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Role of the *Bacillus subtilis gsiA* Gene in Regulation of Early Sporulation Gene Expression

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The *Bacillus subtilis gsiA* operon was induced rapidly, but transiently, as cells entered the stationary phase in nutrient broth medium. A mutation at the *gsiC* locus caused sporulation to be defective and expression of *gsiA* to be elevated and prolonged. The sporulation defect in this strain was apparently due to persistent expression of *gsiA*, since a *gsiA* null mutation restored sporulation to wild-type levels. Detailed mapping experiments revealed that the *gsiC82* mutation lies within the *kinA* gene, which encodes the histidine protein kinase member of a two-component regulatory system. Since mutations in this gene caused a substantial blockage in expression of *spoIIA*, *spoIIG*, and *spoIID* genes, it seems that accumulation of a product of the *gsiA* operon interferes with sporulation by blocking the completion of stage II. It apparently does so by inhibiting or counteracting the activity of KinA.

During the transition from vegetative growth to the stationary phase, *Bacillus subtilis* induces several adaptive responses, including genetic competence, degradative enzyme synthesis, nutrient transport, antibiotic production, and chemotaxis and motility. Under prolonged starvation conditions, *B. subtilis* cells activate a complex morphogenetic pathway leading to endospore formation. To enable the cell to sense and react appropriately to dramatic changes in the environment, the regulatory genes responsible for activating and coordinating these responses are organized in a large, integrated signal transduction network (4, 6, 9). Many of the genes of the adaptive response pathways are dispensable for sporulation but control or are under the control of essential sporulation genes (7, 37). Whether a particular pathway is chosen may depend on the availability of various nutritional sources. Accordingly, there may be both global and specific regulatory mechanisms to ensure that the most favorable processes are adopted in a given environment. If the cell fails to adapt successfully to growth-limiting conditions and must therefore commit itself to sporulation, some gene products involved in adaptation would become unnecessary or may even be inhibitory to sporulation, and their expression may need to be shut down for successful completion of the developmental pathway.

One mechanism by which *B. subtilis* apparently controls the diverse range of responses to environmental stimuli is by using members of the two-component (sensor-regulator) family of signal-transducing transcriptional regulatory systems (36, 39). In general, one component of the protein pair functions as a kinase, which phosphorylates itself in response to an extracellular or intracellular signal, and then transfers the phosphate residue to a response regulator. This phosphorelay ultimately results in the activation or repression of specific gene sets in response to the environmental challenge. A diverse group of stationary-phase and sporulation-specific genes is thought to respond to changes in the phosphorylation state of the response regulator Spo0A (4, 9, 21). Genetic and biochemical studies imply that there is a cascade of phosphorylation events in the signaling pathway and that Spo0A is a target, indirectly, of the histidine kinase KinA (3, 4, 20, 21, 38). Phosphorylated Spo0A seems to have two activities that control stationary-phase events. It acts as a repressor of the *abrB* gene (41), whose product is itself a repressor of many genes expressed in the stationary phase (28, 35, 42, 46). Spo0A also acts as a direct transcriptional activator of some essential sporulation genes, including *spoIIA* (4, 25) and *spoIIG* (32, 32a). This latter activity of Spo0A is particularly important because it permits synthesis of σ^E, a product of the *spoIIG* operon, and a key component in a cascade of sporulation-specific gene expression (10, 12, 13, 32a).

In the accompanying report, we characterized the *B. subtilis gsiA* and *gsiB* genes, which are induced in response to glucose starvation and other forms of nutritional limitation (17). Transcription from the *gsiA* promoter is induced transiently at the start of the stationary growth phase. The *gsiA* operon is probably transcribed by the σ^A form of RNA polymerase in conjunction with one or more positively acting transcription factors. In fact, induction of *gsiA* expression is dependent on the two-component signal transduction system ComP-ComA, which also controls expression of competence genes (5, 6, 43, 44). We show here that turnoff of *gsiA* transcription is dependent on the product of the *spo0A* gene. In addition, we have identified another locus, *gsiC*, whose product is necessary to turn off expression of *gsiA*. Failure to turn off *gsiA* prevents transcription of the *spoIIA*, *spoIIG*, and *spoIID* genes and reduces sporulation by approximately 90%. We also show that *gsiC82* is an allele of the *kinA* gene, suggesting a connection between KinA-mediated sporulation initiation (3, 21) and inhibition of *gsiA* synthesis.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this work are listed in Table 1.

General methods. Preparation and transformation of competent *B. subtilis* cells were done as previously described (17). Preparation and transformation of *Escherichia coli* by electroporation were done as described by Dower et al. (4a), by using a Bio-Rad GenePulser apparatus (Bio-Rad Labora-

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TABLE 1. *B. subtilis* strains used in this study

Strain	Trait or genotype	Derivation or reference
SMY	Prototrophy	Laboratory stock
EU8743	*trpC2 pheA1* SPβ*c2del2*::Tn*917*::*spoIIG-lacZ cat*	C. Moran; 12
JH642	*trpC2 pheA1*	J. Hoch; 35
JH646	*trpC2 pheA1 spo0A12*	J. Hoch; 35
JH646Tn	*trpC2 pheA1 abrB*::Tn*917*	C. Mathiopoulos; 35
JH12575	*trpC2 pheA1 spo0A12 abrB*::Tn*917*	J. Hoch; 35
KS19	*kinA*::Tn*917*ΩHU19	A. Grossman; 3
MB4	Φ(*spoIID-lacZ*) *cat*	S. Rong (SR10); 29
MB25	Φ(*gsiA-lacZ*)*27 cat*	17
MB30	*glnA73*	S. Brown
MB45	JH642 Φ(*gsiA-lacZ*)*27 cat*	MB25→JH642[a]
MB46	JH646 Φ(*gsiA-lacZ*)*27 cat*	MB25→JH646
MB47	JH646Tn Φ(*gsiA-lacZ*)*27 cat*	MB25→JH646Tn
MB48	JH12575 Φ(*gsiA-lacZ*)*27 cat*	MB25→JH12575
MB61	Δ*gsiA69*::*cat*	17
MB82	Δ*gsiB60*::*neo*	17
MB83	Δ*gsiA69*::*cat* Δ*gsiB60*::*neo*	17
MB146	Δ*gsiB60*::*neo gsiC82*	Spontaneous Spo⁻ mutant of MB82
MB155	*kinA*::Tn*917*ΩHU19	KS19→SMY
MB157	MB155 Δ*gsiA69*::*cat*	MB61→MB155
MB166	Δ*orfW107*::*neo*	pJPM107→SMY
MB170[b]	*gsiC82*	MB146→MB30
MB172	*gsiC82* Δ*orfW107*::*neo*	MB166→MB170
MB173	*orfY109*::*ermC*	pJPM109→SMY
MB176	*orfZ110*::*cat*	pJPM110→SMY
MB182	Δ*gsiA69*::*cat gsiC82*	pJPM69→MB170
MB186	*trpC2* SPβ[s]	17
MB188	MB170 Φ(*gsiA-lacZ*)*27 cat*	MB25→MB170
MB190	*gsiC82 spoIID-lacZ cat*	MB4→MB170
MB218	SPβ*c2del2*::Tn*917*::*spoIIG-lacZ cat*	SPβ::*spoIIG-lacZ*[c] × SMY
MB219	MB170 SPβ*c2del2*::Tn*917*::*spoIIG-lacZ cat*	SPβ::*spoIIG-lacZ*[c] × MB170
MB235	Δ*gsiA114*::*neo*	pJPM114→SMY
MB236	MB170 Δ*gsiA114*::*neo*	pJPM114→MB170
MB238	MB236 SPβ*c2del2*::Tn*917*::*spoIIG-lacZ cat*	SPβ*spoIIG-lacZ*[c] × MB236
MB275	MB182 SPβ*c2del2*::Tn*917*::pSK10Δ6::pJPM117	SPβ*gsiA*$^{+c}$ × MB182
MB284	SPβ*c2del2*::Tn*917*::*spoVG'-'lacZ cat*	SPβ*spoVG'-'lacZ*[c] × SMY
MB285	MB170 SPβ*c2del2*::Tn*917*::*spoVG'-'lacZ cat*	SPβ*spoVG'-'lacZ*[c] × MB170
MB296	*spoIIA-lacZ cat*	pPP81→SMY
MB297	MB170 *spoIIA-lacZ cat*	pPP81→MB170
MB340	*trpC2 spoIIF96*	BGSC[d] 1S59
MB352	Δ*orfW107*::*neo spoIIF96*	MB166→MB340
MB353[e]	*spoIIF96*	MB352→SMY
MB358	MB353 Δ*gsiA69*::*cat*	pJPM69→MB353
MB413	MB155 SPβ*c2del2*::Tn*917*::pSK10Δ6::pJPM149	SPβ*kinA*$^{+c}$ × MB155
MB414	MB170 SPβ*c2del2*::Tn*917*::pSK10Δ6::pJPM149	SPβ*kinA*$^{+c}$ × MB170
MB415	MB353 SPβ*c2del2*::Tn*917*::pSK10Δ6::pJPM149	SPβ*kinA*$^{+c}$ × MB353
ZB307A	SBβ*c2del2*::Tn*917*::pSK10Δ6	P. Zuber; 46
ZB480	*trpC2 pheA1 spo0H*Δ*Hind chr*::Tn*917*ΩHU146	P. Zuber; 46

