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A Small Protein Required for the Switch from σ^F to σ^G during Sporulation in *Bacillus subtilis*▽

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A cascade of alternative sigma factors governs the program of developmental gene expression during sporulation in *Bacillus subtilis*. Little is known, however, about how the early-acting sigma factors are inactivated and replaced by the later-acting factors. Here we identify a small protein, Fin (formerly known as YabK), that is required for efficient switching from σ^F- to σ^G-directed gene expression in the forespore compartment of the developing sporangium. The *fin* gene, which is conserved among *Bacillus* species and species of related genera, is transcribed in the forespore under the control of both σ^F and σ^G. Cells mutant for *fin* are unable to fully deactivate σ^F and, conversely, are unable to fully activate σ^G. Consistent with their deficiency in σ^G-directed gene expression, *fin* cells are arrested in large numbers following the engulfment stage of sporulation, ultimately forming 50-fold fewer heat-resistant spores than the wild type. Based in part on the similarity of Fin to the anti-σ^G factor CsfB (also called Gin), we speculate that Fin is an anti-σ^F factor which, by disabling σ^F, promotes the switch to late developmental gene expression in the forespore.

Complex, multistep cell differentiation pathways are typically orchestrated by the activation of sets of regulatory genes in an ordered sequence. In bacteria, such developmental programs are sometimes driven by cascades of RNA polymerase (RNAP) sigma (σ) factors, as in the paradigmatic example of spore formation by *Bacillus subtilis* (17, 23, 28, 34). Sporulation takes place in a two-compartment sporangium that arises by a process of asymmetric division (Fig 1A). The smaller, forespore compartment develops into the spore, whereas the larger mother cell nurtures the developing forespore. Initially, the forespore and mother cell lie side by side; subsequently, the mother cell engulfs the forespore in a phagocytosis-like process that results in a cell-within-a-cell configuration (Fig. 1A). The engulfed forespore is then encased in protective peptidoglycan cortex and protein coat layers and ultimately released into the environment by lysis of the mother cell.

Gene expression after asymmetric division is driven chiefly by four compartment-specific sigma factors—σ^F, σ^E, σ^G, and σ^K—that direct RNAP to distinct sets of developmental genes (15, 32, 40). The σ^F and σ^E factors are early-acting regulatory proteins that control gene expression in the forespore and mother cell, respectively. At later times, σ^G replaces σ^F in the forespore, whereas σ^K replaces σ^E in the mother cell (Fig. 1A). Importantly, this switch to late developmental gene expression requires not only mechanisms to synthesize and activate σ^G and σ^K but also mechanisms to inactivate and/or remove σ^F and σ^E. The regulation of σ^G and σ^K synthesis and activation at the appropriate time and place has been studied extensively and is known in some detail (albeit more for σ^K than for σ^G) (reviewed in references 17 and 28). However, it remains poorly understood how σ^F and σ^E are inactivated at the transition to late gene expression. Indeed, little overlap between σ^F and σ^G activities in the forespore or between σ^E and σ^K activities in the mother cell is detected, indicating that one or more controls must exist to temporally segregate them (21). Furthermore, evidence shows that the late-acting sigma factors directly or indirectly trigger negative-feedback loops that inactivate their predecessors: deletion of the gene for σ^G or σ^K results in inappropriately sustained σ^F or σ^E activity, respectively (4, 6, 13, 20, 43).

Further clues have emerged regarding replacement of σ^E by σ^K in the mother cell: the σ^K-dependent negative-feedback loop appears to operate at the level of transcription of the σ^E structural gene and specifically requires that σ^K is transcriptionally active (43, 44). The latter finding, which was obtained using a variant of σ^K that binds RNAP but is transcriptionally inactive, eliminates a simple model in which the σ^E-to-σ^K transition is driven by competition for RNAP (18) and instead indicates that one or more target genes of σ^K are involved (44). In contrast, almost nothing is known of the nature of the mechanisms that mediate the switch from σ^F to σ^G in the forespore.

Here we present evidence that a small, conserved protein that we named Fin (previously annotated YabK) is expressed in the forespore and is required for the efficient transition from σ^F- to σ^G-directed gene expression. Remarkably, *fin* mutant cells are deficient for spore formation and progress slowly, if at all, past the engulfment stage (III) of sporulation, a phenotype consistent with a defect in σ^G activation. Thus, *fin* represents a previously unrecognized and uncharacterized sporulation gene. Given the similarity of Fin to the anti-σ^G factor CsfB (also called Gin) (7, 11, 19, 29), as presented herein, we speculate that Fin functions as an anti-σ^F factor which, by antag-

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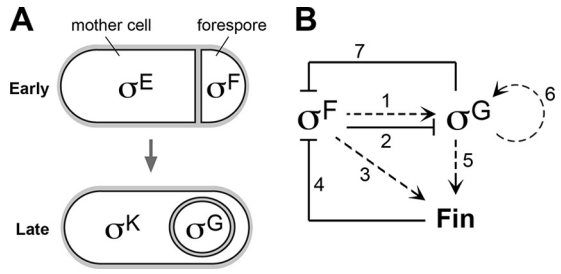


FIG. 1. A role for Fin (YabK) in sigma factor switching during sporulation in *B. subtilis.* (A) Cartoon depicting the sigma factors directing compartment-specific gene expression in sporangia at early (top) and late (bottom) stages of development. At early times, the sigma factors σ^F and σ^E direct gene expression in the forespore and mother cell, respectively. At later times, after the forespore is engulfed by the mother cell, σ^F is replaced by σ^G and σ^E is replaced by σ^K. (B) Model for the switch from σ^F to σ^G. To begin, σ^F activates transcription of the gene (*sigG*) for σ^G (arrow 1); however, σ^G activation is delayed through poorly understood mechanisms that likely involve σ^F (barred line 2) (see Discussion). To trigger the switch to σ^G, σ^F also directs synthesis of its own inhibitor, Fin (arrow 3). Once σ^F is inactivated by Fin (barred line 4), σ^G becomes active. This transition is reinforced by two mechanisms. First, σ^G continues to direct *fin* synthesis, resulting in sustained σ^F inhibition (arrow 5). Second, σ^G autoregulates its own gene, leading to large amounts of the late sigma factor (arrow 6). σ^G also inhibits σ^F by an unknown, Fin-independent pathway (barred line 7) (see Discussion). In the absence of Fin, unchecked σ^F activity prevents σ^G activation, likely due to the same mechanisms represented by barred line 2. Dashed arrows indicate transcriptional regulation. Lines with barred ends indicate inhibition by currently unknown mechanisms.

onizing σ^F, facilitates the switch to σ^G and promotes the transition to late developmental gene expression in the forespore.

