Temporal Regulation and Forespore-Specific Expression of the Spore Photoproduct Lyase Gene by Sigma-G RNA Polymerase during *Bacillus subtilis* Sporulation

MARIO PEDRAZA-REYES,¹ FELIX GUTIÉRREZ-CORONA,¹ AND WAYNE L. NICHOLSON^{2*}

Institute of Investigation in Experimental Biology, Faculty of Chemistry, University of Guanajuato, Guanajuato, Gto., 36060, Mexico,¹ and Departments of Microbiology and Immunology and Biochemistry and Molecular Biology, University of North Texas Health Science Center, Fort Worth, Texas 76107²

Received 18 January 1994/Accepted 29 April 1994

Bacterial spores are highly resistant to killing by UV radiation and exhibit unique DNA photochemistry. UV irradiation of spore DNA results in formation of spore photoproduct (SP), the thymine dimer 5-thymine-5,6-dihydrothymine. Repair of SP occurs during germination of *Bacillus subtilis* spores by two distinct routes, either by the general nucleotide excision repair (*uvr*) pathway or by a novel SP-specific monomerization reaction mediated by the enzyme SP lyase, which is encoded by the *spl* gene. Repair of SP occurs early in spore germination and is independent of de novo protein synthesis, suggesting that the SP repair enzymes are synthesized during sporulation and are packaged in the dormant spore. To test this hypothesis, the expression of a translational *spl-lacZ* fusion integrated at the *spl* locus was monitored during *B. subtilis* growth and sporulation. β -Galactosidase expression from the *spl-lacZ* fusion was silent during vegetative growth and was not DNA damage inducible, but it was activated at morphological stage III of sporulation specifically in the forespore compartment, coincident with activation of expression of the stage III marker enzyme glucose dehydrogenase. Expression of the *spl-lacZ* fusion was shown to be dependent upon the sporulation-specific RNA polymerase containing the sigma-G factor ($E\sigma^G$), as *spl-lacZ* expression was abolished in a mutant harboring a deletion in the *sigG* gene and restored by expression of the *sigG* gene in *trans*. Primer extension analysis of *spl* mRNA revealed a major extension product initiating upstream from a small open reading frame of unknown function which precedes *spl*, and it revealed two other shorter minor extension products. All three extension products were present in higher quantities during sporulation and after *sigG* induction. The three putative transcripts are all preceded by sequences which share homology with the consensus $E\sigma^G$ -type promoter sequence, but *in vitro* transcription by purified sigma-G RNA polymerase was detected only from the promoter corresponding to the major extension product. The open reading frame-*spl* operon therefore appears to be an additional member of the sigma-G regulon, which also includes as members the small, acid-soluble spore proteins which are in large part responsible for spore DNA photochemistry. Therefore, sporulating bacteria appear to coordinately regulate genes whose products not only alter spore DNA photochemistry but also repair the major spore-specific photoproduct during germination.

Dormant bacterial spores are 1 to 2 orders of magnitude more resistant to the killing effects of UV radiation than are vegetatively growing cells of the same organism (42, 49). The mechanisms underlying this phenomenon have to date been most extensively studied in the case of the gram-positive soil bacterium *Bacillus subtilis*; at present, two related phenomena have been found to account for the high resistance of *B. subtilis* spores to UV light.

First, DNA from UV-irradiated spores has photochemistry very different from that of DNA from UV-irradiated vegetative cells (5). In contrast to the *cis-syn* cyclobutane pyrimidine dimers which are produced in DNA as a result of UV irradiation of vegetative cells, UV irradiation of *B. subtilis* spores results in the production of a novel DNA photoproduct informally called spore photoproduct (SP) (5). The chemical structure of SP has been elucidated to be the thymine dimer 5-thymine-5,6-dihydrothymine (51). SP has been isolated in nature only from UV-irradiated bacterial spores (5), although this photoproduct can also be formed *in vitro* (32). During

sporulation bacteria produce specific proteins which upon binding to spore DNA determine in large part its unique photochemistry. DNA inside spores is found associated with a group of DNA-binding proteins called small, acid-soluble spore proteins (SASPs) of a class known in the case of *B. subtilis* as the α/β -type (12, 47). Deletion of the genes encoding the major α/β -type SASP in *B. subtilis* results in strains whose spores are very sensitive to UV radiation (22). Studies *in vitro* have demonstrated that α/β -type SASPs bind DNA (31) and promote a DNA helical change from the B conformation to an A-like conformation (25); UV irradiation of these SASP-DNA complexes *in vitro* results in formation of SP and suppression of cyclobutyl dimer formation (32).

Second, SP which accumulates in dormant spores is eliminated from spore DNA at the onset of spore germination in part through the activity of nucleotide excision repair mediated by the *uvr* pathway (28, 29). In addition to nucleotide excision repair, however, *B. subtilis* possesses a unique, SP-specific DNA repair enzyme called SP lyase (26, 28, 29), encoded by the *spl* gene (7, 26). SP lyase appears to act by direct *in situ* monomerization of SP in DNA to two thymines (28, 29, 52). Taken together, the *uvr* and *spl* repair pathways probably account for most of the activity which removes UV damage

* Corresponding author. Phone: (817) 735-2120. Fax: (817) 735-2118.

TABLE 1. Bacterial strains used in this study

Strain	Genotype and/or phenotype	Source (reference) ^a
<i>B. subtilis</i>		
168	<i>trpC2</i>	Laboratory stock
WN118	<i>sigGΔ1 trpC2</i>	P. Setlow (50)
WN119	<i>trpC2 spl-lacZ</i> Cm ^r	pWN116 → 168
WN126	<i>sigGΔ1 trpC2 spl-lacZ</i> Cm ^r	pWN116 → WN118
WN127	<i>sigGΔ1 trpC2 spl-lacZ</i> Cm ^r [pDG298; P _{spac} - <i>sigG</i> Km ^r] ^b	pDG298 → WN126
YB5176	Tn917 <i>dinA76-lacZ</i> Cm ^r	R. Yasbin (3)
<i>E. coli</i>		
JM83	<i>ara Δ(lac-proAB) rpsL φ80</i> <i>lacZΔM15</i>	Laboratory stock (53)
GM161	F ⁻ <i>thr-1 leuB6 dam-4 thi-1 hsdS1</i> <i>lacY1 tonA21 λ⁻ supE44</i>	Laboratory stock (7)

^a →, transformation.^b Brackets contain the plasmid and marker.

from DNA during spore germination, as it has been observed that mutant strains of *B. subtilis* lacking both repair pathways produce spores which are highly sensitive to UV radiation (26, 27).