[a] An arrow indicates construction by transformation.
[b] Strain MB170 was constructed by congression with MB30, a Gln⁻ derivative of SMY.
[c] SPβ-mediated transduction.
[d] *Bacillus* Genetic Stock Center, Ohio State University, Columbus.
[e] The *spoIIF96* mutation was moved into SMY by transformation using selection for the *neo* gene integrated downstream of *gsiA* (Fig. 1). The sporulation deficiency associated with the *spoIIF96* mutation was cotransferred with the Neo^r marker at a frequency of 10%.

tories). Isolation and manipulation of plasmid DNA for cloning and analysis were done by using standard protocols (31). To introduce the *gsiA-lacZ* fusions into other wild-type and mutant strains, chromosomal DNA was purified from strain MB25 and used to transform competent cells of the isogenic strains to chloramphenicol resistance (Cm^r; 2.5 μg/ml; Table 1).

Growth and sporulation conditions. *B. subtilis* cells were induced to sporulate by nutrient exhaustion. A culture in nutrient broth sporulation (DS) medium (33) in the exponential growth phase was diluted to give an A_{600} of 0.05. Culture growth was monitored by measuring A_{600}; T_0 was defined as the end of the exponential growth phase. Heat resistance was measured by heating samples at 80°C for 20 min and plating serial dilutions on nutrient agar plates (17).

Construction of isogenic strains containing *spo-lacZ* fusions. The *spoVG-lacZ* and *spoIIG-lacZ* fusions were introduced into strains SMY and MB170 by transduction, by using SPβ lysates of strains ZB308 and EU8743, respectively (12, 46). The *spoIIA-lacZ* fusion was introduced by integration of plasmid pPP81 (27) into the chromosome. The *spoIID-lacZ* fusion was introduced by transformation with chromosomal DNA from an SMY derivative in which plasmid pSR10 had been integrated (29). Chloramphenicol resistance was used

as the selectable marker for transfer of each fusion to the recipient strains.

Measurement of enzyme activity. Samples were taken at different times during growth and sporulation in DS medium, and β-galactosidase activity, expressed as Miller units (16), was determined as previously described (17). Alkaline phosphatase activity was assayed from cells grown to T_5 in DS medium as described by Seki et al. (34).

***kinA* plasmid constructions.** pJPM146 was created by subcloning the 1.3-kb neomycin resistance cassette from pBEST501 (35) into the unique *Eco*RI site of pDG581 (3; see Fig. 4). pJPM148 was created by cloning into the *cat*-bearing integrational plasmid pJPM1 (17) a DNA segment from pDG581 extending from the *Hpa*I site near the 5′ terminus of Tn*917* to a *Sac*I site upstream from *kinA* (see Fig. 4). pJPM149 was created by transforming strain SMY with pJPM148 and then selecting for Cm^r. Chromosomal DNA from one such transformant was cut with *Hin*dIII, which released the plasmid and adjacent chromosomal DNA extending from the *Sac*I site upstream from *kinA* to the *Hin*dIII site at the downstream end of the gene (see Fig. 4). The *Hin*dIII-digested chromosomal DNA was ligated at a low DNA concentration (3.3 μg/ml) to favor intramolecular recombination and used to transform *E. coli* DH5α; this was followed by selection for ampicillin resistance. pJPM149 was isolated from one such ampicillin-resistant transformant. Plasmid pJPM152 was created by subcloning the 1.7-kb end-filled *Cla*I-to-*Acc*I fragment of pDG581 into the *Sma*I site of plasmid pJPM1. pJPM152 contains DNA between the *Cla*I site in the adjacent *orfX* gene and an *Acc*I site (see Fig. 4) within *kinA*.

Construction of a strain bearing a wild-type copy of the *kinA* gene at the SPβ locus. We used a method developed by Nakano and Zuber (19) to introduce a copy of the *kinA* gene into the genome of specialized transducing bacteriophage SPβ. Plasmid pJPM149 contains a wild-type copy of the *kinA* gene which extends from an upstream *Sac*I site to a *Hin*dIII site just downstream from the gene (see Fig. 4). Plasmid pJPM149 was used to transform ZB307A, and this was followed by selection for Cm^r. Transformants bearing a copy of *kinA* at the SPβ locus were expected to arise by single-crossover recombination between pJPM149 DNA and the chromosomal copy of pSK10Δ6 present in the prophage genome (19). Plasmid insertions in SPβ were identified by production of a heat-induced SPβ phage transducing lysate of the pool of Cm^r transformants and infection of SPβ-sensitive strain MB186 with selection for Cm^r. A Cm^r lysogen of MB186 was used as a source of specialized transducing phage SPβ*kinA*$^+$. For complementation analysis, a pure SPβ*kinA*$^+$ transducing lysate was prepared and used to transfect the appropriate recipient strains; this was followed by selection for Cm^r.

Sequencing of *kinA* mutant alleles. To sequence *spoIIF96*, we constructed a *spoIIF96*-bearing plasmid by use of pJPM148 (see Fig. 4). Integration of plasmid pJPM148 into the chromosome of *spoIIF96* mutant strain MB353 gave rise to only Spo$^+$ transformants, suggesting that the mutation was located very close to the *Sac*I site (see Fig. 4 and Results). A Spo$^+$ transformant arising from integration of pJPM148 was used to create a plasmid called pJPM150 that contained DNA extending from the upstream *Sac*I site to the Tn*917*ΩHU19 insertion junction contained on plasmid pJPM148 (see Fig. 4) by use of the method described above to create pJPM149. pJPM150 is identical to pJPM148, except that it contains the *spoIIF96* mutation. When used to transform SMY, plasmid pJPM150 gave rise exclusively to Spo$^-$ integrants. A DNA fragment extending from the upstream *Sac*I site to the *Nsi*I site in *kinA* was subcloned from pJPM150 into pSK$^+$ and pSK$^-$ for nucleotide sequence analysis as previously described (17).

To sequence *gsiC82*, plasmid pJPM148 was integrated into the chromosome of mutant strain MB170 (*gsiC82*). pJPM148 generated approximately equal numbers of Spo$^+$ and Spo$^-$ transformants (see Fig. 4 and Results). A plasmid (pJPM151) bearing the *gsiC82* mutation was created from a Spo$^-$ transformant as described above for the construction of pJPM149. pJPM151 is identical to pJPM149 (Fig. 4), except that it contains the *gsiC82* mutation. The presence of the *gsiC82* mutation in the plasmid was confirmed by demonstrating that plasmid pJPM151 gave rise to Spo$^-$ integrants only when used to transform strain MB170 (*gsiC82*). The nucleotide substitution corresponding to the *gsiC82* mutation was identified by DNA sequence analysis of the 1.0-kb *Nsi*I-*Dra*I segment of pJPM151 (see Fig. 4) subcloned into pSK$^+$ and pSK$^-$.

