samples were taken for analysis of DNA synthesis and β-galactosidase as described in Materials and Methods. β-Galactosidase was assayed with MUG, and specific activities were expressed relative to the OD_{600} of the culture. No symbol, OD_{600}; ●, thymidine incorporation; ○, β-galactosidase specific activity.

Although the data noted above indicated that the expression of DNA repair genes began soon after the initiation of spore germination, the large increases in the specific activities of β-galactosidase from the various *lacZ* fusions were somewhat misleading, as (i) there was little, if any, β-galactosidase in spores; and (ii) the method normally used for calculating β-galactosidase specific activity involves the expression of this value relative to the OD_{600} of the culture. Consequently, we turned to expressing β-galactosidase specific activities during spore germination and outgrowth relative to the amounts of protein accumulated during these periods. By using this method, we found that with untreated wild-type spores, β-galactosidase specific activities from all three *lacZ* fusions were essentially constant throughout spore outgrowth (Fig. 2 to 4); for the *recA*- and *dinR-lacZ* fusions, the latter values were identical to those measured in growing cells (data not shown). With wild-type spores which were subjected to DNA-damaging UV irradiation, the specific activities of β-galactosidase from all three fusions rose to levels 4- to 15-fold higher than those in untreated spores (Fig. 2 to 4). This induction by UV irradiation was expected, as UV does induce DNA repair genes, such as *recA*, in growing cells (17).

The data given above indicated that DNA damage could induce expression of DNA repair genes during spore outgrowth and further indicated that the induced level of expression was maintained until at least the time when the OD_{600} of an outgrowing culture began to increase. Consequently, in order to assess the effect of different treatments of spores on expression of DNA repair genes, we subsequently analyzed samples taken only 95 to 350 min after the start of spore germination. When this analysis was carried out with untreated wild-type and $\alpha^-\beta^-$ spores, we found that $\alpha^-\beta^-$ spores showed a significantly slower return to growth (Fig. 5), although both $\alpha^-\beta^-$ and wild-type strains exhibit growth rates identical to those of vegetative cells (19, 34). The slower outgrowth of $\alpha^-\beta^-$ spores has been seen previously and is due at least in part to the extremely low generation of amino acids through proteolysis in $\alpha^-\beta^-$ spores (19, 34). Strikingly, $\alpha^-\beta^-$

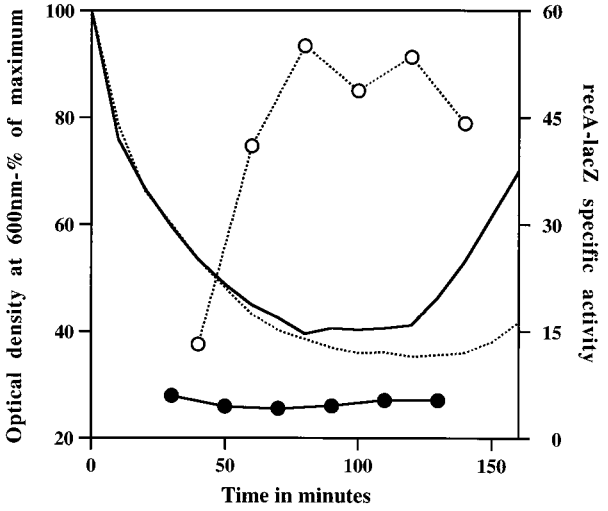

FIG. 2. Expression of *recA-lacZ* during germination and outgrowth of wild-type spores with and without UV treatment. Spores of strain PS2271 (*recA-lacZ*) were either treated with UV (53% killing) or not subjected to this treatment as described in Materials and Methods. Spores were added to germination medium containing [^{3}H]leucine, and at various times, samples were taken for analysis of protein synthesis and of β-galactosidase (with MUG as the substrate). β-Galactosidase specific activities were expressed relative to protein accumulated in the culture. The initial OD_{600}s for the cultures were as follows: unirradiated, 0.749; irradiated, 0.594. No symbol, OD_{600}; circles, β-galactosidase specific activity; dashed line, UV irradiated; solid line, nonirradiated.

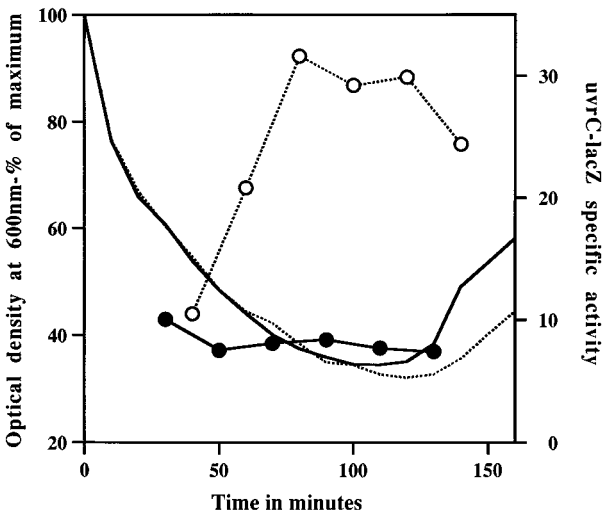

FIG. 4. Expression of *uvrC-lacZ* during germination and outgrowth of wild-type spores with and without UV treatment. Spores of strain PS2320 (*uvrC-lacZ*) were either treated with UV (12% killing) or not subjected to this treatment as described in Materials and Methods. Spores were added to germination medium containing [^{3}H]leucine, and at various times, samples were taken for analysis of protein synthesis and of β-galactosidase (with MUG as the substrate). β-Galactosidase specific activities were expressed relative to protein accumulated in the culture. The initial OD_{600}s for the cultures were as follows: unirradiated, 0.754; irradiated, 0.505. No symbol, OD_{600}; circles, β-galactosidase specific activity; dashed line, UV irradiated; solid line, nonirradiated.