MATERIALS AND METHODS

General methods. Bacterial strains were propagated in Luria-Bertani medium. When appropriate, antibiotics were included at the following concentrations: chloramphenicol (5 μg/ml), erythromycin plus lincomycin (1 μg/ml and 25 μg/ml, respectively), spectinomycin (100 μg/ml), kanamycin (5 μg/ml), phleomycin (0.4 μg/ml), and ampicillin (100 μg/ml). To measure sporulation efficiency, cells were induced to sporulate by nutrient exhaustion for 24 h at 37°C in Difco (Schaeffer's) sporulation medium (DSM) (27, 30). The number of CFU that survived heat treatment (80°C for 20 min) was determined and normalized to the number of heat-resistant CFU obtained in parallel from the wild-type strain. For all other experiments, sporulation was induced at 37°C by the Sterlini-Mandelstam resuspension method (27, 33). β-Galactosidase activity was measured as previously described (6).

Strain and plasmid construction. *B. subtilis* strains used in this study were derived by transformation of the prototrophic laboratory strain PY79 (42) or derivatives thereof with chromosomal DNA, plasmids, or PCR products. The genes utilized to confer resistance of *B. subtilis* to antibiotics were as follows: *cat* (chloramphenicol), *erm* (erythromycin plus lincomycin), *spc* (spectinomycin), *kan* (kanamycin), and *phleo* (phleomycin). Competent *B. subtilis* cells were prepared as previously described (41). Unless otherwise noted, PY79 chromosomal DNA served as a template for PCR amplification. Plasmids were cloned and propagated in the *Escherichia coli* strain DH5α. Plasmid mutagenesis was performed with a QuikChange II XL site-directed mutagenesis kit (Stratagene). The genotypes, features, and sources of strains and plasmids used in this study are listed in Table 1. The sequences of primers utilized in strain and plasmid construction are provided in Table 2.

The Δ*fin*::*phleo* deletion strain (AHB1931) was generated by the long-flanking-homology PCR (LFH-PCR) method (39), using primer pairs AH493/AH494 and AH495/AH496, with the plasmid pAH247 [*phleo* in pBluescript KS(+); see below] as the source for the phleomycin resistance gene.

Plasmids were constructed as follows. pAH247 [*phleo* in pBluescript KS(+)] was constructed by subcloning a BamHI/SalI fragment harboring the *phleo* resistance gene from pKM080 (a gift of D. Rudner) into BamHI/SalI-digested

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Genotype or description	Source or reference
B. subtilis strains[a]		
PY79	Prototrophic wild type	42
RL1275	Δ*sigF*::*erm*	Laboratory stock derived from MO173 (gift of P. Stragier)
AHB98	Δ*sigG*::*kan*	7
AHB199	Δ*csfB*::*tet*	7
AHB1931	Δ*fin*::*phleo*	This study
AHB1983	Δ*fin*::*phleo sacA*::P_{fin}-*fin kan*	This study
AHB1933	*amyE*::P_{fin}-*lacZ cat*	This study
AHB1956	*amyE*::P_{fin}-*lacZ cat* Δ*sigF*::*erm*	This study
AHB1934	*amyE*::P_{fin}-*lacZ cat* Δ*sigG*::*kan*	This study
AHB2085	Δ*fin*::*phleo amyE*::P_{fin}-*gfp-fin spc*	This study
AHB881	*amyE*::P_{spoIIQ}-*lacZ cat*	6
AHB1953	*amyE*::P_{spoIIQ}-*lacZ cat* Δ*fin*::*phleo*	This study
AHB1985	*amyE*::P_{spoIIQ}-*lacZ cat* Δ*fin*::*phleo sacA*::P_{fin}-*fin kan*	This study
AHB882	*amyE*::P_{spoIIQ}-*lacZ cat* Δ*sigG*::*kan*	6
AHB1954	*amyE*::P_{spoIIQ}-*lacZ cat* Δ*sigG*::*kan* Δ*fin*::*phleo*	This study
AHB324	*ywrK*::Tn*917*::*amyE*::P_{sspB}-*lacZ cat*	6
AHB1952	*ywrK*::Tn*917*::*amyE*::P_{sspB}-*lacZ cat* Δ*fin*::*phleo*	This study
AHB1984	*ywrK*::Tn*917*::*amyE*::P_{sspB}-*lacZ cat* Δ*fin*::*phleo sacA*::P_{fin}-*fin kan*	This study
AHB1879	*ywrK*::Tn*917*::*amyE*::P_{sspB}-*lacZ cat* Δ*csfB*::*tet*	This study
AHB2112	*ywrK*::Tn*917*::*amyE*::P_{sspB}-*lacZ cat* Δ*fin*::*phleo* Δ*csfB*::*tet*	This study
Plasmids		
pAH247	*phleo* in pBluescript KS(+)	This study
pAH515	*amyE*::P_{fin}-*lacZ cat*	This study
pAH537	*sacA*::P_{fin}-*fin kan*	This study
pAH585	*amyE*::P_{fin}-*gfp-fin spc*	This study

[a] All *B. subtilis* strains are isogenic with PY79.