RESULTS

Isolation of a spontaneous *gsiC* mutant. Transcription of *gsiA* is induced transiently at the beginning of the stationary phase in cells growing in nutrient broth sporulation medium (17). A sporulation mutation, isolated serendipitously, caused inability to shut off *gsiA* expression (see below). We had noticed that strains containing a *gsiB* deletion-insertion mutation (17) segregated sporulation-deficient colonies. These were isolated by streaking a 5- to 7-day-old colony on DS agar plates: sporulation-deficient segregants (detected by altered colony morphology) appeared among the sporulation-proficient colonies. Insertion mutations just upstream or downstream of the *gsiB* coding region, as in strains MB173 and MB176, respectively, did not result in the appearance of spontaneous Spo$^-$ segregants at a detectable frequency (Fig. 1B and Table 1). Our interpretation of this behavior is that a *gsiB* null mutation is deleterious to some aspect of cell growth and/or sporulation, even though no dramatic alteration in colony size, growth rate, or sporulation frequency was seen (17; Table 1). One spontaneous *spo* mutation, designated *gsiC82*, was studied further. An isogenic derivative of strain SMY carrying the *gsiC82* mutation was constructed by transformation of auxotrophic mutant MB30 (Gln$^-$) to prototrophy at high concentrations of chromosomal DNA prepared from strain MB146. The Gln$^+$ transformants were screened for acquisition of the Spo$^-$ phenotype by congression. A single Gln$^+$ Spo$^-$ transformant was purified for further analysis. The resultant strain, MB170, was indistinguishable from the original *gsiC* mutant, MB146, in terms of sporulation phenotype (see below). No linkage between *gsiC* and *gsiB* was observed in transformation crosses.

The efficiency of sporulation of *gsiC82*-containing strains was approximately 10% of that observed for parental strain SMY (Table 2). The frequency of sporulation of the strain carrying only the *gsiC82* mutation was similar to that of *gsiB gsiC* double-mutant strain MB146, suggesting that the *gsiC* mutation was solely responsible for the oligosporogenous phenotype (Table 2). The *gsiC* mutants produced early-stationary-phase-associated protease and antibiotics active against cells of a *spo0A* strain of *B. subtilis* (data not shown). Alkaline phosphatase activity (a late stage II marker) was induced in the stationary phase to a level approximately 30 to 40% of that seen in wild-type (SMY) cultures, suggesting

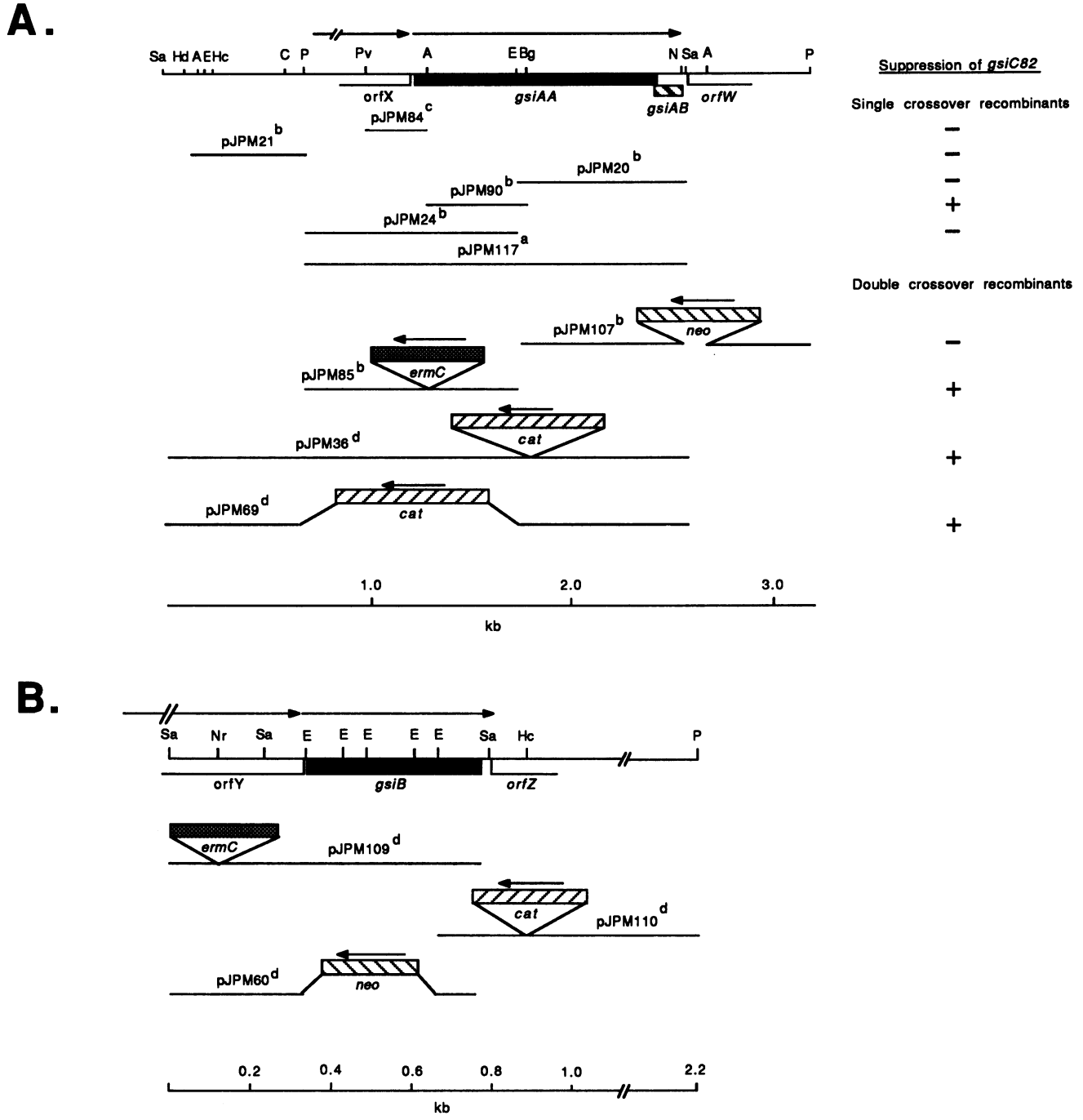

FIG. 1. Physical map of the *gsiA* (A) and *gsiB* (B) regions of the *B. subtilis* chromosome. The chromosomal inserts contained in the plasmids used in this study are indicated as lines below the abbreviated restriction maps. The locations of the *gsi* genes are shown. The regions designated *orfX*, *orfY*, *orfW*, and *orfZ* represent uncharacterized open reading frames adjacent to the *gsiA* and *gsiB* transcriptional units. The positions and structures of the various insertions in the chromosome are also indicated, as is the direction of transcription of the antibiotic resistance cassette. The phenotypes of progeny resulting from integration by homologous recombination into strain MB170 are noted at the right. A plus sign indicates that integration of a plasmid carrying the indicated DNA fragment by a single-crossover recombination event or insertion of an antibiotic resistance cassette via a double-crossover recombination event led to suppression of the sporulation defect caused by the *gsiC82* mutation. Restriction enzyme cleavage sites: E, *Eco*RI; Hd, *Hin*dIII; Pv, *Pvu*II; B, *Bgl*II; A, *Acc*I; Hc, *Hin*cII; C, *Cla*I; N, *Nru*I; Nr, *Nar*I; Sa, *Sau*3A; P, *Pst*I. Vectors are indicated by the following superscripts: a, pBEST501; b, pJPM1; c, pJPM3; d, pSK$^-$.

a partial block in the transition from stage II to stage III of morphogenesis.

The *gsiC82* mutation results in increased *gsiA* transcription. We asked whether the *gsiC82* mutation would have any effect on *gsiA* transcription by introducing a *gsiA-lacZ* fusion into the chromosome of strain MB170 (*gsiC82*). In a GsiC$^+$ strain, β-galactosidase activity began to appear at T_0 and declined after $T_{1.0}$, as is normal for *gsiA* expression (17;

TABLE 2. Effect of a *gsiA* deletion mutation on sporulation in *gsiC*, *spoIIF*, and *kinA* mutant strains

Strain[a]	Trait or relevant genotype	CFU/ml[b]		Frequency[c]
		Total	Heat resistant	
SMY	Prototrophy	6.9×10^8	5.1×10^8	0.74
MB61	Δ*gsiA69*::*cat*	4.8×10^8	3.7×10^8	0.77
MB82	Δ*gsiB60*::*neo*	5.8×10^8	4.3×10^8	0.74
MB83	Δ*gsiA69*::*cat* Δ*gsiB60*::*neo*	4.7×10^8	3.4×10^8	0.72
MB146	*gsiC82* Δ*gsiB60*::*neo*	3.6×10^8	3.0×10^7	0.08
MB155	*kinA*::Tn*917*ΩHU19	7.4×10^8	1.6×10^8	0.22
MB157	MB155 Δ*gsiA69*::*cat*	7.7×10^8	6.7×10^8	0.87
MB166	Δ*orfW107*::*neo*	6.7×10^8	5.4×10^8	0.81
MB170	*gsiC82*	3.3×10^8	3.0×10^7	0.09
MB172	*gsiC82* Δ*orfW107*::*neo*	2.5×10^8	2.0×10^7	0.08
MB182	*gsiC82* Δ*gsiA69*::*cat*	4.9×10^8	2.3×10^8	0.47
MB275	MB182 SPβ*gsiA*$^+$	3.2×10^8	3.0×10^7	0.09
MB353	MB166 *spoIIF96*	2.5×10^9	2.1×10^8	0.08
MB358	MB353 Δ*gsiA69*::*cat*	7.8×10^8	6.5×10^8	0.83

[a] All of the strains listed are isogenic derivatives of strain SMY constructed by transformation.
[b] The strains indicated were induced to sporulate by exhaustion of DS medium, and samples were taken 24 h after the onset of sporulation. Appropriate dilutions were plated on DS agar before and after heat treatment (80°C, 20 min) to determine the proportions of viable and heat-resistant CFU.
[c] Heat-resistant CFU divided by the total. The values given are averages of at least two independent experiments.

