Regulation of Transcription of the Cell Division Gene *ftsA* during Sporulation of *Bacillus subtilis*

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Three distinct 5' ends of *ftsA* mRNA were identified by S1 mapping and by primer extension analysis. These are thought to represent three transcription start sites. The transcripts from the downstream and upstream sites were detected throughout growth. The transcript from the middle site was not detected during exponential growth but was detected within 30 min of the start of sporulation, when it was the predominant transcript. Insertion of a *cat* cassette in the middle promoter, *ftsAp*₂ (p2), did not affect vegetative growth but prevented postexponential symmetrical division and spore formation. Transcription from p2 was dependent on RNA polymerase containing σ^H , and promoter p2 resembled the consensus σ^H promoter. Transcription from p2 did not require expression of the *spo0A*, *spo0B*, *spo0E*, *spo0F*, or *spo0K* loci. Northern (RNA) blot analysis indicated that *ftsA* is cotranscribed with the adjacent *ftsZ* gene. Multiple promoters provide a mechanism by which essential vegetative genes can be subjected to sporulation control independent of control during vegetative growth. In the case of *ftsA*, *Z*, the promoters provide a mechanism to permit septum formation in conditions of nutrient depletion that might be expected to shut down the vegetative division machinery.

The formation of spores by *Bacillus subtilis* is a primitive system of cellular differentiation (25). It is initiated by nutrient depletion, and there has been intense interest in how this leads to the carefully orchestrated program of gene expression during spore formation. As a consequence, there is now compelling evidence that transcription is activated at the start of sporulation by a phosphorelay system (11). There is also extensive evidence that either depletion of GTP (and/or GDP) or alteration of some metabolite closely associated with GTP may be a critical signal that initiates spore formation (18). Despite these and other advances, the determinants of the early morphometric events during spore formation are poorly understood. For example, little is known about the generation of cellular asymmetry, which is a crucial process in differentiation (31).

The formation of an asymmetrically sited division septum is an early event of sporulation. The asymmetric division results in two distinct cells, the mother cell and the prespore, which have radically different developmental fates. This "sporulation" division (defined as stage II of sporulation) contrasts with division during vegetative growth of *B. subtilis*, in which the septum is symmetrically situated with respect to the ends of the dividing cell. In addition to the asymmetric division during sporulation, there is likely to be a symmetrical division after sporulation has been triggered in bacteria that were growing in rich medium, where chromosome replication was dichotomous (24, 31). This final symmetrical division would facilitate segregation of a single completed chromosome into the prespore. The postexponential symmetrical and asymmetrical divisions occur in starvation conditions that might be expected to be inimical to the normal vegetative division process. However, it would seem reasonable that much of the machinery required to

form division septa during spore formation would be the same as that required for septum formation during vegetative growth. It would seem likely that there are, in addition, controls that are unique to spore formation and that are likely to be critical to spore formation. We explore here the regulation of transcription of genes associated with septum formation.

Several genes thought to be involved in the formation of the vegetative septum (27) in *B. subtilis* have been identified, but their role in spore formation has not been extensively studied. The *B. subtilis* homologs of the *Escherichia coli* cell division genes *ftsA* and *ftsZ* have been cloned and sequenced (5). As in *E. coli*, the genes are adjacent to each other. They appear to have roles in vegetative cell division similar to those of their *E. coli* counterparts. Expression of the *B. subtilis* *ftsA* or *ftsZ* gene in *E. coli* leads to filamentation and cell death (5). The *B. subtilis* mutation *ts-1*, which causes temperature-sensitive filament formation during vegetative growth, has been shown to map to *ftsZ* (21). Beall and Lutkenhaus (7) have recently shown that in *B. subtilis*, *ftsZ* has a role in formation of the sporulation septum as well as of the vegetative septum. As a mutation causing temperature-sensitive spore formation, *spoIIN279*, maps in *ftsA* (Leighton; quoted in reference 6), it seems likely that *ftsA* is also involved in spore septum formation as well as in formation of the vegetative septum. We address here the question of how the expression of *ftsA* and *ftsZ* might be controlled during spore formation. We show that *ftsA* and *ftsZ* are transcribed from a distinct promoter during sporulation.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* strain used was DH5 α [F[−] *endA1 hsdR17* (r_K^- m_K^+) *supE44 thi-1 recA1 gyrA96 relA1* Δ (*lacZYA-argF*)*U169* ϕ 80*dlacZ* Δ M15] except where otherwise indicated. *E. coli* MM294(pMI1101 Δ P), kindly provided by P. Youngman (University of Georgia), was the source of

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TABLE 1. *B. subtilis* strains used