TABLE 2. Primers used in this study

Primer	Sequence (5′→3′)[a]	Description
AH493	cgtactgcagcagccgttatatg	Δ*fin* LFH-PCR primer (P1)
AH494	caattcgccctatagtgagtcgtgccatacaagggtcctcctgatg	Δ*fin* LFH-PCR primer (P2)
AH495	ccagcttttgttccctttagtgagtaaaaagctttggtgtagacactagacc	Δ*fin* LFH-PCR primer (P3)
AH496	gagagatacttcacgagctcctgatc	Δ*fin* LFH-PCR primer (P4)
AH497	gatcgaattcgacagtcatttacgacgaccttg	Forward primer, P_{fin} upstream sequence (EcoRI site)
AH498	gatcaagcttcatacaagggtcctcctgatg	Reverse primer, P_{fin} downstream sequence including RBS and *fin* start codon (HindIII site)
AH499	gatcggatcccctctgtttcctgcgagaacag	Reverse primer, downstream of *fin* (BamHI site)
AH521	catcaggaggacccttgtatgGCTAGCgctttgcattattattgtcg	Mutagenesis primer to insert NheI site downstream of *fin* ATG start codon, top strand
AH522	cgacaataataatgcaaagcGCTAGCcatacaagggtcctcctgatg	Mutagenesis primer to insert NheI site downstream of *fin* ATG start codon, bottom strand
AH387	gatcgctagcagtaaaggagaagaacttttcactggag	Forward primer, *gfp* upstream sequence (starting with second codon) (NheI site)
AH388	gatcgctagctttgtatagttcatccatgccatgtg	Reverse primer, *gfp* downstream sequence (omitting stop codon) (NheI site)

[a] Restriction endonuclease recognition sites are underlined. Insertions in mutagenesis primers are indicated with uppercase letters.

pBluescript KS(+) (Stratagene). pAH515 (*amyE*::P_{fin}-*lacZ cat*) was constructed by cloning a HindIII/EcoRI PCR fragment containing the *fin* promoter (P_{fin}), ribosome-binding site (RBS), and start codon (amplified with primers AH497 and AH498) into HindIII/EcoRI-digested pAH124 (*amyE*::*lacZ cat*) (6). pAH537 (*sacA*::P_{fin}-*fin kan*) was generated by cloning a HindIII/BamHI PCR product harboring the *fin* open reading frame (ORF) and its upstream promoter sequences (amplified with primers AH497 and AH499) into HindIII/BamHI-digested pSac-Kan (*sacA*::*kan*) (26). pAH585 (*amyE*::P_{fin}-*gfp-fin spc*) was built in three steps. First, a HindIII/BamHI PCR product harboring the *fin* ORF and its upstream promoter sequences (the same as that used for construction of pAH537; amplified with primers AH497 and AH499) was ligated with HindIII/BamHI-digested pDG1731 (*amyE*::*spc*) (16), yielding pAH540. Second, pAH540 was mutagenized with primer pair AH521/AH522 to insert an in-frame NheI restriction site downstream of the *fin* start codon, yielding pAH581. Finally, an NheI/NheI PCR fragment containing the *gfp* ORF (amplified from pAC172 [12] with primers AH387 and AH388) was ligated into NheI-digested pAH581, yielding pAH585.

Microscopy. Cells expressing the *gfp-fin* fusion gene were collected by brief, gentle centrifugation at hour 3.5 of sporulation and were resuspended in phosphate-buffered saline (PBS) containing 1 μg/ml of the membrane stain FM4-64 (Invitrogen). Fluorescence microscopy was performed with an Olympus BX61 microscope fitted with an Olympus UPlanF1 100× phase-contrast objective. Green fluorescent protein (GFP) fluorescence was visualized using filter set U-M41001 (excitation filter, 455 to 495 nm; dichroic mirror, 505 nm; and emission filter, 510 to 555 nm), while FM4-64 fluorescence was visualized using filter set U-MWG2 (excitation filter, 510 to 550 nm; dichroic mirror, 570 nm; and barrier filter, >590 nm). Images were captured with an Orca-R2 digital charge-coupled device (CCD) camera using Simple PCI imaging software, version 6.0 (Hamamatsu Corporation). Exposure times were typically 500 to 1,000 ms for both GFP and FM4-64. Images were false-colored, overlaid, and adjusted for brightness and contrast by use of ImageJ software (1).

To evaluate the developmental status of *fin* mutant and control cells, cells were collected by brief, gentle centrifugation at hour 6 of sporulation and resuspended in PBS containing 1 μg/ml FM4-64 and 10 μg/ml Mitotracker green FM (MTG; Invitrogen). The microscope setup and image acquisition/processing procedure were the same as those described above, except that the U-M41001 filter was used to image MTG fluorescence, with a typical exposure time of 200 ms. Dual labeling of cell membranes with FM4-64, which cannot permeate membranes, and MTG, which can permeate membranes, allows for identification of sporangia that have completed forespore engulfment, as previously described (31). In brief, the membranes of fully engulfed forespores stain only with MTG, while those of unengulfed forespores stain with both FM4-64 and MTG. Cells were additionally observed by phase-contrast microscopy to identify sporangia that had progressed to postengulfment stages of sporulation (cortex and coat assembly), as indicated by increased forespore refractility (i.e., "phase-bright" forespores).

Identification and alignment of Fin and CsfB orthologs. Fin (YabK) and CsfB orthologs were identified by BLAST searches (2; http://www.ncbi.nlm.nih.gov/) with *B. subtilis* protein sequences. The following proteins were chosen as representative orthologs (GenBank accession numbers are given in parentheses): *B. subtilis* 168 CsfB (NP_387905), *Bacillus licheniformis* ATCC 14580 CsfB (YP_077310), *Geobacillus kaustophilus* HTA426 CsfB (YP_145875), *Oceanobacillus iheyensis* HTE831 CsfB (NP_690955), *Bacillus anthracis* Ames CsfB (NP_842595), *Bacillus halodurans* C-125 CsfB (NP_240906), *B. subtilis* 168 Fin (NP_387935), *B. licheniformis* ATCC 14580 YabK (YP_089740), *G. kaustophilus* HTA426 YabK (YP_145900), *O. iheyensis* HTE831 YabK (NP_690983), *B. anthracis* Ames YabK (NP_842620), and *Bacillus clausii* KSM-K16 YabK (YP_173586). CsfB from *B. clausii* and YabK from *B. halodurans* were not found annotated in databases; however, manual inspection of the relevant regions of their respective genomes (*B. clausii* KSM-K16 and *B. halodurans* C-125) revealed the presence of their encoding genes.