Fig. 2A). In GsiC$^-$ mutant strain MB188, induction of *gsiA* transcription occurred normally as cells entered the stationary phase but was maintained at an elevated level for at least the next 3 h (Fig. 2A). Expression of *gsiB* was also enhanced in a *gsiC* mutant (data not shown). These results suggest that a normal function of GsiC is to prevent *gsiA* and *gsiB* from being highly expressed during sporulation. The sporulation deficiency of *gsiC* mutants might therefore result from persistent expression of *gsiA* or *gsiB* or both.

Turn off of *gsiA* gene expression requires Spo0A. Since *spo0A* controls several stationary-phase responses, we tested whether the decrease in *gsiA* expression after the first hour of the stationary phase was dependent on Spo0A. We introduced the *gsiA-lacZ* transcriptional fusion into isogenic *spo0A* mutant and wild-type strains by transformation of competent cells with chromosomal DNA from wild-type cells bearing an integrated copy of the fusion. The fusion-bearing strains were grown in DS medium and assayed for *gsiA*-directed β-galactosidase synthesis at various times during growth and sporulation. Activation of *gsiA* transcription was not dependent upon *spo0A*. On the contrary, in a *spo0A* mutant, the activity of the *gsiA-lacZ* fusion in response to nutrient exhaustion was elevated and prolonged during the stationary phase, indicating that in the absence of the *spo0A*-encoded protein, transcription of *gsiA* failed to be shut off at the normal time (Fig. 2B). Induction occurred at the usual time, but β-galactosidase activity reached a level severalfold higher than that in wild-type cells. This suggests that the Spo0A protein negatively regulates *gsiA* expression during an early stage of the sporulation process. Mutations in *abrB* only partially suppressed the enhancing effect of a *spo0A* mutation on *gsiA* (Fig. 2B). In an *abrB* single mutant (*abrB*::Tn*917*), transcription from the *gsiA* promoter was unaffected (Fig. 2B). Elevated and persistent levels of *gsiA*-directed β-galactosidase activity were also observed in isogenic strains carrying a null mutation in *spo0H* (data not shown).

Deletion of *gsiA* restores sporulation to the *gsiC* mutant. If prolonged expression of *gsiA* or *gsiB* were solely responsible for the sporulation defect of the *gsiC82* mutant strain, mutations in the former genes would be expected to suppress the sporulation phenotype. When strain MB170 was transformed with *Bam*HI-linearized plasmid pJPM69, carrying a deletion-insertion of the *gsiA* operon (Fig. 1A), all of the chloramphenicol-resistant transformants appeared to be sporulation proficient. One of these transformants, strain MB182, and various control strains were grown in liquid medium under sporulation conditions, and spore production was quantitated (Table 2). The *gsiA* mutation restored sporulation to approximately wild-type levels in a *gsiC82* mutant strain (Table 2). We did not determine whether both the promoter-proximal (*gsiAA*) and promoter distal (*gsiAB*) cistrons are needed for manifestation of the sporulation inhibition phenotype in *gsiC* strains (17). When a *gsiB* deletion-insertion mutation was introduced into a *gsiC82*-bearing strain, no suppression of the Spo$^-$ phenotype was seen (Table 2). (This is consistent with the original conditions under which *gsiC82* was isolated.)

The suppression of the block in sporulation in a *gsiC82*Δ*gsiA69* double mutant might be due either to loss of a functional *gsiA* product or to a polar effect of the *gsiA* mutation on expression of a downstream gene. SPβ-mediated complementation (19) was used to show that the DNA fragment carried on plasmid pJPM117 (17; Fig. 1A), containing the entire *gsiA* operon, could restore the Spo$^-$ phenotype to the *gsiC gsiA* double mutant strain. Cells of the *gsiC gsiA* strain were lysogenized with a specialized transducing phage bearing an intact copy of the *gsiA* operon (17). Neo^r transductants containing the SPβ*gsiA*$^+$ prophage were Spo$^-$ (Table 2; MB275). As a control, a SPβ::*gsiA*::*cat* prophage failed to restore the sporulation deficiency phenotype of *gsiC gsiA* mutant cells (data not shown). A polar effect is, therefore, incompatible with the observation of complementation by pJPM117. As an independent test of polarity, a neomycin resistance cassette was inserted just downstream of the *gsiA* transcriptional unit in the *B. subtilis* chromosome by marker replacement (Fig. 1A). Since cells containing the *gsiC82* mutation and the Δ*orfW107*::*neo* insertion-deletion were oligosporogenous (Table 2), we conclude that the *gsiA* transcriptional unit does not contain a downstream gene whose interruption is required to suppress the sporulation defect of a *gsiC* mutant. In further support of this, only insertions or deletions within the *gsiA* transcriptional unit resulted in suppression of *gsiC82* (Fig. 1A). These results

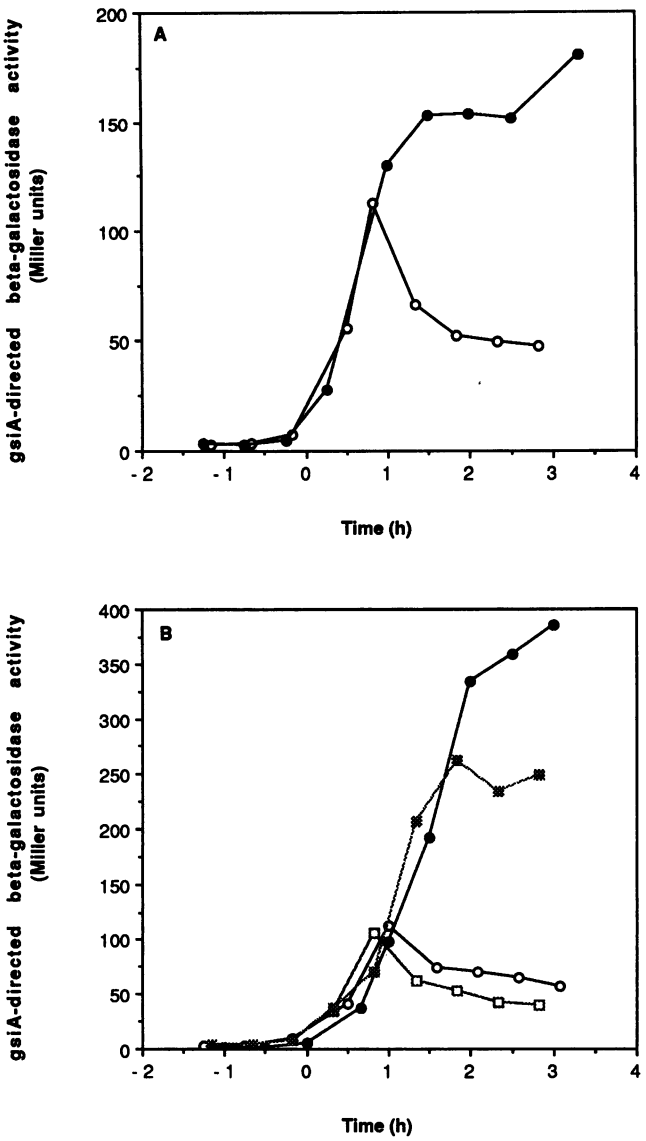

FIG. 2. Expression of β-galactosidase in *gsiC* and *spo0* mutant strains carrying a *gsiA-lacZ* fusion. The cells were grown in DS medium. The abscissa is divided into hours preceding and following the end of exponential growth, which was designated time zero. Symbols in panel A: ○, *gsi*$^+$; ●, *gsiC82*. Symbols in panel B: ○, *spo*$^+$; ●, *spo0A12*; □, *abrB*::Tn*917*; ■, *spo0A12 abrB*::Tn*917*.

indicate that the sporulation-deficient phenotype caused by a mutation at the *gsiC* locus can be suppressed by *gsiA* null mutations and suggest that persistent synthesis of *gsiA* is the primary cause of the sporulation defect in the *gsiC82* strain.