Strain ^a	Relevant characteristics	Origin or reference
JH642	<i>trpC2 phe-1</i>	J. A. Hoch
JH646	<i>trpC2 phe-1 spo0A12</i>	J. A. Hoch
JH646MS	<i>trpC2 phe-1 spo0A12 abrB15</i>	J. A. Hoch
JH647	<i>spo0E11 trpC2 phe-1</i>	J. A. Hoch
JH651	<i>trpC2 phe-1 spo0H81</i>	J. A. Hoch
MB24	<i>trpC2 metC3 rif-2</i>	41
SL513	<i>spo0H17 trpC2 rif-2</i>	41
SL741	<i>spo0K141 trpC2</i>	14
SL964	<i>spo0B136 metC3 tal-1</i>	41
SL965	<i>spo0F221 metC3 tal-1</i>	41
SL4133	<i>spo0H17 trpC2 rif-2 SPβftsAp₂-lacZ^b</i>	This study
SL4134	<i>trpC2 metC3 rif-2 SBβftsAp₂-lacZ^b</i>	This study
SL4156	<i>trpC2 metC3 rif-2 ftsAp₂::cat(+)^c</i>	This study
SL4512	<i>trpC2 metC3 rif-2 ftsAp₂::cat(-)^c</i>	This study
ZB307	SPβ <i>c2del2::Tn917::pSK10del6</i>	46

^a The JH series and the MB24 and SL series represent distinct sets of isogenic strains.

^b Derivatives of SPβ *c2del2::Tn917* (46); see text.

^c +, *cat* in same orientation as *ftsA*; -, *cat* in opposite orientation.

the *cat* (chloramphenicol acetyltransferase) cassette. The *B. subtilis* 168 strains used are listed in Table 1.

Plasmids. All plasmids were maintained in *E. coli* DH5α unless otherwise stated. The structure of all plasmids was confirmed by analysis of appropriate restriction endonuclease digests. Plasmid pPP215 was prepared by ligating a 1.64-kbp *HindIII-EcoRV* fragment from the Charon 4A clone 7.3.2 (5, 32) into pUC18 that had been digested with *HindIII* and *SmaI*. It contained the 5' end of the *B. subtilis* *ftsA* gene and the region extending approximately 900 bp upstream from the *ftsA* open reading frame (Fig. 1) (6).

Plasmid pPP220 contained a 554-bp *PvuII-Sau3A* fragment from pPP215 cloned into pUC18 that had been digested with *BamHI* and *SmaI* (Fig. 1).

Plasmid pPP221 was derived from pPP220 by exonuclease III digestion. pPP220 was digested with *PstI* and *SalI*, which cut in polylinker sites flanking the inserted *B. subtilis* DNA. The double-digested plasmid was treated with phenol, precipitated with ethanol, washed, dissolved in 10 mM Tris-HCl-1 mM EDTA, pH 8.0, and digested with exonuclease III at 4°C. Samples were taken at intervals and treated with S1 nuclease. The ends were filled in with the Klenow fragment of DNA polymerase, and the resulting DNA fragments were ligated. Ligated plasmids were transformed into *E. coli*, selecting for resistance to ampicillin. Among the transformants, one contained a 495-bp *B. subtilis* insert that contained the presumed promoters *ftsAp₂* (p2) and p3 but not the promoter p1. The structure of the insert was confirmed by DNA sequencing, and the plasmid was designated pPP221. Plasmid pPP222 was constructed by removing the insert in pPP221 by digestion in flanking sites with *HindIII* and *EcoRI* and ligating it to the promoterless *lacZ* transcriptional fusion plasmid pGV34 (45) previously cut with the same enzymes.

Plasmids pPP223 and pPP224 were constructed by ligating a partial *XmnI* digest of pPP215 to the *SmaI* fragment from pMI1101ΔP that contained the *cat* cassette. Restriction analysis established that they contained two copies of the *cat* cassette inserted in tandem into the *XmnI* site in *ftsAp₂*. The *cat* inserts in pPP223 were in opposite orientation to those in pPP224.

Plasmid pPP306 was constructed by ligating a 1.6-kb *EcoRI-HindIII* fragment of pPP215 into pDH32 (ptrpBGI [37]) that had been digested with *BamHI*; in each case, the

FIG. 1. Restriction map of the *ftsA* region of the *B. subtilis* chromosome. The top shows the approximate locations of open reading frames in the vicinity of *ftsA* (5, 6, 38, 43). The lower part shows the regions cloned in plasmids used in the study. The regions used to probe Northern blots are indicated as A and B. P3, P2, and P1 indicate the transcription start points for *ftsA* (see text). Restriction sites: H, *HindIII*; Hc, *HincII*; P, *PstI*; Pv, *PvuII*; S, *Sau3A*; V, *EcoRV*; X, *XmnI*. Not all *Sau3A* sites are indicated. CAT indicates the insertion of a *cat* cassette (see text).

ends were filled in with DNA polymerase I Klenow fragment.