Multiple sequence alignments of CsfB and YabK/Fin orthologs were generated with ClustalW (37).

RESULTS

***fin* (*yabK*) is expressed in the forespore under the control of σ^F and σ^G.** The *yabK* gene, which we renamed *fin*, is located at an origin-proximal position on the chromosome (5.1° relative to the origin, at 0°/360°), between the genes *pth* (formerly named *spoVC*), which encodes peptidyl-tRNA hydrolase (25), and *mfd*, which encodes a transcription repair coupling factor (3) (Fig. 2A). The *spoVT* gene, which encodes a regulator of σ^G-directed gene expression in the forespore (5), is also in close proximity to *fin* on the chromosome (downstream of *mfd*). Interestingly, this gene organization (like *fin* itself [see below]) is conserved among *Bacillus* species and species of related genera.

Close inspection of the *fin* upstream sequence revealed a nearly perfect match to the consensus −35 and −10 elements of promoters recognized by σ^F (40) (Fig. 2B). This is consistent with a study that assigned *fin* to the σ^F regulon by microarray analysis (32). We note, however, that the same elements are also excellent matches for the σ^G recognition consensus (40) (Fig. 2B). Another sequence immediately upstream of the *fin* ORF displays strong similarity to the optimal *B. subtilis* RBS (38) (Fig. 2B).

To investigate the expression of *fin*, we fused its upstream sequences, including the putative promoter (P_{fin}), RBS, and ATG start codon, in frame to the *lacZ* reporter gene. β-Galactosidase production in cells harboring this P_{fin}-*lacZ* fusion (integrated at the nonessential *amyE* locus) began at hour 2 of

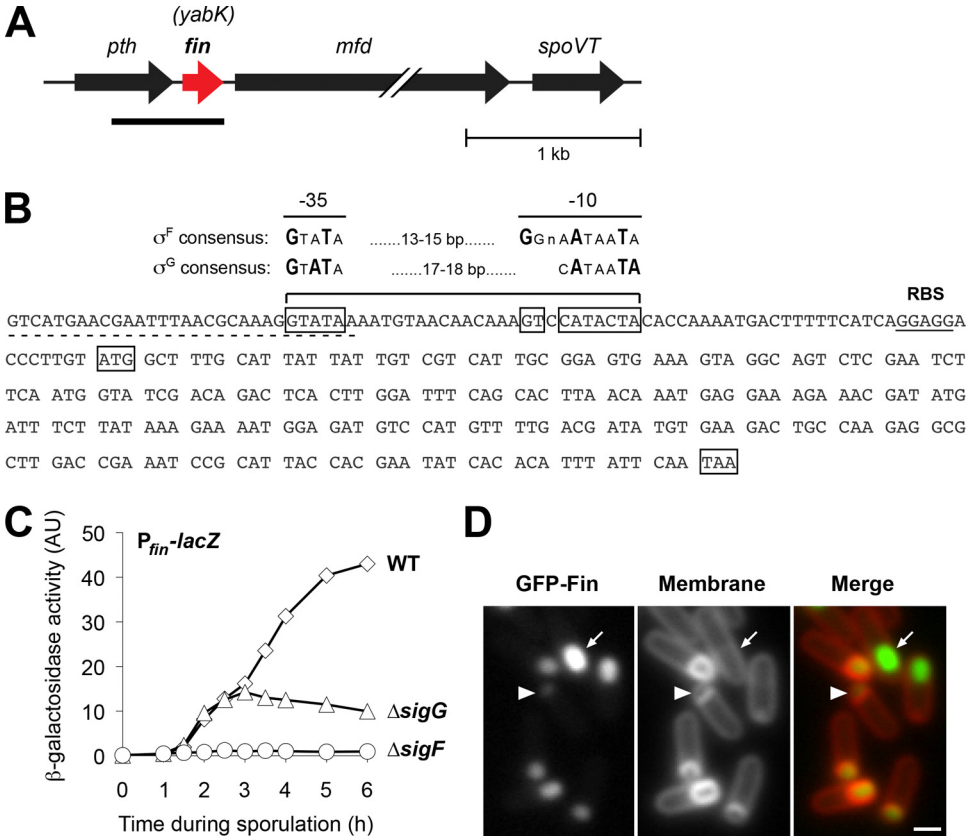


FIG. 2. *fin* (*yabK*) is a small gene that is expressed in the forespore under the control of σ^F and σ^G. (A) The *fin* gene (previously annotated *yabK*), depicted within its chromosomal context. To our knowledge, this gene synteny is conserved in all *Bacillus* genomes completed to date. The black bar indicates the *fin*-containing region that was inserted at the *sacA* locus to complement the mutation of *fin*. The drawing is to scale (as indicated), except that 2 kb of the *mfd* gene is not shown. (B) Sequence of the *fin* coding and upstream promoter regions. The putative −10 and −35 promoter sequences are boxed, with the consensus σ^F- and σ^G-recognized sequences shown above (40). Also boxed are the *fin* ATG start codon and TAA stop codon. The RBS for *fin* is underlined, while the dashed line indicates the 3′ end of the upstream *pth* gene, which overlaps the predicted *fin* promoter elements. (C) The *fin* promoter (P_{fin}) is activated by σ^F and σ^G during sporulation. The accumulation of β-galactosidase from a P_{fin}-*lacZ* reporter gene was measured during sporulation of wild-type cells (WT; ◇), cells deleted for *sigF* (Δ*sigF*; ○), and cells deleted for *sigG* (Δ*sigG*; △) (strains AHB1933, AHB1956, and AHB1934, respectively). AU, arbitrary units. (D) GFP-Fin localizes diffusely throughout the forespore during early and late sporulation. Cells deleted for the native *fin* gene and harboring a functional *gfp-fin* gene fusion at the *amyE* locus (strain AHB2085) were observed by fluorescence microscopy at hour 3.5 of sporulation. GFP fluorescence is shown in grayscale (GFP-Fin) or false-colored green (merge). Membrane fluorescence from the dye FM4-64 is shown in grayscale (membrane) or false-colored red (merge). White arrowheads indicate a forespore at an early stage of sporulation (immediately following asymmetric division), while the white arrows indicate a forespore at a later stage of sporulation (after engulfment; note that membranes surrounding engulfed forespores are not stained due to membrane impermeability for the FM4-64 dye). Bar = 1 μm.