The SP lyase repair system was originally defined by a mutation now called *spl-1* (26). To better understand *spl*-mediated DNA repair, the wild-type *spl* gene from *B. subtilis* 168 was recently cloned because of its ability to restore UV resistance to spores of a *B. subtilis* strain which harbors mutations in both the *uvr* and *spl* pathways (7). The physical organization of the *spl* locus has been deduced by nucleotide sequence analysis of the region, and comparison by FASTA analysis (35) of the deduced amino acid sequences of SP lyase and other DNA repair proteins revealed that the carboxy-terminal region of SP lyase shares sequence homology with those of DNA photolyases from a number of organisms (7). This observation suggests that these enzymes may have descended from a common ancestral protein, even though SP lyase is not dependent on visible light for its function (7).

Early indirect studies of SP repair during *B. subtilis* germination indicated that both the *uvr* and *spl* repair systems were operational when spores were germinated in the presence of either chloramphenicol or rifampin, compounds which inhibit de novo synthesis of protein and RNA, respectively (29). The results of these experiments suggest that the components of the *spl* and *uvr* pathways are synthesized during the previous round of either vegetative growth or sporulation and are packaged in the mature dormant spore. With the recent molecular cloning of the *spl* gene, this hypothesis can now be

tested by direct assay of *spl* expression. In this article we report the construction of a transcriptional and translational fusion between the *spl* gene and the *Escherichia coli lacZ* gene to study the regulation of expression of the *spl* gene during the developmental cycle of *B. subtilis* and the characterization of *spl* transcription both in vitro and in vivo.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. *B. subtilis* and *E. coli* strains used in this study are listed in Table 1, and plasmids used in this study are described in Table 2. Media used were Difco sporulation medium (DSM) (41) and Luria-Bertani (LB) medium (24). Preparation of competent *B. subtilis* cells and their transformation with plasmid DNA were performed as previously described (2). When appropriate, antibiotics were added to media as follows: chloramphenicol, 3 μg/ml; ampicillin, 50 μg/ml; and kanamycin, 10 μg/ml. Cells were grown in liquid media with vigorous aeration or on solid media at 37°C. The optical density of liquid cultures was monitored with either a Klett-Summerson colorimeter fitted with a no. 66 (red) filter or a Perkin-Elmer model 565 spectrophotometer set at 600 nm.

Construction and integration of the *spl-lacZ* gene fusion. Construction of an in-frame translational fusion between the *spl* gene and the *E. coli lacZ* gene was carried out in the integrative plasmid pJF751 (11) by inserting an 846-bp *EcoRI*-*BclI* fragment from plasmid pWN87, corresponding to a region from nucleotide (nt) 527 to 1373 in the published *spl* sequence (7), into *EcoRI*-*Bam*HI-cleaved plasmid pJF751, as illustrated in Fig. 1. Identification of the desired plasmid construction was performed by restriction enzyme analysis of small-scale plasmid preparations (1) digested with *EcoRI* and *Pvu*II. The resulting plasmid containing the *spl-lacZ* fusion, designated pWN116 (Fig. 1), was propagated in *E. coli* JM83. Competent cells of *B. subtilis* were transformed with plasmid pWN116, and transformants were selected on solid DSM containing chloramphenicol. Chromosomal DNA was isolated (4) from one of these transformants, designated strain WN119, and integration of the *spl-lacZ* fusion at the chromosomal *spl* locus was confirmed by Southern blot analysis (48). Briefly, genomic DNA isolated from *B. subtilis* 168 and WN119 was restricted with *Hind*III and separated by electrophoresis through a 1% agarose gel. The DNA fragments were transferred to a nitrocellulose membrane and were probed with an agarose gel-purified 2.3-kb *EcoRI*-*Hind*III fragment from plasmid pWN41, which encodes the entire *spl* sequence (Table 2) (7). The probe was labeled by the random oligonucleotide priming technique (10) using digoxigenin-dUTP. Detection of hybrids was carried

TABLE 2. Plasmids used in this study

Plasmid	Description	Source (reference)
pDG298	IPTG-inducible <i>sigG</i> gene; Km ^r	P. Setlow (50)
pJF751	Integrational <i>lacZ</i> fusion vector; Cm ^r	P. Setlow (11)
pPS395	<i>sspA</i> gene cloned in pJH101	P. Setlow (3a)
pWN41	2.3-kb <i>EcoRI</i> - <i>Hind</i> III fragment of <i>spl</i> from pWN35 cloned in pBGSC6	This study (7)
pWN68	1,357-bp <i>EcoRI</i> - <i>Sph</i> I fragment of <i>spl</i> gene (from nt 527 to 1884) cloned in pBGSC6	Laboratory stock (7)
pWN78	839-bp <i>Pst</i> I- <i>Eco</i> RI fragment of <i>spl</i> (from nt 117 to 956) cloned in pUC18	P. Fajardo-Cavazos, this laboratory
pWN81	678-bp <i>Pst</i> I- <i>Eco</i> RI fragment of <i>spl</i> (from nt 117 to 795) cloned in pUC18	P. Fajardo-Cavazos, this laboratory
pWN87	pWN68 propagated in <i>E. coli</i> GM161; lacks <i>Dam</i> methylation	This study
pWN116	846-bp <i>EcoRI</i> - <i>Bcl</i> I fragment (from nt 527 to 1373) of <i>spl</i> from pWN87 cloned into <i>EcoRI</i> - <i>Bam</i> HI site of pJF751	This study ^a
pWN204	292-bp <i>Hind</i> III- <i>Eco</i> RI fragment of <i>sspA</i> 5' end from pPS395 cloned in pUC18	This study

^a Shown in Fig. 1.

FIG. 1. Construction of plasmid pWN116 containing an in-frame translational *spl-lacZ* gene fusion. See Materials and Methods for details. MCS, multiple cloning site; oriE, *E. coli* origin of replication.

out by using antidigoxigenin antibodies conjugated to alkaline phosphatase and the lumigen substrate 4-methoxy-4-(3-phosphatphenyl)spiro(1,2dioxetane-3,2'-ada-mantane) with the Dig-luminescent Detection kit (Boehringer-Mannheim Biochemicals) as described by the manufacturer. The presence of the *spl-lacZ* fusion integrated at the *spl* locus was confirmed by visualization of an expected 11.3-kb hybridizing band in the sample from strain WN119, whereas an expected 3.7-kb hybridizing band was detected in the sample from strain 168 (data not shown).