Since we observed persistent stationary-phase expression of *gsiA* in *spo0A* and to a lesser extent in *spo0H* and *spo0A abrB* mutants (Fig. 2B) (18), and since *gsiA* mutations could suppress the sporulation-deficient phenotype of a *gsiC82* mutation, we tested the possibility that *gsiA* mutations could also suppress the sporulation defect exhibited by *spo0A*, *spo0H*, and *spo0A abrB* mutations. *Bam*HI-linearized plasmid pJPM69 was introduced into competent cells of strains JH646, ZB480, and JH12575, and selection was made for Cm^r. All of the colonies from each cross maintained the Spo$^-$ phenotype of the parental strain, indicating that the *gsiA* mutation did not suppress the requirement for *spo0A*, *spo0H*, and *abrB* gene products in sporulation. Mutations in *gsiA* also failed to suppress the sporulation-deficient phenotype caused by *spo0B*, *spo0E*, *spo0F*, *spo0J*, and *spo0K* mutations (data not shown).

Effect of a *gsiC* mutation on expression of various *spo* genes. As a first approach to defining the temporal role of the *gsiC* product in the sporulation process, we examined the effect of the *gsiC82* mutation on expression of several *spo* genes. Fusions of the *lacZ* gene with the *spoVG*, *spoIIA*, *spoIIG*, and *spoIID* promoters were introduced into strains SMY and MB170. The *gsiC82* mutation had no effect on expression of *spoVG-lacZ* during growth or during the first 3 h of sporulation (Fig. 3). In contrast, expression of the *spoIIA-lacZ*, *spoIIG-lacZ*, and *spoIID-lacZ* fusions, which are normally expressed later in development, was significantly lower in the *gsiC* mutant than in the parental strain (Fig. 3). The reduced level of *spoIID* promoter activity was expected, since expression of *spoIID* is dependent on σ^E (a product of the *spoIIG* operon) and the posttranslational processing of pro-σ^E is dependent on *spoIIA* (10, 30). Disruption of *gsiA* restored *spoIIG*-directed β-galactosidase activity in a *gsiC82* background (Fig. 3), consistent with the suppression by *gsiA* mutations of the sporulation defect of a *gsiC* mutant. Thus, lowered transcription of the *spoIIA*, *spoIIG*, and *spoIID* operons in a *gsiC* mutant seems to reflect overexpression of *gsiA*.

Genetic location of the *gsiA* and *gsiC* loci. The *gsiA* locus was initially localized to the SF14 fragment of the *B. subtilis* chromosome by pulsed-field gel electrophoresis (1, 2). The chromosomal location of *gsiA* was determined more precisely by transformation using a phenotypically silent *neo* insertion (Δ*orfW107*::*neo*) very close to *gsiA* (approximately 98% cotransformation with Δ*gsiA69*::*cat*) as a selectable genetic marker (Fig. 1). Δ*orfW107*::*neo* was found to be cotransformed with auxotrophic mutation *metC3* at a frequency of 12% and with sporulation mutations *spoIIF96* and *gsiC82* at a frequency of 10%. These results and those of other crosses are consistent with the gene order *pro-1–metC3–gsiA–(spoIIF96/gsiC82)* and indicate a location at about 115°C on the *B. subtilis* genetic map (26).

The *gsiC82* and *spoIIF96* mutations are alleles of *kinA*. Mutations in several *B. subtilis spoII* genes prevent expression of *spoIIG* and block development of the spore at an early stage (10). Isogenic strains carrying the *spoIIF96*, *kinA*::Tn*917*ΩHU96, or *gsiC82* mutation are phenotypically indistinguishable. Each mutant strain is oligosporogenous, reduces transcription of *spoIIG*, and is located at approximately the same position on the genetic map (3, 13, 21, 44, 45). These findings suggested the possibility that *gsiC82* is an allele of the *spoIIF* or *kinA* gene. Previous unpublished reports have indicated that *spoIIF* and *kinA* are allelic (cited in reference 26). To test the above hypothesis, we first measured the ability of a *gsiA* null mutation to suppress the sporulation defect of *spoIIF96* and *kinA*::Tn*917*ΩHU19 mutant strains. The *spoIIF96* Δ*gsiA69*::*cat* and *kinA*::Tn*917* ΩHU19 Δ*gsiA69*::*cat* double mutants sporulated as efficiently as isogenic wild-type and Δ*gsiA69*::*cat* strains (Table 2), indicating that the absence of *gsiA* suppresses the sporulation defects of both *spoIIF96* and *kinA*::Tn*917*ΩHU19.

To investigate whether the *spoIIF96* and *gsiC82* mutations were *kinA* alleles, we used integrational plasmid pJPM148 to transform strains containing *spoIIF96* and *gsiC82* mutations and then selected for Cm^r. Plasmid pJPM148 contains an amino-terminal truncated copy of the wild-type *kinA* gene (Fig. 4). If the wild-type alleles of the *gsiC82* and *spoIIF96*