sporulation, consistent with the timing of σ^F activation in the forespore (Fig. 2C). P_{fin}-*lacZ* also displayed a second wave of activity at later times (starting after hour 3), most consistent with the timing of σ^G activity (Fig. 2C). To test the dependence of *fin* expression on σ^F and σ^G, we introduced deletions of their encoding genes (*sigF* and *sigG*, respectively) into the P_{fin}-*lacZ* strain. As shown in Fig. 2C, deletion of *sigG* mostly eliminated the late phase of P_{fin} expression, while deletion of *sigF* blocked both the early and late phases (note that σ^G activation requires σ^F; as such, *sigF* cells lack σ^F- and σ^G-directed gene expression). In all, these findings suggest that *fin* is activated in the developing forespore first by σ^F and later by σ^G. Importantly, the deletion of genes encoding other sporulation sigma factors (including σ^E and σ^K), either individually or pairwise with *sigF* or *sigG*, yielded results that were consistent with this conclusion (data not shown).

To visualize the compartmentalization of *fin* expression, we inserted the coding sequence of *gfp* in frame downstream of the ATG start codon of the full-length *fin* gene, leaving the upstream promoter and RBS intact. The resulting fusion gene (P_{fin}-*gfp-fin*; henceforth called *gfp-fin* for simplicity) was integrated at the *amyE* locus in a strain deleted for native *fin*. Importantly, *gfp-fin* was able to complement the *fin* mutant phenotypes (data not shown; see below), indicating that the encoded GFP-Fin fusion protein is functional. As shown in Fig. 2D, GFP-Fin localized diffusely within the forespore compartment of sporangia at both early and late stages of sporulation. Indeed, GFP-Fin was detectable in the forespore compartment beginning very soon after asymmetric septation (Fig. 2D, white arrowheads) and continuing through the completion of forespore engulfment (Fig. 2D, white arrows). We therefore concluded that P_{fin} is expressed in the forespore compartment

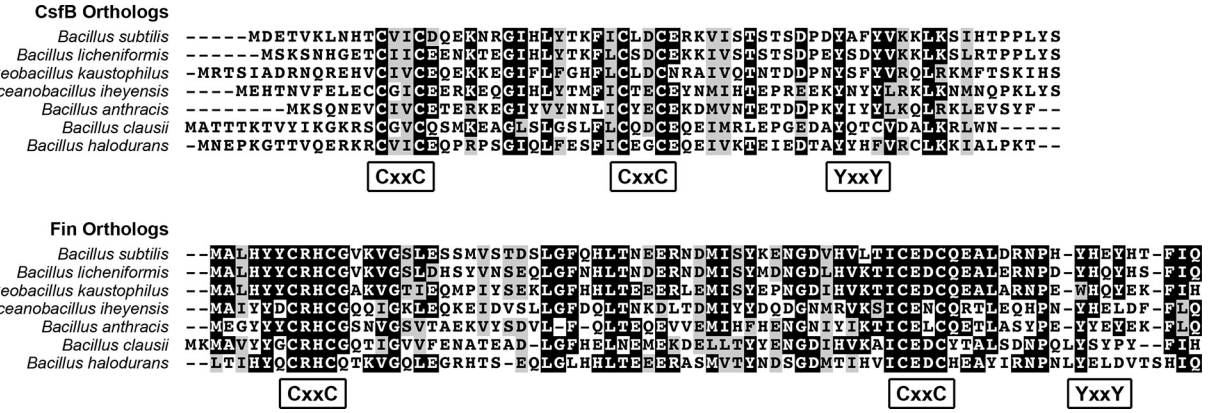


FIG. 3. The Fin protein is conserved among *Bacillus* species and displays similarity to the anti-σ^G factor CsfB. Multiple sequence alignments are shown for CsfB orthologs (top) and Fin (YabK) orthologs (bottom) from various *Bacillus* and related species. The full amino acid sequence for each protein is shown. Identical or similar residues present in more than 50% of the sequences are shaded black or gray, respectively. Conserved Cys-X-X-Cys (CxxC) and Tyr-X-X-Tyr (YxxY) motifs present in Fin and CsfB ortholog families are indicated. Accession numbers for protein sequences are listed in Materials and Methods. Note that CsfB is also conserved more widely in endospore formers, including *Clostridium* species, and that multiple sequence alignments of the entire CsfB family have been published previously (19, 29).

throughout development and, moreover, that Fin appears to be a soluble, cytosolic protein.

Fin is conserved among *Bacillus* species and is similar to the anti-σ^G factor CsfB. The *fin* ORF encodes a small protein of 76 amino acids with a predicted molecular mass of 8.8 kDa. A BLAST search against a nonredundant protein database revealed that Fin is encoded in most, if not all, genomes sequenced to date from *Bacillus* species and species of other closely related genera (2). One exception appeared to be *B. halodurans*; however, manual inspection of the *pth-mfd* intergenic region from this species revealed an unannotated ORF corresponding to *fin*. In contrast, our searches failed to identify *fin* in *Listeria* species, which are closely related to *Bacillus* but are unable to sporulate, or in the more distantly related endospore formers of the genus *Clostridium*.