spores (~2 weeks old) initially exhibited an approximately twofold higher *recA-lacZ*-driven β-galactosidase specific activity than did wild-type spores, with the value for $\alpha^-\beta^-$ spores falling to that found in wild-type spores (Fig. 5). We believe that the initial difference between the *recA-lacZ*-driven β-galactosidase specific activities of the $\alpha^-\beta^-$ and wild-type spores is the result of a slight induction of *recA-lacZ* by small amounts of DNA damage accumulated during sporulation and in the 2 weeks needed to purify the $\alpha^-\beta^-$ spores. Indeed, use of $\alpha^-\beta^-$ *recA-lacZ* spores which were purified in only 5 days (but which

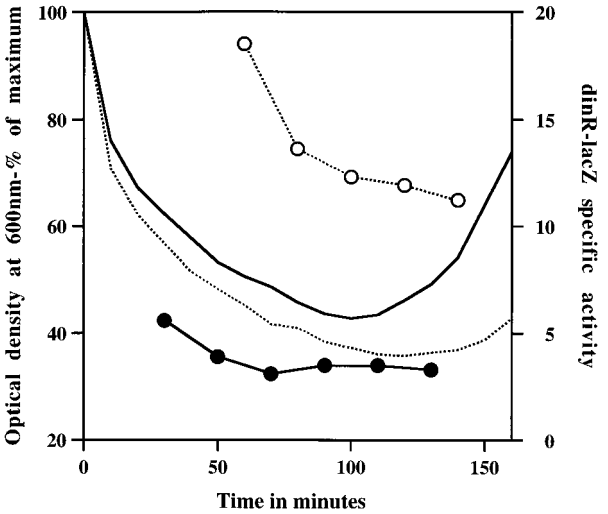

FIG. 3. Expression of *dinR-lacZ* during germination and outgrowth of wild-type spores with and without UV treatment. Spores of strain PS2332 (*dinR-lacZ*) were either treated with UV (22% killing) or not subjected to this treatment as described in Materials and Methods. Spores were added to germination medium containing [^{3}H]leucine, and at various times, samples were taken for analysis of protein synthesis and of β-galactosidase (with ONPG as the substrate). β-Galactosidase specific activities were expressed relative to protein accumulated in the culture. The initial OD_{600}s for the cultures were as follows: unirradiated, 0.759; irradiated, 0.593. No symbol, OD_{600}; circles, β-galactosidase specific activity; dashed line, UV irradiated; solid line, nonirradiated.

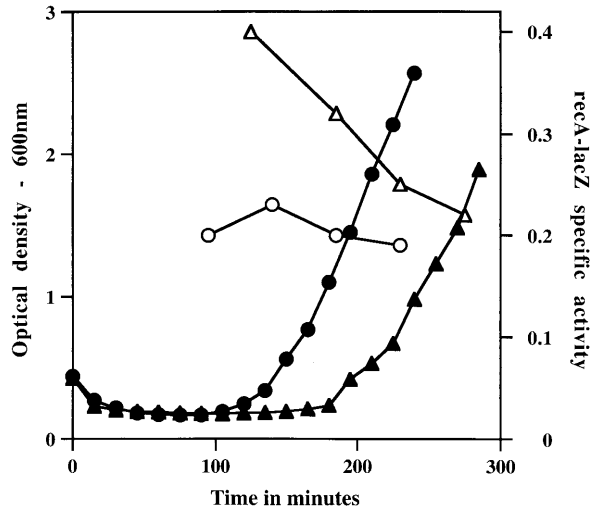

FIG. 5. Specific activities of β-galactosidase from *recA-lacZ* fusion during outgrowth and subsequent cell growth of untreated wild-type and $\alpha^-\beta^-$ spores. Spores of strains PS2271 (*recA-lacZ*) and PS2272 ($\alpha^-\beta^-$ *recA-lacZ*) were germinated in medium containing [^{3}H]leucine, samples were analyzed for protein accumulation and assayed for β-galactosidase with ONPG, and β-galactosidase specific activity was expressed relative to protein accumulation in the culture. The values for percentage of total leucine incorporated at each time point for which β-galactosidase specific activities were determined are given in Table 3. ○, PS2271 β-galactosidase specific activity; ●, PS2271 OD_{600}; △, PS2272 β-galactosidase specific activity; ▲, PS2272 OD_{600}.