Cell growth and enzymatic assays. *B. subtilis* strains carrying the *spl-lacZ* fusion were grown and allowed to sporulate in liquid DSM containing chloramphenicol. Samples (1 or 1.5 ml each) were collected periodically during vegetative growth and throughout sporulation. Cell samples were washed with cold 0.25 M Tris-HCl buffer (pH 7.5), and the cell pellets were stored at -20°C . Cell extracts were prepared from mother cell and forespore fractions as previously described (21, 33) and were assayed for β -galactosidase (24, 33) and glucose dehydrogenase (GDH) (13, 33) activities. Briefly, washed cell samples were first disrupted with lysozyme and subjected to centrifugation. β -Galactosidase activity present in the supernatant was measured and assigned to the "mother cell" fraction (which actually consisted of mother cells plus lysozyme-sensitive forespores [21]). The pellet, which consisted of lysozyme-resistant forespores containing spore coats, was subjected to spore coat removal, washing in buffer, and a second round of lysozyme treatment (21). β -Galactosidase activity after this treatment was assigned to the "forespore" fraction. Total β -galactosidase activity expressed by the cells is the sum of the activities in the mother cell and forespore fractions.

Because of the small amount of β -galactosidase expressed by the *spl-lacZ* fusion and the endogenous ONPGase activity expressed during *B. subtilis* sporulation, in each experiment the strain harboring the *spl-lacZ* fusion was grown and sporulated in parallel with a culture of the corresponding parental strain lacking the *spl-lacZ* fusion; the endogenous ONPGase activity of the parental strain was then subtracted from the β -galactosidase activity of the fusion-containing strain at each time point.

Induction experiments. Expression of the *spl-lacZ* fusion following induction of *sigG* expression in *B. subtilis* WN127 was performed as follows. Cells were grown to an optical density at 600 nm (OD_{600}) of 0.3. The culture was divided into two subcultures of equal volume, and isopropyl- β -D-thiogalactopyranoside (IPTG) was added to one subculture to a final concentration of 1 mM; the second subculture served as a control. Samples of cells harvested before and after IPTG addition were assayed for β -galactosidase and GDH activities as described above. To analyze the DNA damage inducibility of *spl-lacZ* expression, *B. subtilis* WN119 was grown in LB medium to an OD_{600} of 0.5, the culture was divided into two equal subcultures, and mitomycin (0.5- $\mu\text{g}/\text{ml}$ final concentration) was added to one of the subcultures. Cells were harvested and assayed for β -galactosidase activity before and after the addition of mitomycin as described above. As a positive control, cells of *B. subtilis* YB5176, containing the *dinA76-lacZ* fusion (kindly provided by R. Yasbin, University of Maryland) were grown and treated identically with mitomycin. In addition, *spl-lacZ* expression was assayed as described above for samples harvested throughout the growth of strain WN119 under conditions which induce genetic competence (2).

Mapping of the 5' ends of *spl* mRNA. The 5' ends of *spl* mRNA were mapped by primer extension (23) of *spl* transcripts produced either during sporulation or in response to induction of *sigG* expression, as follows. Total RNA was isolated from sporulating cells of *B. subtilis* WN119 or from either IPTG-induced or uninduced cells of strain WN127 as previously reported (20) by using the expression of β -galactosidase activity directed by the *spl-lacZ* fusion to determine the time at which to harvest the cells to obtain the maximum amount of *spl* transcripts (20, 34). The synthetic 17-mer oligonucleotide 5'-dGCTGCGGAACAAATGGG-3', complementary to *spl* mRNA between 9 and 26 nt downstream from the putative *spl* translation initiation codon (7), was end labeled by using [γ - 32 P]ATP and phage T4 polynucleotide kinase and was hybridized to 10 μ g of total RNA from each sample. The primer was extended with Moloney murine leukemia virus reverse transcriptase, and the extended products were separated by electrophoresis through a 6% polyacrylamide DNA sequencing gel. The positions of the radioactive products relative to a DNA sequencing ladder (40) generated from plasmid pWN41 (Table 2) (7) were determined by using the oligonucleotide described above as the sequencing primer with the Sequenase version 2.0 kit (U.S. Biochemical Corp., Cleveland, Ohio).

RNA polymerase purification and in vitro transcription. RNA polymerase containing the sigma-G factor was isolated as follows. *B. subtilis* WN127 was cultivated in 250 ml of LB medium containing chloramphenicol and kanamycin to an OD₆₀₀ of 0.25. IPTG was added to the culture to a final concentration of 5 mM, and the culture was incubated for 2 h. During this time period, samples were withdrawn from the culture and assayed for β -galactosidase activity encoded by the *spl-lacZ* fusion as described above, to ensure induction of *sigG* expression. Cells were harvested by centrifugation and washed, and RNA polymerase was isolated as described previously (34, 50) through the heparin-agarose column step. The resulting protein preparation was assayed for sigma-G RNA polymerase activity by runoff transcription utilizing the *sspA* promoter carried on plasmid pWN204 cleaved with *EcoRI*. To assay in vitro transcription from the putative *spl* promoters, the RNA polymerase preparation was used in runoff transcription experiments with *EcoRI*-linearized plasmids pWN78 and pWN81. In vitro transcription reactions were performed as previously described (34), with the exception that the labeled nucleotide used was [α - 35 S]UTP (Dupont, New England Nuclear). Transcription products were separated by electrophoresis through a 6% polyacrylamide sequencing gel, and the dried gel was subjected to autoradiography. Sizes of observed transcripts were estimated by comparison of their mobilities with a DNA sequencing ladder generated by using the phage M13 DNA template supplied with the Sequenase version 2.0 kit and by correction for the slightly decreased mobility of RNA relative to that of DNA (approximately 3% difference under the electrophoresis conditions used [38a]).

RESULTS

Temporal and compartmental expression of an *spl-lacZ* fusion. *B. subtilis* WN119, containing a single copy of the *spl-lacZ* fusion integrated at the *spl* locus, was used to study the expression of *spl* throughout growth and sporulation. The kinetics of appearance of β -galactosidase activity directly extracted from the cells by lysozyme treatment revealed that *spl* was not expressed during vegetative growth; β -galactosidase activity first appeared 4 h after the onset of the stationary

FIG. 2. Forespore-specific expression of *spl*-directed β -galactosidase activity during stage III of *B. subtilis* sporulation. *B. subtilis* WN119 was grown and induced to sporulate in liquid DSM as described in Materials and Methods (circles). Samples collected at the indicated times were treated with lysozyme, and the extracts were assayed directly for β -galactosidase activity (open squares) or GDH (triangles). β -Galactosidase activity present in the lysozyme-resistant forespore fraction (solid squares) was assayed as described in Materials and Methods.

phase (i.e., at T_4) and peaked at T_5 , and then the enzyme levels subsequently decreased (Fig. 2).