Plasmid pPP308 was constructed from pPP295. Plasmid pPP295 was a derivative of plasmid pPP221 obtained by exonuclease III digestion from the *AccI* site at nucleotide 282 (numbering of Beall et al. [5]) so that it contained nucleotides 308 to 495 of the *ftsA* upstream region (confirmed by DNA sequencing). This region, as part of a *HindIII* (filled in)-*EcoRI* fragment, was ligated into pDH32 previously digested with *Bam*HI (filled in) and *EcoRI* in order to construct pPP308.

Plasmid pPP319 was constructed by ligating a 543-kb *Mbo*II fragment from a derivative of pPP215 into pDH32 that had been digested with *Bam*HI and then treated with Klenow fragment.

Construction of a bacteriophage SP β derivative containing an *ftsAp₂-lacZ* fusion. Plasmid pPP222 was linearized with *Bal*I and used to transform *B. subtilis* ZB307, which contains the heat-inducible prophage SP β C2del2::Tn917::pSK10del6 (46). Transformants showing chloramphenicol resistance (Cm^r) were selected, in which the *ftsAp₂-lacZ* transcriptional fusion had integrated into the prophage. Isolated transformant clones were checked for the transposon-determined erythromycin resistance (Em^r). A transducing lysate was prepared by heat induction of a culture that had been grown from an isolated clone (46). Transductants of target strains were obtained by selection for Cm^r and Em^r.

DNA sequencing. DNA sequencing was done by the dideoxy chain termination method of Sanger et al. (35). A Sequense kit was used according to the protocol of the manufacturer (United States Biochemical Corp., Cleveland, Ohio).

Three oligonucleotide primers designed to be complementary to *ftsA* mRNA were synthesized with an Applied Biosystems model 380B DNA synthesizer and kindly provided to us by J. K. de Riel (Temple University). The first primer, 5'-CATTCTCCGACGATCAC-3', corresponded to bases 652 to 635 in the numbering of Beall et al. (5), 99 to 82 bp downstream from the deduced vegetative transcription start site p1. The second primer, 5'-GAGCCTTTT TCAACCC-3', corresponded to nucleotides 717 to 701 (5). The third primer, 5'-GTGGCTTTACAAGTGTG-3', corresponded to bases 513 to 497 (5).

RNA analysis. RNA was prepared as described previously (42). Primer extension analysis was done essentially as described by Wu et al. (42). Electrophoresis of RNA in agarose-formaldehyde gels and Northern (RNA blot) hybridizations were performed as described by Maniatis et al. (26). S1 mapping was performed essentially as described by Ausubel et al. (4); mRNA was hybridized with a reverse transcript of the promoter region that had been generated in vitro by using a synthetic oligonucleotide primer.

Sporulation. Bacteria were induced to sporulate in modified Schaeffer's sporulation medium (MSSM) essentially as described previously (34). *B. subtilis* strains are prone to grow as chains in rich medium, particularly during early exponential growth. To reduce chain formation, cultures in late exponential growth were diluted 10-fold in warm MSSM and reincubated. This procedure was repeated two to three times before the cultures were permitted to grow beyond the exponential phase and so, by definition, to start sporulating. The procedure did not affect the growth rate but was effective in reducing chain formation. The start of sporulation was defined as the end of exponential growth. In experiments involving Spo⁻ mutants, control cultures of Spo⁺ strains were included to ensure that the particular

FIG. 2. Determination of the 5' end of *ftsA* mRNA by primer extension analysis. Primer extension analysis was carried out on mRNA preparations as described in Materials and Methods. RNA was extracted from MB24 during vegetative growth (sample a), and at intervals during sporulation (0.5, 1.5, 2.5, 3.5, and 4.5 h after the start of sporulation; samples b, c, d, e, and f, respectively). Each sample contained 50 ng of RNA. A sequencing ladder with the same primer is also shown. The letters above the lanes indicate which dideoxynucleotide was used to terminate the sequencing reaction. The sequence indicated is that of the nontranscribed strand and is the complement of the sequence that can be read from the sequencing ladder. The 5' ends of the mRNA are indicated with arrows; the 5' end corresponding to p1 was apparent as a faint band on the original autoradiograph but is difficult to see here.

batch of medium used permitted good (i.e., greater than 60% of all organisms are phase-bright spores) sporulation. Growth was followed by measuring the A_{600} and converting this to milligrams of bacteria (dry weight) per milliliter with a standard calibration curve.

Microscopy. Cultures were fixed in 1% Formalin, washed in water, and attached to polylysine-coated coverslips. They were visualized and photographed by differential interference contrast microscopy.

All other methods have been described previously (33, 42).