As shown in Fig. 3, the amino acid sequence of Fin is conserved throughout the length of representative orthologs (>30% identity, >50% similarity). Notably, Fin harbors two absolutely conserved Cys-X-X-Cys motifs, often found in zinc-binding proteins, that are spaced ~40 amino acids apart (Fig. 3). As drawn to our attention by A. Henriques and P. Stragier (personal communications), this makes Fin similar to the anti-σ^G factor CsfB (also known as Gin) (7, 11, 19, 29). CsfB is also a small, highly conserved protein harboring two invariant Cys-X-X-Cys motifs (Fig. 3). Furthermore, the CsfB and Fin protein families both contain an imperfectly conserved Tyr-X-X-Tyr motif at their C termini (Fig. 3). Like *fin*, *csfB* is expressed in the forespore under the control of σ^F (13). Unlike P_{fin}, however, P_{csfB} does not appear to be activated by σ^G (A. H. Camp and R. Losick, unpublished results). Finally, the *csfB* gene is located in the same origin-proximal chromosomal region as *fin* (approximately 25 kb apart). In all, these similarities suggest that Fin may be evolutionarily, structurally, and/or functionally related to CsfB.

***fin* is required for the switch from σ^F- to σ^G-directed gene expression.** Given the presence of Fin in the forespore and its similarity to the anti-σ^G factor CsfB (see above), we wondered whether Fin is a regulator of forespore gene expression. To test this, we first monitored σ^F activity in the absence of *fin* by using a *lacZ* reporter gene under the control of the σ^F-dependent *spoIIQ* promoter (P_{spoIIQ}) (22). As shown in Fig. 4A, P_{spoIIQ}-*lacZ* activity commenced at hour 2 of sporulation in both wild-type and *fin* cells, consistent with the timing of σ^F activation in the forespore. Strikingly, however, σ^F-directed β-galactosidase production in *fin* cells reached higher overall levels (approximately twice that seen in the wild type) and persisted to later times of sporulation (peaking at hour 4 versus hour 3 in the wild type) (Fig. 4A). Importantly, reintroduction of a wild-type copy of the *fin* gene at the *sacA* locus complemented the effect of the mutation, confirming that this phenotype was due to the absence of *fin* and not a polar effect on the adjacent, downstream gene (*mfd*) (Fig. 4A). Furthermore, we confirmed that the effect of *fin* deletion was not specific to P_{spoIIQ}, as *fin* mutant cells displayed hyperactivity of other σ^F-dependent promoters, including P_{csfB}, P_{csfC}, and P_{yyaC} (data not shown) (13, 32, 40).

Next, we tested the effect of a *fin* mutation on σ^G activity by using a *lacZ* reporter gene under the control of the *sspB* promoter (P_{sspB}), which is recognized exclusively by σ^G (35). Remarkably, σ^G-directed β-galactosidase production from the P_{sspB}-*lacZ* reporter was significantly decreased in *fin* mutant cells (≤25% of wild-type levels by hour 6) (Fig. 4C). As described above, we determined that σ^G activity was restored to wild-type levels when *fin* was reintroduced (Fig. 4C). In all, we concluded that Fin facilitates the switch from σ^F to σ^G: σ^F does not shut off and σ^G does not turn on fully in the absence of *fin*.

We and others previously reported that deletion of *sigG* causes overexpression of σ^F target genes (including *spoIIQ*) at late times during sporulation, suggesting that σ^G directly or indirectly inhibits σ^F (4, 6, 13). This raised the possibility that Fin functions primarily to promote σ^G activity and that the σ^F hyperactivity phenotype of cells mutant for *fin* is caused secondarily, by reduced σ^G activity. If this were true, then *fin* should have no effect on σ^F activity in the absence of *sigG* (i.e., the effect of a *sigG* mutation should be epistatic to a *fin* mutation). However, as shown in Fig. 4B, we found that σ^F activity

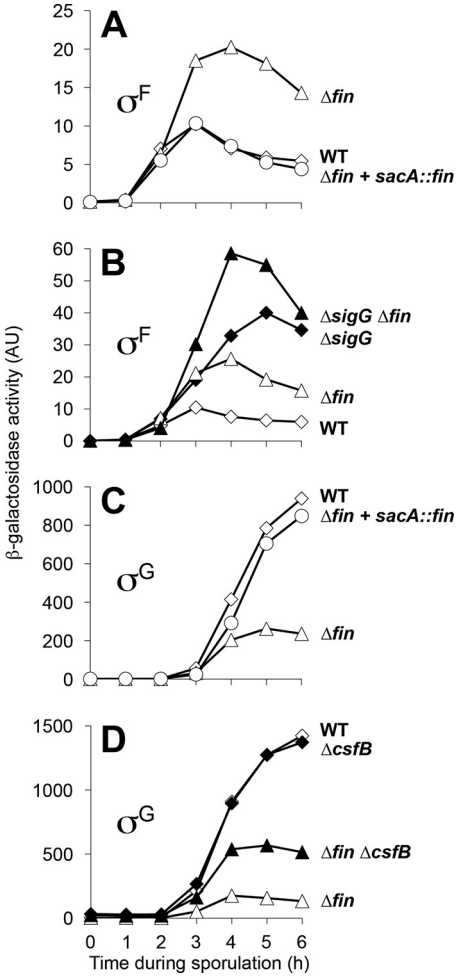
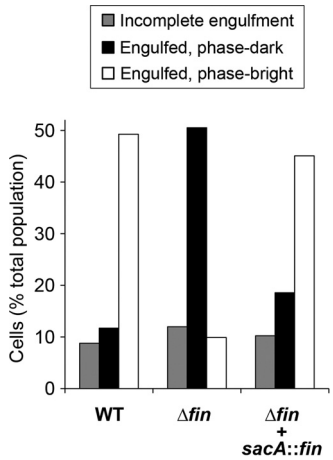