Sporulating *B. subtilis* cells undergo an asymmetric cellular division which results in the establishment of two cell compartments, a forespore enveloped within a mother cell (36). The observed increase and subsequent decline of *spl-lacZ*-encoded β -galactosidase activity directly extractable by lysozyme (Fig. 2) were reminiscent of genes which are expressed in the developing forespore compartment at an intermediate stage of development (44), as it has been observed previously that during the sporulation process the forespore becomes resistant to direct disruption by lysozyme as a result of deposition of spore coat proteins on its surface (21). To test this notion, cellular fractionation experiments were performed to assay compartment-specific expression of β -galactosidase directed by the *spl-lacZ* fusion (Fig. 2). During sporulation of strain WN119, β -galactosidase activity extracted from the lysozyme-resistant forespore compartment was detected at T_5 , and forespore β -galactosidase activity continued to accumulate until at least T_{10} (Fig. 2). The increase in forespore enzyme activity was paralleled by a decrease in β -galactosidase activity extracted from the lysozyme-sensitive mother cell fraction, characteristic of forespore-specific gene expression (Fig. 2) (9, 13, 21).

To further investigate the timing of forespore-specific expression of the *spl-lacZ* fusion, the same cell extracts used in the β -galactosidase assays (Fig. 2) were also assayed for GDH activity encoded by the stage III, forespore-specific *gdh* gene (13, 21, 30). GDH activity and β -galactosidase from the *spl-lacZ* fusion were expressed with essentially identical kinetics (Fig. 2), strongly indicating that *spl* gene expression is activated at stage III of sporulation specifically in the developing forespore compartment.

Sigma-G dependence of *spl-lacZ* fusion expression. It has been reported that the *gdh* operon (30) and several other sporulation-associated genes including the *ssp* family (21), the *spoVA* operon (6, 21), and the *gerA* operon (9), among others, form a forespore-specific regulon of genes whose coordinate expression is controlled both temporally and compartmentally by RNA polymerase containing the forespore-specific sigma-G

FIG. 3. Expression of *spl*-directed β -galactosidase activity (triangles) during growth and sporulation (circles) of wild-type strain WN119 (closed triangles) and *sigG* Δ 1 mutant strain WN126 (open triangles) in DSM. Samples were harvested and assayed as described in Materials and Methods. Cell extracts were also assayed for GDH activity, with essentially identical results (data not shown).

FIG. 4. Growth (circles) and β -galactosidase (triangles) or GDH (squares) activity in *B. subtilis* WN127 with (closed symbols) or without (open symbols) the addition of IPTG. Cells were grown in LB medium. At zero hours, the culture was divided equally and IPTG was added to one subculture. Cell samples collected at the indicated times were treated with lysozyme and assayed for β -galactosidase and GDH activities as described in Materials and Methods.

factor ($E\sigma^G$) (34, 44). The pattern of expression of the *spl-lacZ* fusion (Fig. 2) strongly suggested that *spl* may be an additional member of the $E\sigma^G$ regulon. Two approaches were used to address this question directly.

First, the *spl-lacZ* fusion was introduced by transformation into strain WN118, a *B. subtilis* strain harboring a deletion of the *sigG* gene, which is unable to transcribe $E\sigma^G$ -dependent genes and in which sporulation is arrested at stage III (14, 50). The resulting strain, WN126, was grown in liquid DSM containing chloramphenicol, and samples were removed from the culture throughout growth and the stationary phase for assay of β -galactosidase and GDH activity. Expression both of β -galactosidase directed by *spl-lacZ* (Fig. 3) and of GDH (data not shown) was almost completely abolished in strain WN126, consistent with *spl* expression being either directly or indirectly under the control of $E\sigma^G$.

Second, to directly test $E\sigma^G$ dependence of *spl* expression, strain WN126 was transformed with plasmid pDG298 carrying the wild-type *sigG* gene under the control of the IPTG-inducible P_{spac} promoter (50), resulting in strain WN127 (Table 1). IPTG induction of *sigG* expression in vegetatively growing cells resulted in activation of the expression of both *spl*-directed β -galactosidase and GDH activities (Fig. 4). Measurements of the maximum induction of β -galactosidase expression indicated that strain WN127 expressed levels of β -galactosidase three to four times higher than those expressed by the wild-type strain, WN119 (compare Fig. 3 and 4). Taken together, these results strongly suggest that *spl* expression is directly dependent on $E\sigma^G$ RNA polymerase.

Mapping the 5' ends of in vivo *spl* mRNA. Examination of the nucleotide sequence surrounding the *spl* gene revealed the presence of a small open reading frame (ORF) situated upstream from *spl*, which could potentially encode a 9-kDa polypeptide (7). Both the ORF and *spl* are preceded by sequences homologous to ribosome binding sites, but the 80 bp of DNA intervening between the two cistrons lacks inverted repeat sequences characteristic of rho-independent transcriptional terminators, suggesting that the two genes may be cotranscribed (7). In contrast, examination of the nucleotide sequences preceding and following the ORF-*spl* region revealed that *ptsI*, the gene preceding the ORF, and *spl* itself are both followed by sequences that could provide rho-indepen-

dent transcriptional termination signals (7). To gain further insights into the transcriptional organization of the *spl* region, primer extension analysis was used to map the 5' ends of mRNAs originating upstream from the *spl* coding sequence. Experiments were carried out by using a 17-nt-long *spl*-specific primer (see Materials and Methods) and total RNA isolated from sporulating cells of *B. subtilis* WN119, harvested at either T_0 , the onset of the stationary phase, or at T_5 , the time of maximum expression of β -galactosidase activity from the *spl-lacZ* gene fusion. Primer extension analysis indicated the existence of a major primer extension product corresponding to coordinates 597 to 598 of the published sequence (7) (Fig. 5), located 15 to 16 bp upstream from the initiation methionine codon of the small ORF preceding *spl*, immediately preceding the ORF ribosome binding site (Fig. 5). In addition, two weak primer extension products were detected, at coordinates 809 (within the ORF) and 906 (9 bp preceding the *spl* ribosome binding site). All three primer extension products were present in higher amounts in the RNA sample obtained from strain WN119 cells harvested at T_5 , the time of maximal *spl-lacZ* expression; a small amount of the major transcript at positions 597 to 598 was also detected in RNA from cells harvested at T_0 (Fig. 5). To determine if these primer extension products resulted from transcription by $E\sigma^G$ RNA polymerase occurring within the ORF-*spl* region, primer extension mapping was also performed on total RNA isolated from cells of strain WN127 which had been cultivated in LB medium either with or without IPTG induction of *sigG* expression from plasmid pDG298 (Fig. 5). Induction of *sigG* expression by IPTG during vegetative growth of strain WN127 also resulted in production of the same three primer extension products seen during sporulation, in approximately the same relative amounts (Fig. 5). The major primer extension product at positions 597 to 598 was also detected at a reduced level in uninduced cells of strain WN127 (Fig. 5).