RESULTS

Analysis of the *ftsA* transcript. The 5' end of the mRNA transcribed from *ftsA* was investigated by primer extension analysis. RNA was extracted from exponentially growing strain MB24 and from MB24 at hourly intervals during spore formation. Reverse transcripts were obtained with an 18-mer primer designed to hybridize to *ftsA* mRNA and corresponding to bases 635 to 652 of the published sequence (5). The reverse transcripts were characterized by electrophoresis with the products of a dideoxy sequencing reaction obtained with the same primer and pPP215 (Fig. 2). A faint band corresponding to nucleotide 554 of Beall et al. (5) was discernible in all samples on the original autoradiograph (promoter designated p1). A strong band corresponding to nucleotide 444 (Fig. 2; band identification based on analysis of a series of gels; promoter p2) was present in samples from sporulating cultures but not in samples from vegetative

250
 TAAGTTTAGCTTTTCTGGGAGTCCATCTTGGTGTAGACTTGTATTAGCAGGTATATTCG
 310
 CATTTGGAGTCAGATTATTTTCAGAAATATAGCCGTTATCAGAAGAAATCTACTAACAAAGT
 370
 GGACTCTTCTAAAAAATAAAAAATGTGATATAAAAGGATATACATAGGATATA
 430
 ACGAATATTTTCAATAAACATAAAATGTGAAAAGCACATAAAATATTCTGTTGTTATTT
 XmnI
 490
 TTTGTTACACACTTGTAAAGCCACATTCACTTGTGTTGTTCCGCAAATAATAGAATAG
 550
 AAATGATCGAAATGTGAGGAGGTGCCATAGAAATGAACAACAACTTACGTCAG
 Sau3A RBS MetAsnAsnAsnGlu....

FIG. 3. DNA sequence of the nontranscribed strand of the *ftsA* promoter region. The sequence is numbered according to Beall et al. (5). The deduced transcription start points are indicated by asterisks. The likely -10 and -35 regions of p_1 , p_2 , and p_3 are underlined. The putative ribosome-binding site (RBS) for *ftsA* and the putative N-terminal sequence of the FtsA protein are shown (5). The *XmnI* and *Sau3A* sites used in the study are indicated under the appropriate sequences. The 3' end of the promoter region in plasmids pPP221, pPP222, and pPP308 is indicated with an arrowhead.

cultures. The same two transcript endpoints were obtained by using a 17-mer primer corresponding to nucleotides 701 to 717 of the published sequence; again, the downstream band was very faint and the upstream band was only present in extracts from sporulating cultures (data not shown). The same results were obtained with several sets of RNA preparations. The positions of the endpoints relative to the *ftsA* coding sequence are indicated in Fig. 3.

With a third primer corresponding to nucleotides 497 to 513, a faint band suggestive of a third transcript end at about nucleotide 296 of Beall et al. (5) was detected. To examine this further, the 5' ends of *ftsA* mRNA were also determined by S1 mapping. The DNA fragment to be protected from S1 nuclease digestion by the mRNA was generated by reverse transcription of denatured pPP215 DNA, using the oligonucleotide primer corresponding to bases 635 to 652; following reverse transcription, the newly synthesized DNA was cleaved by *PvuII* (nucleotide 0 of Beall et al. [5]), generating a 652-nucleotide fragment for hybridization to mRNA. The DNA protected from S1 digestion was fractionated by electrophoresis, and its mobility was compared with that of a sequencing ladder obtained with the same oligonucleotide primer. A band is clearly visible at about nucleotide 296 (Fig. 4). It is most abundant in extracts from exponentially growing bacteria. The band at nucleotide 444 was again predominant in post-exponential-phase samples. A band corresponding to p_1 is just detectable at the bottom of the gel (Fig. 4).

It is thought likely that these 5' ends indicate transcription start points, although we have not excluded the possibility of RNA processing. With this reservation, it is tentatively concluded that there are vegetative transcription start points at about nucleotides 554 and 296 and a sporulation-associated start point at 444 (Fig. 3). The corresponding promoters are designated p_1 (position 554), p_2 (position 444), and p_3

FIG. 4. Determination of the 5' end of *ftsA* mRNA by S1 mapping. S1 mapping was carried out as described in Materials and Methods. RNA was extracted from MB24 during vegetative growth (sample a) and at intervals during sporulation (0, 0.5, 1, and 2 h after the start of sporulation; samples b, c, d, and e, respectively). Each sample contained 50 ng of RNA. A sequencing ladder is shown that was obtained with the same primer as was used to synthesize the DNA for RNA hybridization and S1 protection. The letters above the lanes indicate which dideoxynucleotide was used to terminate the sequencing reaction. The sequences indicated are of the nontranscribed strand and are the complement of the sequences read from the sequencing ladder. The 5' ends of the mRNA are indicated with arrows.

(position 296). The vegetative transcripts were detectable at reduced levels in sporulating cultures of the parent strain. Therefore, it is possible that they were still being synthesized in sporulating bacteria after sporulation-specific transcription had started. However, it is also possible that the vegetative transcripts were only present in those cells in the cultures that were not sporulating.