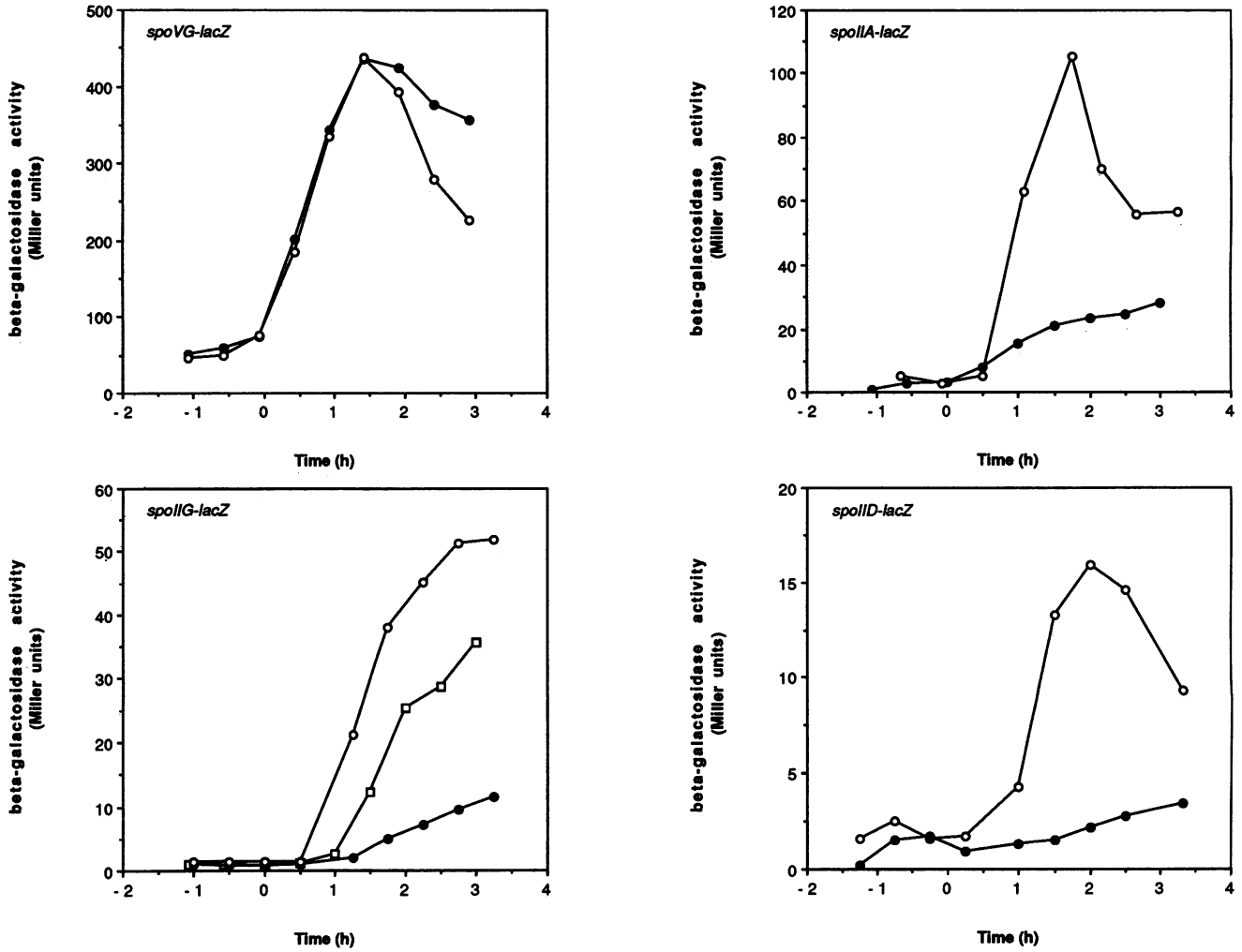

FIG. 3. Effect of the *gsiC82* mutation on *spoVG-lacZ*, *spoIIA-lacZ*, *spoIIG-lacZ*, and *spoIID-lacZ* expression. The activity of β-galactosidase in strain SMY (*gsi*$^+$) (○), MB170 (*gsiC82*) ●, or MB236 (*gsiC82* Δ*gsiA114*::*neo*) □ carrying the indicated *lacZ* fusion was monitored during growth and sporulation in DS medium. The abscissa is divided into hours preceding and following the end of exponential growth, which was designated time zero.

mutations reside within the chromosomal *kinA* fragment on the plasmid, integration of plasmid pJPM148 into the chromosome of mutant strains MB170 (*gsiC82*) and MB353 (*spoIIF96*) should give rise to Spo$^+$ and Spo$^-$ transformants, depending on whether the site of single, reciprocal recombination between the plasmid insert and the chromosome occurs upstream or downstream of the mutation. When used to transform strain MB353, plasmid pJPM148 gave rise exclusively to Spo$^+$ integrants (100 total). To localize the mutation further, we used plasmid pJPM152 (Fig. 4) to transform MB353. Plasmid pJPM152 gave rise to equal numbers of Spo$^+$ and Spo$^-$ transformants, consistent with the location of *spoIIF96* to the right of and very close to the *Sac*I site near the 5′ end of the gene. This location of the *spoIIF96* mutation has been determined independently by Perego and Hoch (24) and confirmed by Stragier and colleagues (40).

Of the 400 Cm^r transformants produced when plasmid pJPM148 was used to transform strain MB170 (*gsiC82*), approximately 56% were Spo$^+$. To localize the mutation further, we used plasmid pJPM152 (Fig. 4) to transform

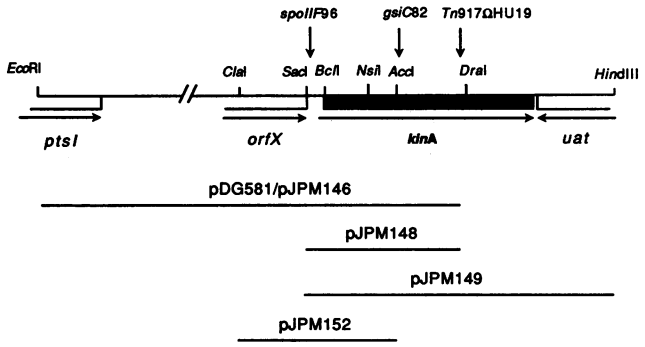

FIG. 4. Integrational plasmids used to analyze the *kinA* locus. The region of DNA cloned in plasmid pDG581 was used for subcloning into integrational plasmid vector pJPM1 (17). Only the restriction sites used in the cloning steps are shown. The solid box represents the extent of the *kinA* open reading frame. The restriction map also shows the approximate positions of *spoIIF96* and *gsiC82*.

TABLE 3. Complementation analysis of *kinA*::Tn*917*ΩHU19, *spoIIF96*, and *gsiC82* mutations

Strain	Relevant genotype	Insertion at SPβ[a]	Spo phenotype[b]
SMY	*kinA*$^+$	None	+
MB155	*kinA*::Tn*917*ΩHU19	None	−
MB413	*kinA*::Tn*917*ΩHU19	*kinA*$^+$	+
MB170	*gsiC82*	None	−
MB414	*gsiC82*	*kinA*$^+$	+
MB353	*spoIIF96*	None	−
MB415	*spoIIF96*	*kinA*$^+$	+

[a] The partial diploids were created by introducing a second copy of the *kinA* gene at the SPβ locus (see Materials and Methods).

[b] The Spo phenotype was assessed by colony color; colonies of cells exhibiting the Spo$^-$ phenotype (indicated by a minus sign) were light brown, whereas colonies exhibiting the Spo$^+$ phenotype (indicated by a plus sign) were dark brown.

competent cells of strain MB170. Plasmid pJPM152 yielded no Spo$^+$ integrants, a finding that places the *gsiC82* mutation very close to and possibly downstream of the *Acc*I site in the *kinA* gene, as shown in Fig. 4.

As a further indication that *spoIIF96* and *gsiC82* are allelic to *kinA*, the *gsiC82* and *spoIIF96* mutations were complemented by a wild-type copy of the *kinA* gene, carried on plasmid pJPM149 (Fig. 4), that had been inserted into the chromosome at the SPβ locus (Table 3). An SPβ prophage carrying only the 5′ end of *kinA* (plasmid pJPM146; Fig. 4) failed to complement (data not shown). These findings confirm that *gsiC82* and *spoIIF96*, which are henceforth designated *kinA82* and *kinA96*, respectively, are allelic to *kinA*.

To determine the nature of the *kinA* mutations and their precise locations within the gene, we cloned both mutant alleles and subjected them to nucleotide sequence analysis (see Materials and Methods). The results showed that the *kinA96* mutation is a 107-bp deletion that removes the ribosome-binding site and the first 23 codons of *kinA* (Fig. 5). The *kinA82* mutation is a T-to-A transversion that converts a tryptophan codon at position 288 to an arginine codon (Fig. 5). It is of interest that the missense mutation in strain MB170 does not lie within the highly conserved motifs among bacterial kinases (3, 21). The *kinA82* mutation may cause a change that impairs the stability of the kinase or a site of interaction with a component of the signal transduction system that activates the kinase.

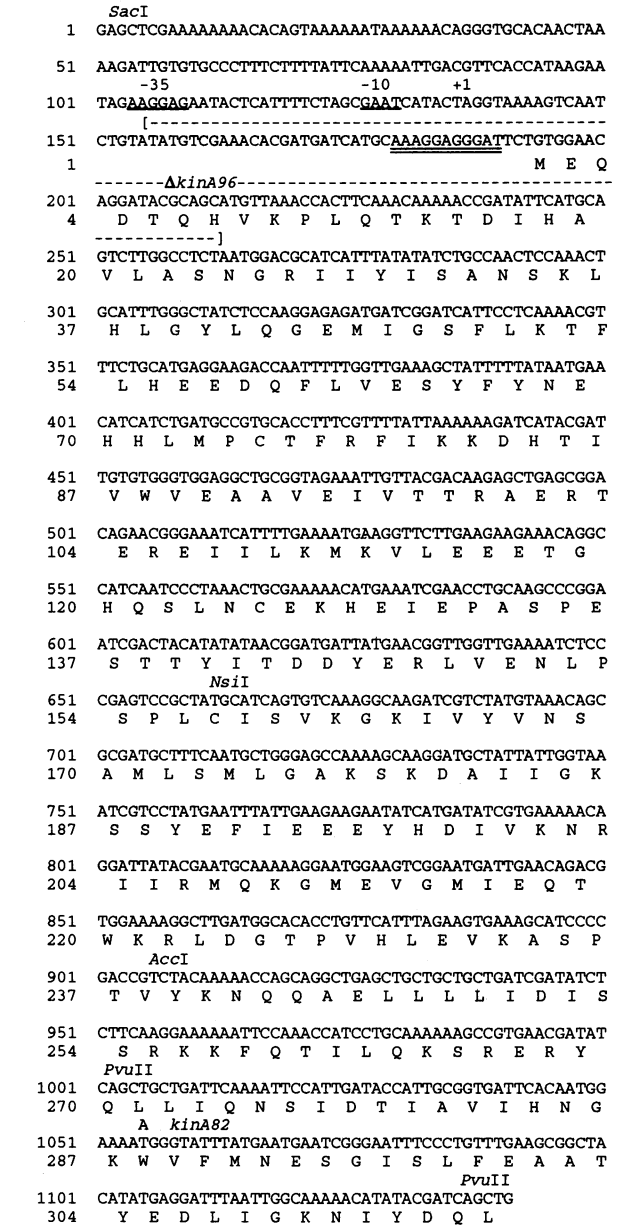

FIG. 5. Positions of the Δ*kinA96* and *kinA82* mutations. The partial nucleotide and amino acid sequence data for the *kinA* gene (GenBank accession number M29450) were taken from Antoniewski et al. (3). The locations of the transcriptional start point and putative σ^H promoter consensus sequences (single underlining) were taken from Predich et al. (27a). The ribosome-binding site is indicated by double underlining. The broken line in brackets shows that the Δ*kinA96* mutation is a deletion of nucleotides 156 to 262.