The three primer extension products observed could actually represent three distinct transcripts or could alternatively be (i) in vivo-processed products of a longer single transcript or (ii) incomplete primer extension products of a longer single transcript. Examination of the nucleotide sequences upstream from each of the three extension products revealed the presence of sequences with similarity to promoters preceding genes

FIG. 5. Primer extension mapping of in vivo *spl* transcripts. Arrows indicate the positions of the primer extension products relative to the nucleotide sequence (lanes G, A, T, and C) generated from plasmid pWN41 (Table 2) (7) by using the same primer. Asterisks indicate the positions of the primer extension products relative to the ORF-*spl* sequence coordinates of Fajardo-Cavazos et al. (7). Primer extension reactions were performed on total RNA isolated from strain WN119 at T_0 (lane 1), strain WN119 at T_5 (lane 2), strain WN127 with IPTG (lane 3), and strain WN127 without IPTG (lane 4).

which are members of the sigma-G regulon (34); these sequences were designated putative promoters P1, P2, and P3 (Fig. 6). Of the three putative promoters preceding *spl*, P1 most closely matches the consensus sigma-G promoter sequence (34), exhibiting a perfect match in the -10 region (Fig. 6). The -35 region of P1 also matched the sigma-G consensus

	-35	-17 or 18 bp	-10	
<i>sigG</i> -type	TGAATA	---	CATACTA	
	:		: :	**
P1	562-TTCATAAGTAAGGGTATAGAAGGACACAATAACATGG-598		: :	*
	: :		: :	
P2	768-CGCAGAGGACGATGCTGTGTTG-CAAGCTATGAAGAAGCTAA-809		: :	*
	:		: :	
P3	870-CTCATATCCTTTCCGCTAGTGA-AAAAGTAAACGTTA-906		: :	*

FIG. 6. Comparison of the consensus $E\sigma^G$ promoter sequence (34) (top line) with the putative promoter sequences (P1, P2, and P3) preceding *spl* (bottom 3 lines), deduced from the primer extension data of Fig. 5. Underlined bases are highly or absolutely conserved in $E\sigma^G$ -type promoters (34). Perfect matches are indicated by vertical lines. Matches in less highly conserved regions of the $E\sigma^G$ consensus promoter sequence are indicated by colons. Asterisks over the sequences denote the positions of the corresponding primer extension products (Fig. 5).

promoter very well, containing only a single mismatch at position -35 , where a highly conserved G residue is replaced in P1 by a T (Fig. 6). The -10 and -35 regions of P1 are separated by a distance of 18 bp, also in agreement with the sigma-G consensus sequence (Fig. 6). Sequence analysis showed that putative promoters P2 and P3 exhibited a lower overall degree of homology to the consensus sigma-G-type promoter sequence and that the -10 region of P2 is in fact located at approximately -15 with respect to the primer extension product at coordinate 809 (Fig. 6).

In vitro transcription of *spl*. To address whether putative promoter sequences P1, P2, and P3 are functional $E\sigma^G$ -type promoters, plasmids which contained DNA spanning the ORF-*spl* region were prepared for use as templates for in vitro runoff transcription reactions with RNA polymerase containing sigma-G. Sporulating cells of *B. subtilis* express two fore-spore-specific forms of RNA polymerase containing either the sigma-F factor or the sigma-G factor, which have very similar promoter specificities in vitro (34, 50). To ensure the absence of $E\sigma^F$ in the preparation, the source of $E\sigma^G$ RNA polymerase was vegetative cells of strain WN127 grown in LB medium with IPTG induction of *sigG* expression. Induction of *sigG* expression by IPTG was monitored by measuring activation of β -galactosidase synthesis encoded by the *spl-lacZ* fusion, in a manner similar to that described in the legend to Fig. 4 (data not shown). The RNA polymerase preparation contained $E\sigma^G$, as it was capable of correctly transcribing plasmid pWN204 linearized with *EcoRI* (Table 2), which contains the known sigma-G-dependent *sspA* promoter (34), generating the expected 112-nt runoff transcript (data not shown). To test for $E\sigma^G$ -dependent runoff transcription from the ORF-*spl* region, two templates were used, plasmids pWN78 and pWN81, each linearized with *EcoRI* (Table 2). Transcription of plasmid pWN78 from P1, P2, or P3 would be expected to generate a runoff transcript of 365, 153, or 56 nt, respectively, while transcription of plasmid pWN81 would be expected to generate a runoff transcript of 204 nt only from P1, as neither P2 nor P3 is contained on this plasmid. In vitro transcription of plasmid pWN78 by $E\sigma^G$ yielded a single runoff transcript of 365 nt, consistent with transcription from P1 (Fig. 7); the shorter transcripts predicted to be generated from P2 and P3 were not detected. When plasmid pWN81 was used as the template, a 204-nt runoff transcript was seen, also consistent with transcription from P1. It therefore appears from this experiment that P1 can function in vitro as an $E\sigma^G$ -dependent promoter and that either P2 and P3 are not functional promoters in vitro or transcription from them is too weak to be detected under the experimental conditions used.

Expression of the *spl-lacZ* fusion following DNA damage or during competence. It has been reported that *B. subtilis* cells exhibit an SOS-like response similar in many aspects to that observed in *E. coli* cells (18). Several DNA damage-inducible genes, collectively called *din* genes, include those whose products are involved in DNA repair, such as *recA* and genes of the *uvr* system (17, 19, 38). In *B. subtilis*, the expression of certain *din* genes such as *recA* can be activated not only by exposure of cells to DNA-damaging agents but also when cells are grown under conditions which induce genetic competence (17, 19). Because the *spl* gene is also involved in DNA repair, the possibility that expression of the *spl-lacZ* gene fusion could be induced either by treatment of *B. subtilis* cells with a DNA-damaging agent or by competence induction was explored. Mid-logarithmic-phase cells of *B. subtilis* WN119 were exposed to the DNA-damaging agent mitomycin at a final concentration of 0.5 $\mu\text{g/ml}$, which was previously used to demonstrate induction of *lacZ* reporter genes fused to the *dinA*, *dinB*, or

FIG. 7. In vitro runoff transcription of *spl*. RNA polymerase containing sigma-G was prepared as described in Materials and Methods and used to transcribe the following templates: plasmid pWN78 linearized with *Eco*RI (lane 1) and plasmid pWN81 linearized with *Eco*RI (lane 2). Lanes marked G, A, T, and C show the results of DNA sequencing reactions of M13 DNA used as molecular size standards. Arrows denote the sizes of the runoff transcripts, corrected for the fact that under the electrophoresis conditions used in the experiment, RNA migrates approximately 3% more slowly than DNA (38a).

dinC promoter region (3). Treatment of strain WN119 with mitomycin did not lead to increased expression of β -galactosidase activity encoded by the *spl-lacZ* fusion (data not shown). As a positive control, treatment of strain YB5176, which contains the *dinA76-lacZ* fusion (3), with mitomycin under identical conditions did induce β -galactosidase expression 20-fold greater than that of untreated cells (data not shown). Likewise, WN119 cells grown to genetic competence (2) failed to induce *spl*-directed β -galactosidase expression (data not shown).