TABLE 3. β-Galactosidase specific activities from *recA-lacZ* in outgrowing wild-type and $\alpha^-\beta^-$ spores given various killing treatments[a]

Treatment of dormant spores	Wild-type spores				$\alpha^-\beta^-$ spores			
	Time[b] (min)	OD_{600}	[^{3}H]leucine incorporation (% of total)	β-Galactosidase specific activity	Time[b] (min)	OD_{600}	[^{3}H]leucine incorporation (% of total)	β-Galactosidase specific activity
None[c]	95	0.172	1.0	0.2	125	0.183	0.6	0.4
	140	0.400	5.1	0.2	185	0.272	2.8	0.3
	185	1.19	21.0	0.2	230	0.750	10.9	0.2
	230	2.30	40.1	0.2	275	1.57	28.4	0.2
UV[d]	155	0.118	0.5	2.6	185	0.143	0.3	3.0
	185	0.146	0.9	1.6	215	0.152	0.6	2.9
	215	0.182	1.4	1.1	245	0.175	0.9	2.6
	245	0.270	2.4	0.6	275	0.204	1.3	1.7
Dry heat[e]	110	0.247	0.6	0.9	170	0.349	0.5	1.7
	140	0.321	1.8	0.7	200	0.367	1.1	1.3
	170	0.537	5.2	0.5	230	0.411	2.2	1.0
	200	1.19	12.3	0.4	260	0.507	3.9	0.6
Wet heat[f]	200	0.210	0.3	0.2	155	0.138	0.5	1.5
	240	0.216	0.4	0.2	185	0.177	1.2	1.3
	275	0.237	0.9	0.1	215	0.262	2.3	0.9
	300	0.262	1.6	0.2	245	0.385	4.5	0.6
H_2O_2[g]	245	0.130	0.5	0.2	260	0.255	0.6	0.4[h]
	275	0.167	1.0	0.2	290	0.286	1.2	0.3
	305	0.280	2.3	0.2	320	0.364	2.4	0.2
	335	0.449	4.8	0.2	350	0.470	4.8	0.2

[a] Spores of strains PS2171 (wild type, *recA-lacZ*) and PS2172 ($\alpha^-\beta^-$ *recA-lacZ*) were germinated, [^{3}H]leucine incorporation was measured, β-galactosidase was assayed with ONPG, and β-galactosidase specific activities were expressed relative to protein accumulated as described in Materials and Methods.

[b] Time zero is the time of addition of spores to the germination medium.

[c] The optical densities of germinating wild-type and $\alpha^-\beta^-$ spores at time zero were 0.442 and 0.427, respectively. This experiment was repeated two other times with essentially identical results.

[d] UV treatment at 254 nm of wild-type and $\alpha^-\beta^-$ spores resulted in 84 and 79% killing, respectively. The initial optical densities of the germinating wild-type and $\alpha^-\beta^-$ spores were 0.373 and 0.389, respectively.

[e] Dry-heat treatment of wild-type and $\alpha^-\beta^-$ spores was for 15 min at 120°C and for 2 min at 90°C, respectively, and yielded 45 and 30% killing, respectively. The initial ODs of the germinating wild-type and $\alpha^-\beta^-$ spores were 0.707 and 0.650, respectively. This experiment was repeated with essentially identical results.

[f] Treatment of wild-type and $\alpha^-\beta^-$ spores in water was at 85°C for 30 min and at 75°C for 30 min, respectively, and yielded 90 and 50% killing, respectively. The initial ODs of the germinating wild-type and $\alpha^-\beta^-$ spores were 0.397 and 0.396, respectively. This experiment was repeated with essentially identical results.

[g] Hydrogen peroxide (15%) treatment of wild-type and $\alpha^-\beta^-$ spores was for 20 and 5 min, respectively, and yielded 25 and 55% killing, respectively. Essentially identical β-galactosidase specific activities were obtained in a second experiment in which 60% of wild-type spores were killed by a longer exposure to hydrogen peroxide (data not shown). The initial ODs of germinating wild-type and $\alpha^-\beta^-$ spores were 0.416 and 0.525, respectively. Hydrogen peroxide treatment of spores greatly retards their outgrowth, even taking spore killing into account.

[h] This value is equivalent to that in untreated $\alpha^-\beta^-$ spores at 125 min, as hydrogen peroxide retards spore outgrowth.

were not as pure as in the usual experiments) gave a β-galactosidase specific activity at 110 min which was only ~70% of that shown in Fig. 5, and this value again fell to that of wild-type spores as outgrowth and growth continued (data not shown). This difference between the wild-type and $\alpha^-\beta^-$ strains was not seen in growing cells, as both strains had identical levels of *recA-lacZ* expression (Table 2) and mitomycin C induced identical *recA-lacZ*-driven β-galactosidase specific activities in the two strains (data not shown).

UV treatment of $\alpha^-\beta^-$ spores resulted in 7- to 11-fold-higher *recA-lacZ*-driven β-galactosidase specific activities during spore outgrowth, values similar to those of UV-treated wild-type spores (Table 3). These high values then decreased significantly as outgrowth and cell growth continued. Similar results were obtained upon analysis of dry-heat-treated spores, although β-galactosidase specific activities were lower than with UV treatment (Table 3). In contrast, wet-heat treatment resulted in *recA-lacZ* induction only with $\alpha^-\beta^-$ spores, while hydrogen peroxide treatment of both wild-type and $\alpha^-\beta^-$ spores gave no induction of *recA-lacZ* (Table 3). Previous work has shown that wild-type spores are not killed by up to 8 cycles of freeze-drying and rehydration while $\alpha^-\beta^-$ spores undergo significant killing during each cycle (10). This killing of $\alpha^-\beta^-$ spores is due to spore desiccation, not freezing, and is caused by DNA damage (10). As expected, freeze-drying of $\alpha^-\beta^-$ *recA-lacZ* spores resulted in significant induction of β-galactosidase synthesis upon subsequent spore outgrowth (Table 4).