Northern blot analysis was used to estimate the size of the *ftsA* transcript. RNA samples were fractionated by electrophoresis in agarose containing formaldehyde and transferred to a nylon membrane. The blot shown (Fig. 5) was probed with an 870-bp *EcoRI-XmnI* fragment from pPP215 that includes most of *ftsA* (probe A, Fig. 1). A band of approximately 2.5 kb was detected in all samples from both vegetative and sporulating bacteria. The size of the band is that of a transcript containing both *ftsZ* and *ftsA* (5). The band was also detected by using a probe (probe B, Fig. 1) for the 5' end of *ftsZ* (data not shown). This would indicate that *ftsZ* as well as *ftsA* is transcribed from the promoters immediately upstream of *ftsA*; Beall and Lutkenhaus (7) have reached the same conclusion through studies with integrative plasmids. A second RNA band was present to variable extents in some extracts of both vegetative and sporulating bacteria. Its size was substantially less than the 1.5-kb (16S mRNA) marker, so that it could not code for the entirety of both *ftsA* and *ftsZ*. Its role is unclear; it is thought to be a breakdown product of the 2.5-kb transcript, but it was not investigated further.

FIG. 5. Northern blot analysis of *ftsA* mRNA. RNA was extracted from MB24 during vegetative growth (sample A) and at intervals during sporulation (0.5, 1.5, 2.5, and 3.5 h after the start of sporulation; samples B, C, D, and E, respectively). The probe used was probe A of Fig. 1. Arrows indicate the positions of 23S and 16S RNA on the same gel.

Fusion of *ftsA* promoters to *lacZ*. To analyze further the sporulation-associated induction of transcription of *ftsA*, a series of transcriptional *lacZ* fusions were constructed (pPPP306 [p1, p2, and p3], pPPP319 [p1 and p2], and pPPP308 [p2]) and inserted into the *amyE* locus by the back-to-front *amy* system of Shimotsu and Henner (37); for each insert, integration by double crossover was confirmed by Southern blots. Transcriptional fusions to *lacZ* of the various promoter combinations and p2 alone all showed the same induction of postexponential expression (Fig. 6). This confirmed the findings by primer extension analysis and by S1 mapping that there is a burst of transcription from p2 beginning within 30 min of the start of sporulation. The presence of p1 or of p1 and p3 did not substantially affect the extent of postexponential expression. The postexponential burst of expression from p2 was not observed in strains that have mutations, *spo0H81* or *spo0H17*, in the structural gene for σ^H (Fig. 6). A low level of *spo0H*-dependent expression from the p2-*lacZ* fusion detected during exponential growth was not observed by primer extension analysis. Neither this difference nor the differences between fusions in the low levels of vegetative expression were investigated further.

The postexponential burst of *ftsA* transcription was also observed with the SP β system of Zuber and Losick (46), using an *ftsAp₂-lacZ* fusion in strain SL4134 (Fig. 7). The burst of expression was not observed in a strain (SL4133) harboring a *spo0H17* mutation (Fig. 7). To check that the *spo0H17* effect was not a consequence of a mutation of the phage upon lysogeny, phage was induced from SL4133 and used to lysogenize the *spo*⁺ strain MB24. The resulting strain gave the same pattern of β -galactosidase induction as did SL4134 (Fig. 7). It should be noted that the absolute level of β -galactosidase obtained for the fusion in SP β was substantially higher than for fusions in *amy* (Fig. 6); this may represent a position effect, as has been described previously (40). The pattern of postexponential induction was similar for fusions at SP β and at *amy*.

No other *spo0* mutation tested (*spo0A12*, *spo0B136*,

FIG. 6. Formation of β -galactosidase by strains containing *ftsA-lacZ* transcriptional fusions inserted into the *amy* locus (37). Specific β -galactosidase activity is expressed as nanomoles of *o*-nitrophenyl- β -D-galactopyranoside hydrolyzed per minute per milligram of bacteria (dry weight). The time is the time after the start of sporulation. The plasmids used for insertion at the *amy* locus were pPPP306 (p1p2p3), pPPP308 (p2), and pPPP319 (p1p2) (see Fig. 1). Symbols: $\circ$, JH642 *amyE::ftsAp₁p₂p₃-lacZ*; $\triangle$, JH642 *amyE::ftsAp₂-lacZ*; $\blacktriangle$, JH642 *amyE::ftsAp₁p₂-lacZ*; $\diamond$, JH642; $\triangle$, JH651 (*spo0H81*) *amyE::ftsAp₂-lacZ*; $\bullet$, SL513 (*spo0H17*) *amyE::ftsAp₂-lacZ*.