DISCUSSION

Bacterial spores must rely on the expression of specific mechanisms to correct damage to their DNA induced as a result of exposure to UV during dormancy. To date, it is well established that the SP which accumulates in the DNA of dormant spores is corrected very specifically and efficiently during early germination through the combined activities of the *uvr* and *spl* pathways (26, 28, 29). Because repair of SP

takes place at the onset of spore germination, it is reasonable to speculate that the product of the *spl* gene could be synthesized during the previous round of sporulation and packaged in the mature spore (7). This speculation is supported by early experiments demonstrating that *spl*-mediated repair of SP can take place when UV-irradiated *B. subtilis* spores are germinated in the presence of antibiotics such as rifampin and chloramphenicol, which either indirectly or directly block de novo protein synthesis (29).

The recent cloning and sequencing of *spl* (7) have allowed direct examination of the molecular mechanisms that regulate its expression. This paper describes the utilization of an in-frame transcriptional and translational *spl-lacZ* fusion to study the temporal and compartmental expression of this novel DNA repair system. Analysis of the expression of the *spl-lacZ* fusion integrated in the *spl* locus during the developmental cycle of *B. subtilis* suggested that the Spl enzyme is produced at an intermediate time during sporulation and specifically in the forespore compartment (Fig. 2). Interestingly, the kinetics of β -galactosidase expression exhibited by the *spl-lacZ* fusion were observed to be similar to those reported for a number of stage III, forespore-specific genes (44). By using GDH activity as a forespore-specific, stage III marker (13, 21, 44), expression of the *spl-lacZ* fusion was observed to be identical to GDH activity with respect to both its kinetics and its compartmentalization (Fig. 2). This important piece of evidence supports the notion that the Spl protein is synthesized during sporulation and is packaged within the dormant spore.

The expression of forespore genes during sporulation in *B. subtilis* is controlled by the sequential action of two types of sporulation-specific RNA polymerases, harboring either the sigma-F factor or the sigma-G factor (reviewed in reference 15). The results in Fig. 2 suggested that $E\sigma^G$ was likely to be the RNA polymerase responsible for the transcription of the *spl-lacZ* fusion; however, transcription of the *spl-lacZ* fusion by $E\sigma^F$ could not at first be ruled out. Such a possibility was tested by measuring the expression of the *spl-lacZ* fusion in a strain harboring a deletion of the *sigG* gene, which lacks $E\sigma^G$ but contains $E\sigma^F$ (34, 50); expression of the *spl-lacZ* fusion and expression of GDH activity were found to be almost totally abolished in the *sigG* mutant, consistent with their strict dependence on sigma-G RNA polymerase (Fig. 3 and data not shown). Furthermore, expression of both the *spl-lacZ* fusion and the *gdh* operon was completely restored to the *sigG* strain WN126 by the introduction into this strain of the wild-type *sigG* gene on an extrachromosomal element (Fig. 4). On the basis of these results it is concluded that *spl* is transcribed during sporulation in the forespore compartment by the direct action of $E\sigma^G$.

RNA polymerase containing the sigma-G factor activates expression of a large regulon of forespore-specific genes including *sspA-E*, *gdh*, *gerA*, *spoIVB*, and *spoVA*, among others (for reviews see references 15 and 44). Transcript mapping and in vitro mutagenesis experiments performed on many of these genes have resulted in the identification of conserved nucleotide sequences characteristic of $E\sigma^G$ -dependent promoters (8, 9, 30, 34, 37). Results obtained as described above with the *spl-lacZ* gene fusion strongly suggest that *spl* is a new member of the $E\sigma^G$ regulon, and results from primer extension mapping of the 5' ends of *spl* mRNA (Fig. 5) support this conclusion. Three primer extension products were identified in the region preceding *spl*, a major product preceding the small ORF upstream from *spl* and two minor products apparently originating from within the ORF and immediately preceding the *spl* ribosome binding site (Fig. 5). Nucleotide sequence analysis identified three putative promoter sequences (here

called P1, P2, and P3) preceding these primer extension products, all of which exhibit homology to the sigma-G consensus promoter sequence (34) (Fig. 6). The P1 promoter was found to be the most similar to the sigma-G consensus promoter sequence, differing from it by only a single base, the highly conserved G at position -35 being replaced in the P1 promoter by a T (Fig. 6). The great similarity between P1 and the $E\sigma^G$ consensus promoter perhaps explains why most of the primer extension products are associated with P1 in vivo (Fig. 5). At present it is unclear whether P2 and P3 actually function in vivo as promoters or whether the primer extension products at these positions represent in vivo-processed mRNA species or incomplete in vitro extension products. The latter two possibilities appear to be supported by results from the in vitro transcription experiment, in which an $E\sigma^G$ -dependent runoff transcript was detected from P1 only (Fig. 7).

On the basis of the observed absence of a putative transcriptional termination sequence between the ORF and *spl*, it was previously proposed that these two genes may form a bicistronic operon (7); this suggestion is supported by the mapping of the major 5' end of the *spl* mRNA upstream from the ORF (Fig. 5) and by the demonstration that the P1 promoter which precedes this transcript is apparently utilized by $E\sigma^G$ in vitro (Fig. 7). Two interesting questions arise from these observations. (i) Is there a structural relationship between the two proteins potentially encoded by this operon? (ii) What is the reason for their spatial arrangement and their coregulation? The deduced Spl protein sequence has been observed to share local amino acid homology with a number of microbial and fungal DNA photolyases (7), and it is interesting to note that in *E. coli*, the *phr* gene which encodes DNA photolyase is also preceded by a small open reading frame (called ORF 169) (39) which is cotranscribed with *phr* but whose product is apparently not needed for photolyase function (16). Indeed, at present a function has not been assigned to either the *E. coli* ORF 169 product or that of the ORF preceding the *B. subtilis spl* gene. Although the possible structural, functional, or regulatory role of the ORF preceding *spl* is at present unknown, experiments that will address these issues are currently in progress.