Analysis of the expression of *lacZ* fusions to the *uvrC* and *dinR* genes gave results that were generally similar to those

TABLE 4. β-Galactosidase specific activities from *recA-lacZ* in outgrowing $\alpha^-\beta^-$ spores treated with four cycles of freeze-drying and rehydration[a]

Time[b] (min)	OD_{600}[c]	[^{3}H]leucine incorporation (% of total)	β-Galactosidase specific activity
75	0.414	0.4	3.8
105	0.437	0.9	2.9
135	0.432	1.7	2.9
225	0.436	4.9	1.6
255	0.462	6.4	1.1
285	0.484	8.0	0.7

[a] Spores of strain PS2172 were subjected to four cycles of freeze-drying and rehydration which yielded 93% killing. The spores were germinated, [^{3}H]leucine incorporation was measured, β-galactosidase was assayed with ONPG, and β-galactosidase specific activities were expressed relative to protein accumulated as described in Materials and Methods.

[b] Time zero is the time of addition of spores to the germination medium.

[c] The OD at time zero was 0.86.

TABLE 5. β-Galactosidase specific activities from *dinR-lacZ* in outgrowing wild-type and $\alpha^-\beta^-$ spores given various killing treatments[a]

Treatment of dormant spores	Wild-type spores				$\alpha^-\beta^-$ spores			
	Time[b] (min)	OD_{600}	[^{3}H]leucine incorporation (% of total)	β-Galactosidase specific activity	Time[b] (min)	OD_{600}	[^{3}H]leucine incorporation (% of total)	β-Galactosidase specific activity
None[c]	62	0.279	0.6	3.1	135	0.165	0.6	3.4
	90	0.303	1.5	3.0	165	0.220	1.4	3.3
	120	0.450	4.4	2.8	195	0.364	3.6	3.6
	150	0.876	12.2	3.0	225	0.828	9.3	3.4
UV[d]	105	0.171	0.4	11	180	0.091	0.5	21
	165	0.181	1.2	12	210	0.118	0.9	22
	195	0.209	2.1	7.2	240	0.158	1.6	16
	225	0.314	3.8	6.0				
Dry heat[e]	120	0.180	0.6	10	165	0.101	0.2	12
	150	0.214	1.4	8.0	210	0.112	0.5	12
	180	0.326	3.3	6.7	240	0.120	0.8	10
	210	0.534	7.8	5.4	270	0.146	1.1	9
Wet heat[f]	135	0.262	0.56	2.3	180	0.146	0.6	10
	165	0.290	1.3	3.2	210	0.176	1.3	7.4
	195	0.418	3.4	3.3	240	0.242	2.4	5.8
	225	0.622	7.9	3.1	270	0.358	4.8	4.0
H_2O_2[g]	225	0.421	0.7	2.2	180	0.207	0.6	3.6[h]
	255	0.508	1.6	2.9	210	0.270	1.5	3.5[h]
	285	0.608	3.7	3.4	240	0.384	3.6	3.8[h]
	315	0.874	8.5	3.1	270	0.664	8.9	2.9

[a] Spores of strains PS2332 (wild type, *dinR-lacZ*) and PS2333 ($\alpha^-\beta^-$ *dinR-lacZ*) were germinated, [^{3}H]leucine incorporation was measured, β-galactosidase was assayed with ONPG, and β-galactosidase specific activities were calculated relative to protein accumulation as described in Materials and Methods.

[b] Time zero is the time of addition of spores to the germination medium.

[c] The OD_{600}s at time zero for wild-type and $\alpha^-\beta^-$ cultures were 0.575 and 0.507, respectively. This experiment was repeated with essentially identical results.

[d] UV treatment at 254 nm of wild-type and $\alpha^-\beta^-$ spores yielded 60 and 91% killing, respectively. The OD_{600}s at time zero for wild-type and $\alpha^-\beta^-$ cultures were 0.414 and 0.298, respectively.

[e] Dry-heat treatment of wild-type and $\alpha^-\beta^-$ spores was for 15 min at 120°C and for 2 min at 80°C, respectively, and yielded 25 and 90% spore killing, respectively. The initial OD_{600}s of the germinating cultures of wild-type and $\alpha^-\beta^-$ spores were 0.438 and 0.436, respectively.

[f] Wet-heat treatment of wild-type and $\alpha^-\beta^-$ spores was for 30 min at 85°C and for 10 min at 80°C, respectively, and yielded 70 and 90% killing, respectively. The ODs at time zero for wild-type and $\alpha^-\beta^-$ cultures were 0.522 and 0.450, respectively. This experiment was repeated with essentially identical results.

[g] Hydrogen peroxide (15%) treatment of wild-type and $\alpha^-\beta^-$ spores was for 20 and 5 min, respectively, and yielded 25 and 50% killing, respectively. The initial OD_{600}s of the cultures of wild-type and $\alpha^-\beta^-$ spores were 0.82 and 0.717, respectively. This experiment was repeated with essentially identical results. Hydrogen peroxide treatment of spores greatly retards their outgrowth, even taking spore killing into account.