DISCUSSION

Our results indicate that expression of *gsiA* is controlled by both positive and negative regulatory factors (Fig. 6). The level of *gsiA* expression appears to be dependent on the interplay of ComP, ComA, KinA, and Spo0A. *gsiA* expression is repressed during rapid exponential growth and is induced by nutrient exhaustion in a ComA-dependent manner (17). Induction is transient, however. The turnoff of *gsiA* transcripts after the first hour of the stationary phase is a developmentally regulated event. Certain early-blocked sporulation mutations (*kinA*, *spo0A*, and *spo0H*) disrupt this negative regulation of *gsiA* in nutrient broth medium. In a *kinA* strain, *gsiA* transcription continues to increase during the stationary phase, which presumably increases the abundance of the *gsiA* gene products. A null mutation in *gsiA* reverses the Spo$^-$ defect of a KinA$^-$ mutant; that is, *kinA gsiA* mutant strains are Spo$^+$. Thus, KinA is required for sporulation, but not in the absence of functional GsiA. We suggest, then, that an essential role of KinA is to shut off *gsiA* during the stationary phase and that failure to do so prevents sporulation.

We do not know how KinA causes repression of *gsiA* during sporulation and whether this is a direct or an indirect effect. Expression of *gsiA* is elevated and prolonged in a *spo0A* mutant during sporulation, suggesting that Spo0A or a Spo0A-dependent factor acts to reduce GsiA synthesis. If so, the action of Spo0A on GsiA synthesis is probably

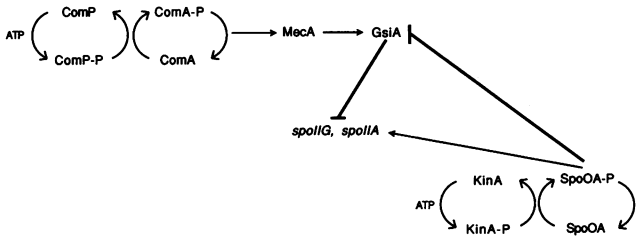

FIG. 6. Proposed pathway for transcriptional regulation of *gsiA* and the possible role of its gene product(s) in the regulation of early sporulation gene expression. This model is based on the data presented in this report and in the accompanying report (17). Lines with arrowheads indicate stimulation; lines with terminal bars indicate inhibition. See the text for a discussion of possible relationships among the gene products.

unrelated to the effect of Spo0A on transcription of *abrB* (41, 42), since prolonged expression of *gsiA* in a *spo0A* mutant was not overcome by a secondary mutation in *abrB*. The finding that certain missense mutations in *spo0A* (i.e., *sof* and *coi* mutations) suppress the sporulation-deficient phenotype of a *kinA* mutant strain suggests that KinA represses transcription from the *gsiA* promoter indirectly, through modulation of Spo0A activity (20, 38). The *gsiA* promoter region has at least one putative Spo0A-binding site between the ComA box and the −35 sequence (17). One possibility is that repression of *gsiA* promoter activity is mediated by a phosphorylated form of Spo0A produced by the activity of KinA (Fig. 6). Alternatively, the role of Spo0A may be to activate σ^H-dependent synthesis of KinA (3, 27a), with subsequent repression of *gsiA* transcription by an unknown KinA-dependent mechanism. It is also possible that KinA activity inhibits the synthesis or activity of a protein (e.g., ComP or ComA) necessary for *gsiA* transcription.

Overexpression of *gsiA* causes reduced expression of the *spoIIA*, *spoIIG*, and *spoIID* operons. This observation is consistent with a postulated negative regulatory role for *gsiA* gene products during sporulation. Thus, shutoff of *gsiA* transcription may normally be required for efficient transcription of the *spoIIA* and *spoIIG* operons and progression through the developmental cycle (Fig. 6). The mechanism by which *gsiA* exerts its control is not known. A *gsiA* gene product(s) could mediate this repression by inhibiting the positive regulatory activity of Spo0A, since transcription of *spoIIA* and *spoIIG* has been shown to be dependent on Spo0A (4, 25, 32, 32a). According to current thinking, phosphorylation of Spo0A must occur for entry into the sporulation pathway (3, 4, 20, 21, 38). One possibility is that GsiA inhibits sporulation by inhibiting a protein kinase or by dephosphorylating Spo0A or one of the proteins required to activate Spo0A. Loss of GsiA in a *kinA* mutant would permit enhanced phosphorylation of Spo0A by kinases other than KinA, including KinB (4, 21, 38). Other interpretations are also consistent with the available results, however.

Some of the characteristics of *gsiA* revealed here are similar to those of the *sin* (8), *hpr* (22), and *spo0E* genes (23) in that the products of all of these genes inhibit sporulation when overproduced. The *sin* and *hpr* genes encode DNA-binding proteins that prevent inappropriate expression of degradative enzymes (8, 11), while the *spo0E* gene product appears to play a negative regulatory role in the sporulation signal transduction cascade (23). It is important to note that a null mutation in *sin* suppresses the sporulation defect of a *kinA* mutant (14, 15). Moreover, overproduction of Sin inhibits transcription of *spoIIA* and *spoIIG* (14). This suggests the possibility that Sin and GsiA are members of the same regulatory pathway.

The transition between exponential growth and the stationary phase may be a period during which alternative responses to nutritional deficiency compete (7, 37). According to this view, sporulation is the final fate of a cell that has exhausted alternate strategies for maintaining balanced growth during a period of nutritional stress. We hypothesize that KinA, Spo0A, Sin, and GsiA function as regulators that direct cells in the stationary phase either to sporulation or to an alternative adaptive response. The relative activities of KinA and GsiA may vary under different nutritional conditions. Successful entry into the sporulation mode would require inhibition of GsiA synthesis or activity. The formidable challenge which remains is to determine how cells sort through the various signals they receive to control the various forms of postexponential gene expression.

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REFERENCES

1. **Amjad, M., J. M. Castro, H. Sandovai, J.-J. Wu, M. Yang, D. J. Henner, and P. J. Piggot.** 1990. An *Sfi*I restriction map of the *Bacillus subtilis* 168 chromosome. Gene **101**:15–21.
2. **Amjad, M., and P. J. Piggot. (Temple University).** 1991. Personal communication.
3. **Antoniewski, C., B. Savelli, and P. Stragier.** 1990. The *spoIIJ* gene, which regulates early developmental steps in *Bacillus subtilis*, belongs to a class of environmentally responsive genes. J. Bacteriol. **172**:86–93.
4. **Burbulys, D., K. A. Trach, and J. A. Hoch.** 1991. Initiation of sporulation in *B. subtilis* is controlled by a multicomponent phosphorelay. Cell **64**:545–552.

4a. **Dower, W. J., J. F. Miller, and C. W. Ragsdale.** 1988. High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res. **16**:6127–6145.

5. **Dubnau, D.** 1991. The regulation of genetic competence in *Bacillus subtilis*. Mol. Microbiol. **5**:11–16.
6. **Dubnau, D.** 1991. Genetic competence in *Bacillus subtilis*. Microbiol. Rev. **55**:395–424.
7. **Fisher, S. H., and A. L. Sonenshein.** 1991. Control of carbon and nitrogen metabolism in *Bacillus subtilis*. Annu. Rev. Microbiol. **45**:107–135.
8. **Gaur, N. K., J. Oppenheim, and I. Smith.** 1991. The *Bacillus subtilis sin* gene, a regulator of alternate developmental processes, codes for a DNA-binding protein. J. Bacteriol. **173**:678–686.
9. **Grossman, A. D.** 1991. Integration of developmental signals and the initiation of sporulation in *B. subtilis*. Cell **65**:5–8.
10. **Jonas, R. M., and W. G. Haldenwang.** 1989. Influence of *spo* mutations on σ^E synthesis in *Bacillus subtilis*. J. Bacteriol. **171**:5226–5228.
11. **Kallio, P. T., J. E. Fagelson, J. A. Hoch, and M. A. Strauch.** 1991. The transition state regulator Hpr of *Bacillus subtilis* is a DNA-binding protein. J. Biol. Chem. **266**:13411–13417.
12. **Kenney, T. J., P. A. Kirchman, and C. P. Moran.** 1988. Gene encoding σ^E is transcribed from a σ^A-like promoter in *Bacillus subtilis*. J. Bacteriol. **173**:3282–3290.
13. **Kenney, T. J., and C. P. Moran.** 1987. Organization and

regulation of an operon that encodes a sporulation-essential sigma factor in *Bacillus subtilis*. J. Bacteriol. **169:**3329–3339.