spo0E11, *spo0F221*, or *spo0K141*) prevented the postexponential induction of *ftsA* transcription. Results for *spo0A12*, as assayed with an *ftsAp₂-lacZ* fusion in *amyE*, are illustrated in Fig. 8 for strain JH646. The pattern was not significantly affected by the presence of an *abrB* mutation (Fig. 8). Strain JH646 *amyE::ftsAp₂-lacZ* was checked by colony morphology, by competence, and by protease production to confirm that it had not inadvertently acquired an *abrB* mutation. In contrast, the *spo0H81* mutation largely prevented the postexponential induction of β -galactosidase from *ftsAp₂-lacZ* (Fig. 8); the basal level in the *spo0H* mutant was higher than for p2 alone (Fig. 6), presumably indicating some *spo0H*-independent postexponential expression from p1. Postexponential expression from p1 (or p3) was not apparent when p2, p1p2, and p1p2p3 fusions in a *spo*⁺ strain were compared (Fig. 6). Studies of p1 (and p3) separated from the other promoters are required to clarify their regulation.

Disruption of the promoter *ftsAp₂*. The -10 region of *ftsAp₂* contains an *Xmn*I site (GAANNNTTC; Fig. 3). This site was used to insert a *cat* cassette, so as to disrupt p2 (and also transcription from p3). To achieve this, a 1.4-kb *cat* cassette from *Sma*I-digested pMI1101 Δ P was ligated to pPP215 that had been partially digested with *Xmn*I. From the

FIG. 7. Formation of β -galactosidase by *spo*⁺ and *spo0H* strains containing an *ftsAp₂-lacZ* transcriptional fusion in phage SP β . Specific β -galactosidase activity is expressed as nanomoles of *o*-nitrophenyl- β -D-galactopyranoside hydrolyzed per minute per milligram of bacteria (dry weight). The time is the time after the start of sporulation. Symbols: $\circ$, SL4134 (*Spo*⁺); $\triangle$, SL4133 (*spo0H17*); $\bullet$, MB24; $\bullet$, MB24 lysogenized with phage from SL4133.

ligation, pPP223 and pPP224 were isolated and shown to contain two copies of the *cat* cassette inserted in tandem into the appropriate *Xmn*I site. In pPP223, the *cat* genes were transcribed in the opposite direction from *ftsA*; in pPP224, they were transcribed in the same direction as *ftsA*. Plasmids pPP223 and pPP224 were linearized with *Sst*I and used to transform strain MB24 to chloramphenicol resistance. Two particular transformant clones, SL4156 from pPP224 and SL4512 from pPP223, were chosen for further study. Analysis of appropriately restricted DNA by Southern hybridization indicated that these clones contained tandem copies of the *cat* cassette inserted into *ftsAp₂* by the expected double-cross-over event.

Strains SL4512, SL4156, and MB24 had very similar growth rates, with doubling times of about 30 min in MSSM. In different experiments, SL4512 gave no detectable spores (less than 10^2 per ml). Strain SL4156 gave 10^4 to 10^5 spores per ml, and MB24 gave 3×10^8 spores per ml. Strain SL513,

FIG. 8. β -Galactosidase formation by *spo0* mutants containing an *ftsAp₂-lacZ* fusion inserted into the *amy* locus (37). Specific β -galactosidase activity is expressed as nanomoles of *o*-nitrophenyl- β -D-galactopyranoside hydrolyzed per minute per milligram of bacteria (dry weight). The time is the time after the start of sporulation. The plasmid used for insertion at the *amy* locus was pPP319 (see Fig. 1). Symbols: $\circ$, JH642 (*spo*⁺) *amyE::ftsAp₁p₂-lacZ*; $\triangle$, JH646 (*spo0A12*) *amyE::ftsAp₁p₂-lacZ*; $\blacktriangle$, JH646MS (*spo0A12 abrB15*) *amyE::ftsAp₁p₂-lacZ*; $\bullet$, JH651 (*spo0H81*) *amyE::ftsAp₁p₂-lacZ*.

a *spo0H17* derivative of MB24, also had the same growth rate and formed no detectable spores. The transition from growth in a rich medium to sporulation involves reduction in cell length, as illustrated for the *Spo*⁺ strain MB24 (Fig. 9a, exponential growth in MSSM; Fig. 9b, 2.5 h after the end of exponential growth in MSSM). This is presumably a consequence of a symmetrical division(s) that reduces cell length. Strains with transcription from *ftsAp₂* and *ftsAp₃* disrupted appear similar to MB24 during exponential growth (illustrated with SL4156, Fig. 9c); however, by 2.5 h after the end of exponential growth, the smallest cells were twice the length of those obtained with MB24 (SL4156, Fig. 9d; MB24, Fig. 9b). No further divisions were apparent in samples taken 1.5 h later. Strain SL513 (*spo0H17*) appeared similar to MB24 during vegetative growth (Fig. 9e) and also 2.5 h after the start of sporulation (Fig. 9f).