Finally, the possible induction of *spl* expression by factors which are able to trigger the *B. subtilis* SOS-like response (18) was investigated. No induction of *spl-lacZ* fusion expression was observed under two conditions reported to induce DNA damage-inducible (*din*) genes, i.e., treatment of cells with mitomycin (3, 18) and growth of cells to genetic competence (19). A probable explanation for these results is the absence in the ORF-*spl* region of the *cis*-acting *din*-regulatory operator sequence GAAC-N₄-GTTC (3). Rather, the results presented in this paper support a model in which the majority of *spl* expression is activated directly during sporulation by $E\sigma^G$.

Most living organisms must handle DNA damage in whichever form it is presented to them. What causes sporulating bacteria to be so unique is the surprising fact that in response to selection for high UV resistance they have evolved both alternative DNA photochemistry and a repair system dedicated to the correction of the resulting spore-specific DNA lesion. In *B. subtilis*, spore UV resistance is governed by two types of gene products which play major roles in (i) determining spore DNA photochemistry during dormancy (i.e., SASPs) and (ii) repair of spore DNA damage during germination (i.e., Spl). The *ssp* genes encoding SASP are transcriptionally activated in the forespore by $E\sigma^G$ RNA polymerase, and SASPs bind to spore DNA and cause it to undergo structural changes which alter its UV photochemistry to favor SP production in the dormant spore (reviewed in references 42 through 47). As

this report demonstrates, the *spl* gene is also transcriptionally activated during sporulation in the forespore by $E\sigma^G$ RNA polymerase and the Spl protein is packaged in the mature spore to correct SP during subsequent spore germination. Because the successful repair of SP by the Spl protein during germination of UV-irradiated spores is tightly linked to the prior activity of SASPs on spore DNA, it seems reasonable to expect that the expression of the genes for all of these proteins is controlled by a common regulatory circuitry.

ACKNOWLEDGMENTS

We thank Peter Setlow and Ron Yasbin for generous donation of strains and plasmids and Tony Romeo and Patricia Fajardo-Cavazos for critical reading of the manuscript.

This work was supported by grants from the National Institutes of Health (GM47461), the American Cancer Society (JFRA-410), and the Texas Advanced Research Program (009768-034) to W.L.N. and by a doctoral fellowship to M.P.-R. from the Consejo Nacional de Ciencia y Tecnología (Mexico).

REFERENCES

1. Birnboim, H. C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* 7:1513-1523.
2. Boylan, R. J., N. H. Mendelson, D. Brooks, and F. E. Young. 1972. Regulation of the bacterial cell wall: analysis of a mutant of *Bacillus subtilis* defective in biosynthesis of teichoic acid. *J. Bacteriol.* 110:281-290.
3. Cheo, D. L., K. W. Bayles, and R. E. Yasbin. 1991. Cloning and characterization of DNA damage-inducible promoter regions from *Bacillus subtilis*. *J. Bacteriol.* 173:1696-1703.
- 3a. Connors, M. J., J. M. Mason, and P. Setlow. 1986. Cloning and nucleotide sequence of genes for three small, acid-soluble proteins from *Bacillus subtilis* spores. *J. Bacteriol.* 166:417-425.
4. Cutting, S. M., and P. B. Vander Horn. 1990. Genetic analysis, p. 27-74. In C. R. Harwood and S. M. Cutting (ed.), *Molecular biological methods for Bacillus*. John Wiley and Sons, Sussex, England.
5. Donellan, J. E., Jr., and R. B. Setlow. 1965. Thymine photoproducts but not thymine dimers are found in ultraviolet irradiated bacterial spores. *Science* 149:308-310.
6. Errington, J., and J. Mandelstam. 1986. Use of a *lacZ* gene fusion to determine the dependence pattern and the spore compartment expression of the sporulation operon *spoVA* in *spo* mutants of *Bacillus subtilis*. *J. Gen. Microbiol.* 132:2977-2985.
7. Fajardo-Cavazos, P., C. Salazar, and W. L. Nicholson. 1993. Molecular cloning and characterization of the *Bacillus subtilis* spore photoproduct lyase (*spl*) gene, which is involved in repair of UV radiation-induced DNA damage during spore germination. *J. Bacteriol.* 175:1735-1744.
8. Fajardo-Cavazos, P., F. Tovar-Rojo, and P. Setlow. 1991. Effect of promoter mutations and upstream deletions on the expression of genes coding for small, acid-soluble spore proteins of *Bacillus subtilis*. *J. Bacteriol.* 173:2011-2016.
9. Feavers, I. M., J. Foulkes, B. Setlow, D. Sun, W. Nicholson, P. Setlow, and A. Moir. 1990. The regulation of transcription of the *gerA* spore germination operon of *Bacillus subtilis*. *Mol. Microbiol.* 4:275-282.
10. Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6-13.
11. Ferrari, E., S. M. H. Howard, and J. A. Hoch. 1985. Effect of sporulation mutations on subtilisin expression, assayed using a subtilisin- β -galactosidase gene fusion, p. 180-184. In J. A. Hoch and P. Setlow (ed.), *Molecular biology of microbial differentiation*. American Society for Microbiology, Washington, D.C.
12. Francesconi, S. C., T. J. MacAlister, B. Setlow, and P. Setlow. 1988. Immunoelectron microscopic localization of small, acid-soluble spore proteins in sporulating cells of *Bacillus subtilis*. *J. Bacteriol.* 170:5963-5967.
13. Fujita, Y., R. Ramaley, and E. Freese. 1977. Location and prop-