14. **Louie, P., A. Lee, K. Stansmore, R. Grant, G. Ginther, and T. Leighton.** 1992. Roles of *rpoD*, *spoIIF*, *spoIIJ*, *spoIIN*, and *sin* in regulation of *Bacillus subtilis* stage II sporulation-specific transcription. J. Bacteriol. **174:**3570–3576.
15. **Mandic-Mulec, I., N. Gaur, U. Bai, and I. Smith.** 1992. Sin, a stage-specific repressor of cellular differentiation. J. Bacteriol. **174:**3561–3569.
16. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Mueller, J. P., G. Bukusoglu, and A. L. Sonenshein.** 1992. Transcriptional regulation of *Bacillus subtilis* glucose starvation-inducible genes: control of *gsiA* by the ComP-ComA signal transduction system. J. Bacteriol. **173:**4361–4373.
18. **Mueller, J. P., and A. L. Sonenshein.** Unpublished data.
19. **Nakano, M. M., and P. Zuber.** 1989. Cloning and characterization of *srfB*, a regulatory gene involved in surfactin production and competence in *Bacillus subtilis*. J. Bacteriol. **171:**5347–5353.
20. **Olmedo, G., E. G. Ninfa, J. Stock, and P. Youngman.** Novel mutations that alter the regulation of sporulation in *Bacillus subtilis*. J. Mol. Biol. **215:**359–372.
21. **Perego, M., S. P. Cole, D. Burbulys, K. Trach, and J. A. Hoch.** 1989. Characterization of a protein kinase which phosphorylates the sporulation-regulatory proteins Spo0A and Spo0F of *Bacillus subtilis*. J. Bacteriol. **171:**6187–6196.
22. **Perego, M., and J. A. Hoch.** 1988. Sequence analysis and regulation of the *hpr* locus, a regulatory gene for protease production and sporulation in *Bacillus subtilis*. J. Bacteriol. **170:**2560–2567.
23. **Perego, M., and J. A. Hoch.** 1991. Negative regulation of *Bacillus subtilis* sporulation by the *spo0E* gene product. J. Bacteriol. **173:**2514–2520.
24. **Perego, M., and J. A. Hoch (Scripps Institute).** 1991. Personal communication.
25. **Perego, M., J.-J. Wu, G. B. Spiegelman, and J. A. Hoch.** 1991. Mutational dissociation of the positive and negative regulatory properties of the Spo0A sporulation transcription factor of *Bacillus subtilis*. Gene **100:**207–212.
26. **Piggot, P. J.** 1989. Revised genetic map of *Bacillus subtilis* 168, p. 1–41. *In* I. Smith, R. Slepecky, and P. Setlow (ed.), Regulation of prokaryotic development. American Society for Microbiology, Washington, D.C.
27. **Piggot, P. J., and C. A. M. Curtis.** 1987. Analysis of the regulation of gene expression during *Bacillus subtilis* sporulation by manipulation of the copy number of *spo-lacZ* fusions. J. Bacteriol. **169:**1260–1266.

27a. **Predich, M., G. Nair, and I. Smith.** 1992. *Bacillus subtilis* early sporulation genes *kinA*, *spo0F*, and *spo0A* are transcribed by the RNA polymerase containing σ^H. J. Bacteriol. **174:**2771–2778.

28. **Robertson, J. B., M. Gocht, M. A. Marahiel, and P. Zuber.** 1989. AbrB, a regulator of gene expression in *Bacillus*, interacts with the transcription initiation regions of a sporulation gene and an antibiotic biosynthesis gene. Proc. Natl. Acad. Sci. USA **86:**8457–8461.
29. **Rong, S.** 1989. Ph.D. thesis. Tufts University, Boston, Mass.
30. **Rong, S., M. S. Rosenkrantz, and A. L. Sonenshein.** 1986. Transcriptional control of the *Bacillus subtilis spoIID* gene. J. Bacteriol. **165:**771–779.
31. **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.
32. **Satola, S., J. A. Baldus, and C. P. Moran.** 1992. Binding of Spo0A stimulates *spoIIG* promoter activity in *Bacillus subtilis*. J. Bacteriol. **174:**1448–1453.

32a. **Satola, S., P. A. Kirchman, and C. P. Moran.** 1991. Spo0A binds to a promoter used by σ^A RNA polymerase during sporulation in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **88:**4533–4537.

33. **Schaeffer, P., J. Millet, and J. P. Aubert.** 1965. Catabolite repression of bacterial sporulation. Proc. Natl. Acad. Sci. USA **54:**704–711.
34. **Seki, T., H. Yoshikawa, H. Takahashi, and H. Saito.** 1987. Cloning and sequence of *phoP*, the regulatory gene for alkaline phosphatase and phosphodiesterase in *Bacillus subtilis*. J. Bacteriol. **169:**2913–2916.
35. **Slack, F., J. P. Mueller, M. A. Strauch, C. Mathiopoulos, and A. L. Sonenshein.** 1991. Transcriptional regulation of a *Bacillus subtilis* dipeptide transport operon. Mol. Microbiol. **5:**1915–1925.
36. **Smith, I.** 1989. Initiation of sporulation, p. 185–210. *In* I. Smith, R. Slepecky, and P. Setlow (ed.), Regulation of prokaryotic development. American Society for Microbiology, Washington, D.C.
37. **Sonenshein, A. L.** 1989. Metabolic regulation of sporulation and other stationary phase phenomena, p. 109–130. *In* I. Smith, R. Slepecky, and P. Setlow (ed.), Regulation of prokaryotic development. American Society for Microbiology, Washington, D.C.
38. **Spiegelman, G., B. VanHoy, M. Perego, J. Day, K. Trach, and J. A. Hoch.** 1990. Structural alterations in the *Bacillus subtilis* Spo0A regulatory protein which suppress mutations at several *spo0* loci. J. Bacteriol. **172:**5011–5019.
39. **Stock, J. B., A. J. Ninfa, and A. M. Stock.** 1989. Protein phosphorylation and regulation of adaptive responses in bacteria. Microbiol. Rev. **53:**450–490.
40. **Stragier, P. (Institut de Biologie Physico-Chimique).** 1991. Personal communication.
41. **Strauch, M., V. Webb, G. B. Spiegelman, and J. A. Hoch.** 1990. The Spo0A protein of *Bacillus subtilis* is a repressor of the *abrB* gene. Proc. Natl. Acad. Sci. USA **87:**1801–1805.
42. **Strauch, M. A., G. B. Spiegelman, M. Perego, W. C. Johnson, D. Burbulys, and J. A. Hoch.** 1989. The transition state transcription regulator *abrB* of *Bacillus subtilis* is a DNA binding protein. EMBO J. **8:**1615–1621.
43. **Weinrauch, Y., N. Guillen, and D. Dubnau.** 1989. Sequence and transcription mapping of *Bacillus subtilis* competence genes *comA* and *comB*, one of which is related to a family of bacterial regulatory determinants. J. Bacteriol. **171:**5362–5375.
44. **Weinrauch, Y., R. Penchev, J. Dubnau, I. Smith, and D. Dubnau.** 1990. A *Bacillus subtilis* regulatory gene product for genetic competence and sporulation resembles sensor protein members of the bacterial two-component signal-transduction systems. Genes Dev. **4:**860–872.
45. **Young, M.** 1975. Genetic mapping of sporulation operons in *Bacillus subtilis* using a thermosensitive sporulation mutant. J. Bacteriol. **122:**1109–1116.
46. **Zuber, P., and R. Losick.** 1987. Role of AbrB in Spo0A- and Spo0B-dependent utilization of a sporulation promoter in *Bacillus subtilis*. J. Bacteriol. **169:**2223–2230.