FIG. 4. *fin* is required for the efficient switch from σ^F to σ^G in the forespore. (A) σ^F remains active at late times during sporulation in the absence of *fin*. The σ^F-dependent activation of a P_{spoIIQ}-*lacZ* reporter gene was monitored during sporulation of wild-type cells (WT; ◇), cells deleted for *fin* (Δ*fin*; △), and cells deleted for *fin* and harboring a *fin* complementation construct at the *sacA* locus (Δ*fin* + *sacA*::*fin*; ○) (strains AHB881, AHB1953, and AHB1985, respectively). (B) Fin and σ^G additively repress σ^F at late times during sporulation. σ^F-Directed β-galactosidase production from a P_{spoIIQ}-*lacZ* reporter gene was measured during sporulation of wild-type cells (WT; ◇), cells deleted for *fin* (Δ*fin*; △), cells deleted for *sigG* (Δ*sigG*; ◆), and cells simultaneously deleted for *fin* and *sigG* (Δ*sigG* Δ*fin*; ▲) (strains AHB881, AHB1953, AHB882, and AHB1954, respectively). (C) Fin is required for full σ^G activation. The σ^G-dependent activation of a P_{sspB}-*lacZ* reporter gene was monitored during sporulation of wild-type cells (WT; ◇), cells deleted for *fin* (Δ*fin*; △), and cells deleted for *fin* and harboring a *fin* complementation construct at the *sacA* locus (Δ*fin* + *sacA*::*fin*; ○) (strains AHB324, AHB1952, and AHB1984, respectively). (D) Deletion of *csfB* partially restores σ^G activity to *fin* mutant cells. P_{sspB}-*lacZ* reporter gene activation by σ^G was monitored during sporulation of wild-type cells (WT; ◇), cells deleted for *fin* (Δ*fin*; △), cells deleted for *csfB* (Δ*csfB*; ◆), and cells simultaneously deleted for *fin* and *csfB* (Δ*fin* Δ*csfB*; ▲) (strains AHB881, AHB1953, AHB1879, and AHB2112, respectively).



Sporulation Efficiency		
1.00	0.02	0.58

FIG. 5. Cells lacking *fin* are defective for spore formation and are arrested after engulfment. The status of sporulation at hour 6 of sporulation was determined for wild-type cells (WT), cells deleted for *fin* (Δ*fin*), and cells deleted for *fin* and harboring a *fin* complementation construct at the *sacA* locus (Δ*fin* + *sacA*::*fin*) (strains PY79, AHB1931, and AHB1983, respectively), using a combination of fluorescence and phase-contrast microscopy (see Materials and Methods) ($n > 190$ for each sample). The remaining, nonsporulating ~25 to 30% of cells from each population are not shown. Also indicated (bottom) are the sporulation efficiencies for the indicated strains. Sporulation efficiency was calculated as the number of heat-resistant spores present after 24 h of growth/sporulation in DSM normalized to the number present in the wild-type sample.

was higher in *sigG fin* mutant cells than in either single mutant alone, indicating that Fin does not influence σ^F activity indirectly through σ^G. As such, the simplest interpretation of the results is that Fin is an anti-σ^F factor which, by antagonizing σ^F, facilitates the switch to σ^G. It is on the basis of this phenotype that we renamed the *yabK* gene *fin*, for σ^F *in*hibitor.

Finally, these results indicate that increased σ^F activity in the absence of *fin* interferes with subsequent σ^G activation. One explanation could be that hyperactive σ^F directs increased transcription of the gene for the anti-σ^G factor CsfB (13). Indeed, the *csfB* promoter (P_{csfB}), like P_{spoIIQ}, was more active in *fin* cells (data not shown). To test whether CsfB contributes to the block in σ^G activation in *fin* cells, we measured σ^G activation in cells lacking *fin* and *csfB*. Consistent with previous reports (7, 11), deletion of *csfB* alone did not appreciably affect the timing or level of activation of the σ^G-dependent reporter P_{sspB}-*lacZ* (Fig. 4D). However, we found that elimination of *csfB* significantly increased σ^G activation in *fin* mutant cells, albeit not to wild-type levels (>3-fold increase over the level in the *fin* single mutant) (Fig. 4D). In all, these findings are consistent with a model in which sustained σ^F activity interferes with σ^G activation, in part due to increased production of the anti-σ^G factor CsfB.

***fin* mutant cells display a sporulation phenotype and are arrested after engulfment.** Our finding that Fin is required for the efficient switch from σ^F to σ^G in the forespore immediately raised the question of whether the *fin* mutant is defective for spore formation. To test this, we determined the number of heat-resistant spores present after 24 h of sporulation in wild-type and *fin* cells. Strikingly, we found that the *fin* mutant displayed a 50-fold reduction in spore formation relative to the wild type (Fig. 5). (We note, however, that *fin* cells still pro-

duced a substantial number of heat-resistant spores [$\sim 1 \times 10^7$ spores per ml, compared to $\sim 5 \times 10^8$ spores per ml for the wild type].) Sporulation was restored to almost wild-type levels by the reintroduction of *fin* at the *sacA* locus (sporulation at ~60% of wild-type efficiency) (Fig. 5). We presume that the incomplete complementation in this sensitive assay was due to subtle differences in *fin* expression from an ectopic locus. Finally, we found that deletion of *csfB* partially rescued spore formation by *fin* cells (approximately 6-fold) (data not shown), consistent with the partial rescue of σ^G activation observed in *fin csfB* cells (see above and Fig. 4D).

Next, we subjected *fin* cells to a fluorescence and phase-contrast microscopy-based assay to determine the stage of sporulation at which they are arrested (see Materials and Methods) (31). We found that cells lacking *fin* entered the sporulation pathway (as indicated by asymmetric septation) and progressed through engulfment in a manner that was nearly indistinguishable from that of the wild type (data not shown). However, we observed a significant defect in the ability of *fin* cells to subsequently form phase-bright forespores, which is typically indicative of the late, postengulfment events of forespore cortex and coat assembly. As shown in Fig. 5, by hour 6 nearly 50% of wild-type sporangia harbored engulfed, phase-bright forespores. At the same time, however, only 10% of *fin* sporangia had reached the same morphological stage. Instead, the majority of *fin* sporangia (51%) harbored engulfed, phase-dark forespores (Fig. 5). Importantly, we found that reintroduction of *fin* at the *sacA* locus restored the ability of the *fin* mutant to progress to later stages of sporulation, similar to the wild type (Fig. 5). In all, these results suggest that *fin* cells proceed slowly, if at all, past the engulfment stage (III) of sporulation, an interpretation that is consistent with a defect in σ^G activation.