- erties of glucose dehydrogenase in sporulating cells and spores of *Bacillus subtilis*. J. Bacteriol. 132:282–293.
14. Karmazyn-Campelli, C., C. Bonamy, B. Savelli, and P. Stragier. 1989. Tandem genes encoding sigma factors for consecutive steps of development in *Bacillus subtilis*. Genes Dev. 3:150–157.
 15. Kroos, L., and S. Cutting. 1994. Intercellular and intercompartmental communication during *Bacillus subtilis* sporulation, p. 155–180. In P. J. Piggot, C. P. Moran, Jr., and P. Youngman (ed.), Regulation of bacterial differentiation. American Society for Microbiology, Washington, D.C.
 16. Lorence, M. C., S. D. Maika, and C. S. Rupert. 1990. Physical analysis of *phr* transcription in *Escherichia coli* K-12. J. Bacteriol. 172:6551–6556.
 17. Love, P. E., M. S. Lyle, and R. E. Yasbin. 1985. DNA damage inducible (*din*) loci are transcriptionally activated in competent *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA 82:6201–6205.
 18. Love, P. E., and R. E. Yasbin. 1984. Genetic characterization of the inducible SOS-like system of *Bacillus subtilis*. J. Bacteriol. 160: 910–920.
 19. Lovett, C. M., Jr., P. E. Love, and R. E. Yasbin. 1989. Competence-specific induction of the *Bacillus subtilis* RecA protein analog: evidence for dual regulation of a recombination protein. J. Bacteriol. 171:2318–2322.
 20. Mason, J. M., P. Fajardo-Cavazos, and P. Setlow. 1988. Levels of mRNAs which code for small, acid-soluble spore proteins and their *lacZ* gene fusions in sporulating cells of *Bacillus subtilis*. Nucleic Acids Res. 16:6567–6583.
 21. Mason, J. M., R. H. Hackett, and P. Setlow. 1988. Regulation of expression of genes coding for small, acid-soluble proteins of *Bacillus subtilis* spores: studies using *lacZ* gene fusions. J. Bacteriol. 170:239–244.
 22. Mason, J. M., and P. Setlow. 1986. Essential role of small, acid-soluble spore proteins in resistance of *Bacillus subtilis* spores to UV light. J. Bacteriol. 167:174–178.
 23. McKnight, S. L., and R. Kingsbury. 1982. Transcription control signals of a eukaryotic protein-coding gene. Science 217:316–324.
 24. Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
 25. Mohr, S. C., N. V. H. A. Sokolov, C. He, and P. Setlow. 1991. Binding of small acid-soluble proteins from *Bacillus subtilis* changes the conformation of DNA from B to A. Proc. Natl. Acad. Sci. USA 88:77–81.
 26. Munakata, N. 1969. Genetic analysis of a mutant of *Bacillus subtilis* producing ultraviolet-sensitive spores. Mol. Gen. Genet. 104:258–263.
 27. Munakata, N., and Y. Ikeda. 1968. A mutant of *Bacillus subtilis* producing ultraviolet-sensitive spores. Biochem. Biophys. Res. Commun. 33:469–475.
 28. Munakata, N., and C. S. Rupert. 1972. Genetically controlled removal of “spore photoproduct” from deoxyribonucleic acid of ultraviolet-irradiated *Bacillus subtilis* spores. J. Bacteriol. 111:192–198.
 29. Munakata, N., and C. S. Rupert. 1974. Dark repair of DNA containing “spore photoproduct” in *Bacillus subtilis*. Mol. Gen. Genet. 130:239–250.
 30. Nakatani, Y., W. L. Nicholson, K.-D. Nietzke, P. Setlow, and E. Freese. 1989. Sigma-G RNA polymerase controls forespore specific expression of the glucose dehydrogenase operon in *Bacillus subtilis*. Nucleic Acids Res. 17:999–1017.
 31. Nicholson, W. L., B. Setlow, and P. Setlow. 1990. Binding of DNA in vitro by a small, acid-soluble spore protein from *Bacillus subtilis* and the effect of this binding on DNA topology. J. Bacteriol. 172:6900–6906.
 32. Nicholson, W. L., B. Setlow, and P. Setlow. 1991. Ultraviolet irradiation of DNA complexed with α/β type small, acid-soluble spore proteins from spores of *Bacillus* or *Clostridium* species makes spore photoproduct but not thymine dimers. Proc. Natl. Acad. Sci. USA 88:8288–8292.
 33. Nicholson, W. L., and P. Setlow. 1990. Sporulation, germination, and outgrowth, p. 391–450. In C. R. Harwood and S. M. Cutting (ed.), Molecular biological methods for *Bacillus*. John Wiley and Sons, Sussex, England.
 34. Nicholson, W. L., D. Sun, B. Setlow, and P. Setlow. 1989. Promoter specificity of σ^G -containing RNA polymerase from sporulating cells of *Bacillus subtilis*: identification of a group of forespore-specific promoters. J. Bacteriol. 171:2708–2718.
 35. Pearson, W. R., and D. J. Lipman. 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA 85:2444–2448.
 36. Piggot, P. J., and J. G. Coote. 1976. Genetic aspects of bacterial endospore formation. Bacteriol. Rev. 40:908–962.
 37. Rather, P. N., and C. P. Moran, Jr. 1988. Compartment-specific transcription in *Bacillus subtilis*: identification of the promoter for *gdh*. J. Bacteriol. 170:5086–5092.
 38. Raymond-Denise, A., and N. Guillen. 1992. Expression of the *Bacillus subtilis* *dinR* and *recA* genes after DNA damage and during competence. J. Bacteriol. 174:3171–3176.
 - 38a. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 39. Sancar, G. B., F. W. Smith, M. C. Lorence, C. S. Rupert, and A. Sancar. 1984. Sequences of the *Escherichia coli* photolyase gene and protein. J. Biol. Chem. 259:6033–6038.
 40. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463–5467.
 41. Schaeffer, P., J. Millet, and J.-P. Aubert. 1965. Catabolic repression of bacterial sporulation. Proc. Natl. Acad. Sci. USA 54:704–711.
 42. Setlow, P. 1988. Resistance of bacterial spores to ultraviolet light. Comments Mol. Cell. Biophys. 5:253–264.
 43. Setlow, P. 1988. Small, acid-soluble spore proteins of *Bacillus* species: structure, synthesis, genetics, function, and degradation. Annu. Rev. Microbiol. 42:319–338.
 44. Setlow, P. 1989. Forespore-specific genes of *Bacillus subtilis*: function and regulation of expression, p. 211–221. In I. Smith, R. Slepecky, and P. Setlow (ed.), Regulation of procaryotic development: structural and functional analysis of bacterial sporulation and germination. American Society for Microbiology, Washington, D.C.
 45. Setlow, P. 1992. DNA in dormant spores is in an A-like conformation. Mol. Microbiol. 6:563–567.
 46. Setlow, P. 1992. I will survive: protecting and repairing spore DNA. J. Bacteriol. 174:2737–2741.
 47. Setlow, P. 1994. DNA structure, spore formation, and spore properties, p. 181–194. In P. J. Piggot, C. P. Moran, Jr., and P. Youngman (ed.), Regulation of bacterial differentiation. American Society for Microbiology, Washington, D.C.
 48. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503–517.
 49. Stuy, J. H. 1956. Studies on the mechanism of radiation inactivation of microorganisms. III. Inactivation of germinating spores of *Bacillus cereus*. Biochim. Biophys. Acta 22:241–246.
 50. Sun, D., P. Stragier, and P. Setlow. 1989. Identification of a new sigma factor which allows RNA polymerase to transcribe the *sspE* gene and other forespore specific genes during sporulation of *Bacillus subtilis*. Genes Dev. 3:141–149.
 51. Varghese, A. J. 1970. 5-Thyminyl-5,6-dihydrothymine from DNA irradiated with ultraviolet light. Biochem. Biophys. Res. Commun. 38:484–490.
 52. Wang, T. C., and C. S. Rupert. 1977. Evidence for the monomerization of spore photoproduct to two thymines by the light-independent “spore repair” process in *Bacillus subtilis*. Photobiol. 25:123–127.
 53. Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 33:103–119.