[h] These values are equivalent to those in untreated $\alpha^-\beta^-$ spores, at earlier times, as hydrogen peroxide greatly slows spore outgrowth.

obtained with the *recA-lacZ* fusion, with two exceptions (Tables 5 and 6). Thus, *uvrC-lacZ* (but not *dinR-lacZ*) expression was slightly higher during outgrowth of untreated $\alpha^-\beta^-$ spores than during outgrowth of wild-type spores. In addition, *dinR-* and *uvrC-lacZ* expression was induced during spore outgrowth by treatment of $\alpha^-\beta^-$ and wild-type spores with dry heat and UV, while wet-heat treatment resulted in induction only with $\alpha^-\beta^-$ spores. As was seen with *recA-lacZ* expression, *dinR-lacZ* expression was not induced during outgrowth of $\alpha^-\beta^-$ or wild-type spores by hydrogen peroxide treatment; *uvrC-lacZ* also was not induced following hydrogen peroxide treatment of wild-type spores. However, hydrogen peroxide treatment of $\alpha^-\beta^-$ spores did result in an ~twofold induction of *uvrC-lacZ* upon subsequent spore outgrowth.

Effect of a *recA* mutation on spore resistance. The data presented above indicated that a number of treatments that kill wild-type or $\alpha^-\beta^-$ spores result in induction of expression of DNA repair genes upon spore outgrowth. This finding suggested that these treatments cause spore DNA damage which then results in a signal inducing expression of DNA repair genes. Indeed, all treatments that resulted in induction of DNA repair genes are thought to kill spores by damaging DNA (9, 10, 39, 45). This correlation further suggested that DNA repair is important in spore resistance to these particular treatments, as has been shown to be the case for UV (45). In order to assess the importance of DNA repair in other types of spore resistance, we analyzed the resistance of spores of *recA* derivatives of our wild-type and $\alpha^-\beta^-$ strains to various treatments. As expected, the *recA* mutation had little effect on the wet-heat and hydrogen peroxide resistance of wild-type spores but significantly reduced their dry-heat resistance and slightly reduced their UV resistance (Table 7). The slight effect of the *recA* mutation on spore UV resistance has been seen previously (14, 25) and is due to the decrease in excision repair in the *recA* mutant; SP-specific repair, however, is unaffected. In contrast to these results, resistance of $\alpha^-\beta^-$ spores to all treatments tested, including freeze-drying, was significantly decreased by the *recA* mutation (Tables 8 and 9). However, the *recA* mutation did not result in sensitivity of $\alpha^-\beta^-$ spores to freezing (Table 9).

Levels of RecA in spores. Given the central role for RecA in the induction of expression of repair genes in response to DNA damage (11, 48), an obvious question is whether RecA is present in spores. β-Galactosidase from the *recA-lacZ* fusion was not found in spores, indicating that this enzyme is degraded during sporulation, at least in the forespore compartment of the sporulating cell. This has been seen previously for other *lacZ* fusions expressed only in vegetative cells (30, 31) but does not mean that the wild-type product of the gene fused to *lacZ* is absent from spores (30, 31). Consequently, we carried out Western blot analysis with anti-RecA serum and both cell and spore extracts. Surprisingly, the level of RecA in spores relative to that of other proteins was much less than that in vegetative cells (Fig. 6, lanes 1, 4, and 5). Western blot

TABLE 6. β-Galactosidase specific activities from *uvrC-lacZ* in outgrowing wild-type and $\alpha^-\beta^-$ spores given various killing treatments[a]

Treatment of dormant spores	Wild-type spores				$\alpha^-\beta^-$ spores			
	Time[b] (min)	OD_{600}	[^{3}H]leucine incorporation (% of total)	β-Galactosidase specific activity	Time[b] (min)	OD_{600}	[^{3}H]leucine incorporation (% of total)	β-Galactosidase specific activity
None[c]	90	0.146	0.6	8.7	105	0.198	0.5	13
	120	0.204	1.7	10	150	0.213	1.5	13
	150	0.353	5.0	8.0	180	0.334	4.0	11
	180	0.774	13.0	7.2	210	0.640	10.0	9.3
UV[d]	120	0.103	0.3	33	135	0.154	0.6	41
	150	0.115	0.6	36	165	0.179	1.4	42
	180	0.142	1.2	28	195	0.248	2.6	36
	210	0.19	2.2	19	225	0.312	4.1	28
Dry heat[e]	135	0.279	1.3	36	150	0.320	1.2	47
	165	0.372	3.4	27	186	0.322	2.1	40
	195	0.660	8.2	19	225	0.356	3.2	32
	225	1.068	16.7	14	270	0.400	5.4	21
Wet heat[f]	165	0.112	0.4	10	152	0.191	0.8	31
	195	0.150	1.1	8.6	180	0.226	1.8	26
	225	0.266	2.7	7.3	210	0.306	3.4	21
	255	0.444	6.0	8.1	240	0.454	6.5	16
H_2O_2[g]	240	0.147	0.4	8.0	195	0.289	0.8	28[h]
	270	0.180	0.9	7.8	225	0.308	1.6	17[h]
	300	0.242	2.1	7.3	255	0.418	3.6	14[h]
					285	0.622	8.1	11[h]

[a] Spores of strains PS2320 (wild type, *uvrC-lacZ*) and PS2321 ($\alpha^-\beta^-$ *uvrC-lacZ*) were germinated, [^{3}H]leucine incorporation was measured, β-galactosidase was assayed with MUG, and β-galactosidase specific activities were expressed relative to protein accumulated as described in Materials and Methods.