DISCUSSION

Three 5' ends for *ftsA* mRNA were detected by S1 mapping and primer extension analysis. We consider that these represent transcription start points, although the possibilities of 5' processing and degradation have not been ruled out. The downstream and upstream transcripts were faint and were present throughout growth; the likely promot-

FIG. 9. Effects of disruption of *ftsA*₂ on cell morphology. Bacteria were fixed in 5% Formalin and attached to polylysine-coated coverslips. They were viewed and photographed by differential interference contrast microscopy. Samples were taken 30 min before (a, c, and e) and 150 min after (b, d, and f) the end of exponential growth in MSSM. (a and b) MB24; (c and d) SL4156; (e and f) SL513. Bars, 5 μ m.

ers (p1 and p3) have potential -35 and -10 regions that resemble promoters recognized by RNA polymerase holoenzyme containing the major vegetative factor σ^A (5, 23) (Fig. 3). The middle transcript was not detected during vegetative growth but appeared within about 30 min of the start of sporulation (defined by the end of exponential growth), when it was the predominant transcript (Fig. 2 and 4). The sporulation-associated induction of expression from this promoter (p2) was also observed by measuring β -galactosidase activity in a strain that contained p2 fused to *lacZ* (Fig. 6). Gonzy-Tréboul et al. (20) and Stragier and Losick (39) have independently found sporulation-associated transcription of *ftsA* by testing various *ftsA::lacZ* transcriptional fusions and have identified the same three transcription start points.

The -35 and -10 regions of the promoter *ftsAp₂* resemble those of promoters recognized by RNA polymerase holoenzyme containing σ^H (41). σ^H is the product of the *spo0H* locus (17), and mutations in *spo0H* prevented transcription from p2, as detected by *lacZ* transcriptional fusions (and by primer extension analysis; data not shown). Furthermore, M. Amjad (3) has found that induction of *spo0H* from the *spac* promoter activates transcription from *ftsAp₂*. We consider the circumstantial evidence strong that $E\text{-}\sigma^H$ transcribes *ftsA* from p2 in vivo, although we have not ruled out the possibility that the role of σ^H is indirect. σ^H is present and is transcriptionally active during vegetative growth (41). There is a substantial increase in σ^H activity at the end of exponential growth (22). This increase could explain the timing of induction from *ftsAp₂*. However, we consider it likely that there is one or more additional control for induction from *ftsAp₂* at or soon after the start of sporulation, as transcription from *ftsAp₂* was not activated during competence development (36), when σ^H is known to be active (1). Expression from *ftsAp₂* was not prevented by mutations in the *spo0A*, *spo0B*, *spo0E*, *spo0F*, or *spo0K* loci. Thus, the transcription determinant is distinct from other known sporulation controls.

The nature of the additional factor(s) required for transcription from *ftsAp₂* remains to be established. Presumably this factor relays information about sporulation-inducing conditions so as to trigger division under conditions of nutrient depletion that shut down the normal vegetative septum formation machinery. An attractive possibility is that it could relay the information by modifying the mechanism(s) that ordinarily couples cell division to vegetative growth rate. Several such mechanisms have been suggested. Any one could potentially be modified in some way so as to allow the sporulation division(s). Four possibilities might be considered. (i) ppGpp has been thought to be a possible mediator of growth rate control and is closely related metabolically to GTP. However, Ochi et al. (29) have shown that a *B. subtilis* mutant unable to make ppGpp in response to nutrient starvation sporulated efficiently, provided there was a fall in the GTP/GDP pool. Moreover, it has recently been shown that an *E. coli* mutant lacking ppGpp shows normal growth rate control (19), so that, at least in *E. coli*, ppGpp cannot be the (sole?) mediator of growth rate control. (ii) The *lov* gene of *E. coli* is a plausible link between ribosome biosynthesis (and hence growth rate) and cell division (10, 15); a homolog in *B. subtilis* could play the same role and also mediate the sporulation divisions. (iii) Termination of chromosome replication must take place in a sporulation medium for sporulation to take place (30). Termination is clearly necessary for subsequent division, and the sporulation medium requirement makes termination an attractive candidate for a causal

role here. A similar causal role for chromosome termination in vegetative division has long been considered but has yet to be established (8, 28). (iv) In *E. coli*, *ftsA* and *ftsZ* are in a gene cluster which shows a complex pattern of gene regulation when analyzed with transcriptional *lacZ* fusions (44). Aldea et al. (2) have recently identified a series of promoters in the region. Most interestingly, one of these appears to belong to a class of promoters for *E. coli* morphogenes that they name "gearbox." These promoters are sensitive to growth rate, so that expression from them increases as growth rate decreases. In this respect, they resemble the *B. subtilis* *ftsAp₂* promoter. However, *ftsAp₂* shows no homology in the -10 and -35 regions to the *E. coli* gearbox promoters. At present, our conclusion must be cautious: some mechanism may link division to sporulation via *ftsAp₂* transcription; this may or may not be related to the mechanism linking vegetative growth rate to vegetative division.