[b] Time zero is the time of addition of spores to the germination medium.

[c] The initial OD_{600}s of wild-type and $\alpha^-\beta^-$ cultures were 0.475 and 0.458, respectively. This experiment was repeated with essentially identical results.

[d] UV treatment at 254 nm of both wild-type and $\alpha^-\beta^-$ spores yielded 70% killing. The initial OD_{600}s of the wild-type and $\alpha^-\beta^-$ cultures were 0.394 and 0.421, respectively.

[e] Dry-heat treatment of wild-type and $\alpha^-\beta^-$ spores was for 15 min at 120°C and for 2 min at 90°C, respectively, and yielded 20 and 90% killing, respectively. The initial OD_{600}s of the cultures of wild-type and $\alpha^-\beta^-$ spores were 0.758 and 0.765, respectively.

[f] Wet-heat treatment of wild-type and $\alpha^-\beta^-$ spores in water was for 20 min at 85°C and for 10 min at 80°C, respectively, and yielded 65 and 80% killing, respectively. The initial OD_{600}s of the cultures of wild-type and $\alpha^-\beta^-$ spores were 0.409 and 0.446, respectively. This experiment was repeated with essentially identical results.

[g] Hydrogen peroxide (15%) treatment of wild-type and $\alpha^-\beta^-$ spores was for 30 and 7.5 min, respectively, and yielded 55 and 65% killing, respectively. The initial OD_{600}s of the cultures of wild-type and $\alpha^-\beta^-$ spores were 0.590 and 0.649, respectively. This experiment was repeated with essentially identical results. Hydrogen peroxide treatment of spores greatly retards their outgrowth, even taking spore killing into account.

[h] Similar values were obtained in a second independent experiment. Note that these values are higher than those in untreated $\alpha^-\beta^-$ spores at earlier times in outgrowth.

analysis of different amounts of wild-type cell extracts as well as a dormant-spore extract indicated that the level of RecA in spores (relative to other proteins) was <15% of that in cells, and experiments in which cells and spores were broken and extracted together showed that the cell RecA was fully recovered in the mixed extracts (data not shown). Although levels of RecA are low in spores, previous work has provided strong evidence for the presence of UvrC in spores (24); there are, however, no data on the relative levels of this protein in spores and cells. As expected from analyses of expression of the *recA-lacZ* fusion, RecA levels rose significantly during spore outgrowth (Fig. 6, lane 6) and even more so during outgrowth of UV-irradiated spores (Fig. 6, lane 7). The latter results indicate that analysis of *recA-lacZ* expression is a good reflection of RecA synthesis and levels during this period of development.

TABLE 7. Effect of *recA* mutation on resistance of wild-type spores to various treatments[a]

Treatment	Time or dose required for 90% killing of[b]:	
	Wild-type spores	*recA* spores
90°C, in water	18 min	25 min
120°C, dry	18 min	2 min
H_2O_2 (15%)	50 min	55 min
UV	330 J/m^2	185 J/m^2

[a] Spores of strains PS2271 (wild type) and PS2318 (*recA*) were prepared and subjected to various treatments, and their viabilities were determined, as described in Materials and Methods.

[b] All treatments gave relatively linear semilogarithmic killing curves. Numbers presented are averages of values obtained from two separate experiments; the experiments gave similar (±15%) values.

TABLE 8. Effect of *recA* mutation on resistance of $\alpha^-\beta^-$ spores to various treatments[a]

Treatment	Time or dose required for 90% killing of[b]:	
	$\alpha^-\beta^-$ spores	$\alpha^-\beta^-$ *recA* spores
75°C, in water	100 min	7 min
90°C, dry	2 min	<0.2 min
H_2O_2 (15%)	15 min	2 min
UV	18.5 J/m^2	1.8 J/m^2

[a] Spores of strains PS2272 ($\alpha^-\beta^-$) and PS2319 ($\alpha^-\beta^-$ *recA*) were prepared and subjected to various treatments, and their viabilities were determined, as described in Materials and Methods.

[b] All treatments gave relatively linear semilogarithmic killing curves. Numbers presented are averages of values obtained from two separate experiments which gave similar (±15%) results.

TABLE 9. Effect of *recA* mutation on sensitivity of $\alpha^-\beta^-$ spores to freezing and freeze-drying[a]

Treatment	% Survival of:	
	$\alpha^-\beta^-$ spores	$\alpha^-\beta^-$ *recA* spores
One freeze-drying	84	11
Four freeze-thawings	118	123
Four freeze-drying and rehydration cycles	12	<0.003

[a] Spores of strains PS2272 ($\alpha^-\beta^-$) and PS2319 ($\alpha^-\beta^-$ *recA*) were prepared and subjected to either freeze-thawing or freeze-drying and rehydration, and survivors were enumerated, as described in Materials and Methods.