Insertion of the *cat* cassette into the -10 region of *ftsAp₂* had no effect on vegetative growth but blocked spore formation. Promoter p1 (but not p3) was still functioning in strains with p2 inactivated and is thought to be essential for cell viability, at least when p2 and p3 are inactive, although this was not tested directly. We consider it unlikely that the effect of the *cat* insert in *ftsAp₂* results from altered transcription of a gene downstream from *ftsZ*, as (i) Northern blot analysis indicated that no transcript that could extend from *ftsAp₂* (or p1) to downstream of *ftsZ*, (ii) the gene immediately downstream of *ftsZ* is *bpf* (43) and disruption of *bpf* has no effect on spore formation (38), and (iii) there is a plausible transcription terminator between *ftsZ* and *bpf* (43). We consider that transcription of *ftsA* and/or *ftsZ* from p2 (and/or p3) is necessary for a final symmetrical division and for spore formation; we presume that it is also necessary for the asymmetric division. We think that the effect on spore formation is a consequence of the effect on division, but a causal relationship has not been established. The low level of sporulation that is obtained with strain SL4156 is presumed to be a consequence of readthrough from the promoter in the *cat* cassette, which somehow permits a small portion of cells to form spores; no sporulation was detected when the *cat* cassette was inserted in the opposite orientation in strain SL4512.

Disruption of the sporulation promoter *ftsAp₂* by insertion of the *cat* cassette prevented a final symmetrical division (Fig. 9). The division occurs after the time (the end of exponential growth) ordinarily defined as the start of sporulation. This would suggest that a final symmetrical division is, or can be, an early stage of spore formation. Such a stage has been suggested previously (24, 31), although it is not ordinarily considered a stage of spore formation. At least one such division seems necessary if bacteria growing in a rich medium, in which DNA replication is dichotomous, are induced to form spores (24, 31) which contain single complete copies of the chromosome (12). In this context, it is very interesting that Dawes et al. (16) had previously deduced that certain sporulation signals occur prior to the final symmetrical division. They had observed a highly significant correlation in the stages of sporulation which sister cells had reached in chemostat populations that were heterogeneous with respect to the stages of sporulation observed.

The morphology of strain SL513, in which σ^H is inactive, is the same as that of the parent strain MB24 2.5 h after the start of sporulation (Fig. 9). However, the morphology of strains in which the *ftsAp₂* promoter, which is apparently utilized by σ^H , is disrupted differs from that of SL513 and MB24. We are unsure of the reason for the difference

between the morphology of SL513 and that of strains with p2 disrupted. One possible explanation is that the insert also disrupts expression from p3 and that expression from p3, or p2 plus p3, rather than p2 alone is required for the final symmetrical division; this possibility remains to be tested. A second possibility is that a balance is required between FtsA and FtsZ and some other moiety that is also dependent, directly or indirectly, on σ^H . A third possibility is that loss of σ^H but not of *ftsAp₂/p₃* transcription is compensated for in some way so as to increase *ftsA/Z* transcription from p1. This last possibility is consistent with the observation that postexponential expression from p1 is apparent in a *spo0H* mutant (Fig. 8), although not in a *spo⁺* strain (compare Fig. 6, p2-*lacZ* and p1p2-*lacZ*).

Transcription of *ftsA/Z* appeared to be substantially increased at the start of sporulation, as judged by S1 mapping, primer extension analysis, and transcriptional *lacZ* fusions. This might suggest that production of FtsA and FtsZ proteins is increased at the start of sporulation. In *E. coli*, mild overproduction of FtsZ yields organisms with asymmetrically sited septa (9). Thus, in *B. subtilis*, overproduction of FtsZ might partly explain the asymmetric location of the sporulation septum, although it would not be a sufficient explanation, as, in *E. coli*, the smaller cells produced are anucleate (9). The generation of asymmetry is one of the most intriguing problems about spore formation, so that even such a partial explanation would be of considerable interest. Unfortunately for this line of reasoning, Beall and Lutkenhaus (7) were unable to detect minicell production when they artificially induced FtsZ overexpression in *B. subtilis*, and they did not detect an increase in the amount of FtsZ protein during sporulation. There are various ways to explain the change in ratio of transcript to protein product at the start of sporulation that this implies, but no clear conclusion can be drawn about the role of transcript or product in the generation of asymmetry. The level of FtsA protein during sporulation has not been investigated, and its role is also unclear.

Regulation from the three promoters p1, p2, and p3 provides an interesting case of modification of the regulation of an essential vegetative function so as to have a role in sporulation. Carter et al. (13) have reported a σ^H promoter of *rpoD*, the structural gene for the major *B. subtilis* σ factor, and this could provide another such case. Separate promoters under vegetative and sporulation control could provide a general way in which to regulate the expression of essential genes, such as those required for septum formation, during spore formation.

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