DISCUSSION

The regulation of the SOS system that controls the expression of a number of DNA repair genes has been fairly well worked out in *E. coli* (11); the SOS system in *B. subtilis* appears to be generally similar (48). Although some of the specific details of the *B. subtilis* system remain to be established, the likely scenario is as follows. In *B. subtilis*, RecA is an initial sensor of DNA damage, which can be generated in cells by a variety of treatments, including UV, hydrogen peroxide, and cross-linking agents (48). The DNA damage caused by these treatments may not be sensed directly, but single-stranded DNA regions that arise upon either replication or repair of damaged DNA appear to be sensed in some fashion (18). However, it is possible that different types of DNA damage may be sensed differently. Whatever the precise nature of the DNA damage which is or which generates the inducing signal, this signal causes the conversion of RecA into an activated form that stimulates the digestion (probably autodigestion) of the DinR protein (11, 18, 48). DinR is probably a repressor of many damage repair genes, including *recA*, *uvrC*, and *dinR* itself, and DinR autodigestion inactivates its repressor function (6, 16, 18, 48). Consequently, DNA damage results in increased expression of many DNA repair genes, and this expression is eventually shut down as DNA damage is repaired, activated RecA is lost, and functional DinR levels rise.

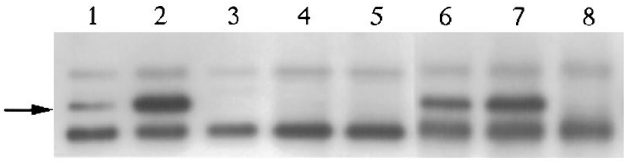

FIG. 6. Levels of RecA in spores and cells. Growing cells were isolated with or without treatment with mitomycin C and extracted as described in Materials and Methods. In parallel experiments, mitomycin C treatment of cells of strain PS2721 resulted in a sixfold increase in *recA-lacZ*-driven β-galactosidase specific activity (expressed in Miller units). Dormant spores or spores germinated for 140 min with or without initial UV treatment (20% killing) were isolated and extracted as described in Materials and Methods. The UV treatment of the spores of strain PS2722 used in this experiment resulted in a threefold increase in *recA-lacZ*-driven β-galactosidase specific activity at 140 min over that in untreated germinated spores; these specific activities were measured relative to protein accumulation. Aliquots of various extracts were subjected to SDS-polyacrylamide gel electrophoresis, proteins were transferred to polyvinylidene difluoride paper, and RecA was detected as described in Materials and Methods. The sources of the extracts (and the amounts of protein applied) are as follows: lane 1, untreated cells of PS832 (1 μg); lane 2, mitomycin C-treated cells of PS832 (1 μg); lane 3, untreated cells of PS2318 (*recA*) (1 μg); lane 4, dormant spores of PS832 (5 μg); lane 5, dormant spores of PS2318 (*recA*) (5 μg); lane 6, germinated spores of PS2272 ($\alpha^-\beta^-$ *recA-lacZ*) (1 μg); lane 7, germinated UV-irradiated spores of PS2722 (1 μg); and lane 8, germinated spores of PS2318 (*recA*) (1 μg). The RecA band is indicated by the horizontal arrow (note the absence of this band in strain PS2318). The band above RecA reacts with nonimmune serum; the band below RecA is presumably reacting with a non-RecA antibody in the polyclonal anti-RecA serum. Lanes 1 to 5 and 6 to 8 are from different gels.

Given what is known about this system in *E. coli* and *B. subtilis*, it is not surprising that treatments causing significant spore DNA damage result in induction of *dinR*-, *recA*-, and *uvrC-lacZ* fusions during spore outgrowth. Presumably the DNA damage is sensed by RecA during outgrowth, resulting in activation of RecA, cleavage of DinR, relief of repression of the *dinR*, *recA*, and *uvrC* genes, and induction of the last three genes, including their *lacZ* fusions. By analogy with the *E. coli* SOS system (11), it is likely that the different degrees of induction (compare the values for wild-type spores with and without UV treatment) of the three DNA repair genes are due to differences in the affinity of DinR for the control regions of these genes. Elevated levels of these and other DNA repair proteins then increase DNA repair, resulting in removal of the original inducing signal, and subsequent cell growth dilutes out the induced level of β-galactosidase from the *lacZ* fusions. Cells carrying *recA* mutations not only lack RecA but also do not show significant induction of many other DNA repair genes (48). Consequently, *recA* cells are defective in DNA repair and are significantly more sensitive to a number of DNA-damaging treatments than are wild-type cells. Although the effects of a *recA* mutation are usually ascribed to effects on the SOS system, there is recent work suggesting that RecA may also modulate the heat shock response in the gram-positive organism *Lactococcus lactis* (8). However, there is no information on the involvement of RecA in the heat shock response in *B. subtilis.*

UV-induced TT formation in cells is thought to result in activation of RecA by generation of single-stranded DNA by either excision of TT or blockage of replication by TT followed by reinitiation of replication after formation of the lesion (18); presumably the induction of DNA repair genes during outgrowth of UV-treated $\alpha^-\beta^-$ spores is due to TT formation. Since UV irradiation of wild-type spores also results in induction of DNA repair genes, SP presumably also causes single-stranded DNA formation during spore outgrowth in a fashion similar to that of TT. Because expression of *recA*, as well as that of *dinR* and *uvrC*, is induced by UV during spore outgrowth at or before the time of initiation of DNA replication in untreated spores, it appears likely that it is excision repair of UV lesions which leads to activation of RecA during this period.

In contrast to the situation with UV, in which the nature of the DNA damage generated is known, for the other spore treatments used in this work, the precise nature of the DNA lesions generated is not clear. However, these lesions appear somewhat less efficient at inducing DNA repair genes than are SP and TT. The initial DNA lesions generated by wet- and dry-heat treatment of $\alpha^-\beta^-$ spores and by dry-heat treatment of wild-type spores may be primarily abasic sites resulting from depurination, although there appear to be further reactions at these abasic sites (9, 40, 45). Unfortunately, the repair process for abasic lesions in *B. subtilis*—including the mechanism for repair of these lesions, the gene products involved, and the regulation of genes involved in this repair pathway—is not known. However, the induction of DNA repair genes during spore outgrowth by wet- and dry-heat treatment of $\alpha^-\beta^-$ spores and by dry-heat treatment of wild-type spores indicates that DNA damage caused by these treatments generates the RecA activation signal. Consequently, genes for repair of this type of damage, which predominantly consists of abasic sites, are at least in part under DinR's control. The large effect of the *recA* mutation on the dry-heat resistance of wild-type spores and the wet- and dry-heat resistance of $\alpha^-\beta^-$ spores is further

evidence that genes for repair of DNA damage generated by these treatments are part of the SOS regulon. This also appears to be the case for damage due to desiccation of $\alpha^-\beta^-$ spores, although there is no precise information on the nature of the initial DNA lesion induced by desiccation other than that it leads to single-strand breaks (10). Interestingly, recent work has indicated that *recA* is also important in the resistance of growing cells of *Deinococcus radiodurans* to desiccation (20), although there are no data available on the role of *recA* in the desiccation resistance of cells of *Bacillus* species.

One surprising result of our analysis of the dependence of DNA repair gene expression on spore treatments is the lack of induction of the *dinR* and *recA* genes by hydrogen peroxide treatment of $\alpha^-\beta^-$ spores. This agent does kill $\alpha^-\beta^-$ spores by damaging DNA (39), and hydrogen peroxide does induce expression of *recA* in *B. subtilis* cells (2). However, the mechanism whereby hydrogen peroxide results in RecA activation in cells is not known, and it is possible that the DNA damage caused by hydrogen peroxide is different in cells and spores. In $\alpha^-\beta^-$ spores, hydrogen peroxide treatment generates no abasic sites in DNA and does generate single-strand breaks (39), but it is not clear if the latter lesion can result in RecA activation. However, the *recA* mutation does significantly reduce the hydrogen peroxide resistance of $\alpha^-\beta^-$ spores, which suggests that the repair of hydrogen peroxide-induced DNA lesions in these spores requires RecA either directly or indirectly, as is the case in growing cells (3). Although *dinR* and *recA* were not induced during outgrowth of hydrogen peroxide-treated $\alpha^-\beta^-$ spores, *uvrC* was induced slightly, although not by hydrogen peroxide treatment of wild-type spores. These data suggest that *uvrC* may be controlled not only by RecA-DinR but also by another regulatory system that responds to the lesion generated in $\alpha^-\beta^-$ spores by hydrogen peroxide. A number of genes involved in inducible resistance of *B. subtilis* cells to hydrogen peroxide are members of a peroxide (*per*) regulon that is distinct from the SOS system (4, 48). However, the precise signal inducing the *per* regulon has not been determined, and it is not known whether *uvrC* is a member of this regulon.

With the exception of the lack of *dinR* and *recA* induction by hydrogen peroxide treatment of $\alpha^-\beta^-$ spores, all treatments that are thought to kill spores by damaging DNA also resulted in induction of DNA repair genes during spore outgrowth. Conversely, treatments that are thought not to kill spores by damaging DNA did not result in induction of DNA repair genes during spore outgrowth. Even in the case of the exception to these correlations, noted above, *uvrC* was induced by hydrogen peroxide treatment of $\alpha^-\beta^-$ spores. We also found that *recA* spores had decreased resistance to any treatment which has been suggested to kill spores by damaging DNA and that the *recA* mutation had no effect on spore resistance to treatments which are thought to kill spores by some means other than DNA damage. Notable among the latter results was the lack of an effect of a *recA* mutation on the hydrogen peroxide resistance of wild-type spores and the significant decrease in hydrogen peroxide resistance in $\alpha^-\beta^-$ *recA* spores. Taken together, these data provide further strong support for our suggestions that DNA damage (i) is a significant mechanism whereby UV and dry heat kill wild-type and $\alpha^-\beta^-$ spores and wet heat, desiccation, and hydrogen peroxide kill $\alpha^-\beta^-$ spores; and (ii) is not important in the killing of wild-type spores by wet heat and hydrogen peroxide.

One of the most significant findings to come from the work reported in this communication is the demonstration of the significant role of DNA repair in spore resistance to some treatments, e.g., dry heat. Spore resistance is dependent on a number of factors, with different factors having differing degrees of importance for any particular treatment. Major factors involved in spore resistance include the relative dehydration of the spore core, the mineralization of the spore core, the relative impermeability of the spore core to potentially DNA-damaging chemicals, the protection of spore DNA against some types of damage, and DNA repair (12, 44). Although the mechanism for repair of spore UV damage has been established at least in part, we currently know very little about the enzymes involved in the repair of other types of *B. subtilis* spore DNA damage. Clearly, a challenge for future work will be to identify repair enzymes that are involved in other aspects of spore DNA repair.

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