DISCUSSION

To date, only limited progress has been made in unraveling the mechanisms that mediate the switch from one sigma factor to another during spore formation. Here we discovered a critical role for a small, conserved protein that we named Fin in the transition from early to late gene expression in the forespore. The *fin* gene is expressed in the forespore throughout development, under σ^F and σ^G control. In the absence of *fin*, the early-acting σ^F protein fails to shut off appropriately and, conversely, the late-acting σ^G protein fails to become activated fully. Concomitantly, *fin* cells are defective for sporulation, producing 50-fold fewer spores than the wild type. During sporulation, *fin* cells are arrested in large numbers following the engulfment stage (III), consistent with a defect in activation of σ^G, which is required for postengulfment events, including cortex and coat assembly.

We note that *yabK* (*fin*) was previously assigned a role in DNA repair, given that null mutations of the gene were observed to act as suppressors of the recombination defect of certain recombination genes (8, 9). On this basis, *yabK* was named *subA*, for suppressor of *recU* and *recB* (9). We think it improbable that *yabK* (*fin*) plays a role in DNA repair, in light of our evidence that it is expressed during sporulation, its orthologs are present only in endospore-forming species closely related to *Bacillus*, and it likely functions to inhibit σ^F (see below). Instead, and given that the previous authors failed to show that the phenotypes attributed to the *yabK* mutation could be reversed by complementation, it seems likely that the observed suppression was due to an effect on the expression of the downstream gene *mfd*, which encodes a transcription repair coupling factor (3).

How does Fin participate in the switch from σ^F to σ^G? One possibility is that Fin helps to promote σ^G activation such that it can surmount σ^F activity in the forespore. In this scenario, deletion of *fin* causes primarily reduced σ^G activity, which in turn permits sustained σ^F activity, as has been observed previously (6). However, this is not supported by our genetic analysis. If Fin were driving the σ^F-to-σ^G switch through activation of σ^G, then *fin* should not influence σ^F activity in the absence of σ^G. In contrast, we found that a *fin sigG* mutant displayed even more σ^F activity than the *sigG* single mutant. As such, we instead favor an alternative explanation in which Fin facilitates the σ^F-to-σ^G switch by inhibiting σ^F. In this model, deletion of *fin* causes primarily derepression of σ^F, which in turn interferes with σ^G activation.

As shown in Fig. 1B, we propose the following comprehensive model for the σ^F-to-σ^G transition. To begin, σ^F activates transcription of the gene (*sigG*) for σ^G (arrow 1) (36). However, activation of σ^G is delayed through poorly understood mechanisms that may include weak transcription of *sigG* by σ^F (Camp and Losick, unpublished data), inhibition by the σ^F-activated anti-σ^G factor CsfB (13, 19), and/or competition between σ^F and σ^G for RNAP (although the latter idea is controversial [10]) (barred line 2). To trigger the switch to σ^G, σ^F also turns on the gene encoding its own inhibitor, Fin (arrow 3). Once σ^F is sufficiently inactivated by Fin (barred line 4), its successor, σ^G, can become active. (The importance of σ^F inhibition for σ^G activation is considered in more detail below.) The transition to σ^G is then reinforced by two mechanisms. First, σ^G continues to direct *fin* synthesis, resulting in sustained σ^F inhibition (arrow 5). Second, σ^G autoregulates its own gene, leading to large amounts of the late sigma factor (arrow 6). Finally, we note that Fin cannot account for all observed σ^F inhibition, given that a *fin sigG* double mutant displayed more σ^F derepression than a *fin* mutant alone. As such, σ^G must additionally inhibit σ^F by an unknown, Fin-independent pathway (barred line 7).

A noteworthy feature of this model is that σ^G is crippled in the presence of sustained σ^F activity, as is the case for *fin* cells. This suggests that σ^F either directly or indirectly inhibits σ^G (indicated by barred line 2 in Fig. 1B). We speculate that this inhibition is mediated by some of the same mechanisms that ordinarily delay σ^G activation, including σ^F-dependent production of the σ^G inhibitor CsfB and competition between σ^F and σ^G for RNAP (see above). Consistent with the former, we found that deleting *csfB* partially rescued the σ^G activation and sporulation defects of *fin* mutant cells. In addition to competing for RNAP, the σ^F and σ^G regulons may also compete for the raw materials required for transcription and translation, such as nucleotides and amino acids. The latter idea is especially intriguing given our recent finding that the forespore loses its self-sufficiency to support macromolecular synthesis at around the time of the switch from σ^F to σ^G (6). Our "feeding tube" model posits that the mother cell restores the metabolic potential of the forespore at this time by providing critical

small molecules through a novel channel apparatus connecting the two cells (7, 14, 24). It is possible, however, that resources may still be limiting in the forespore even in the presence of the feeding tube.

How might Fin inhibit σ^F? One possible clue comes from the similarity of Fin to the anti-σ^G factor CsfB. CsfB is a potent inhibitor of σ^G and is likely to accomplish this by binding to the sigma factor (19, 29), although a direct biochemical interaction between CsfB and σ^G has not yet been demonstrated. CsfB was proposed to be the key target of an intercellular signaling pathway controlling σ^G activity in the forespore (19), but other work has convincingly indicated that the anti-σ^G factor instead plays an auxiliary role in preventing premature σ^G activation (see above) (7, 11). Like Fin, CsfB is a small protein that harbors two absolutely conserved Cys-X-X-Cys motifs and a less-conserved Tyr-X-X-Tyr motif (19, 29). Importantly, in the case of CsfB, these motifs are critical for σ^G inhibition and binding to σ^G in a yeast two-hybrid assay (29). It is tempting to speculate, therefore, that Fin binds to and inhibits σ^F analogously to the case for CsfB and σ^G. However, efforts to demonstrate Fin-dependent inhibition of σ^F activity in vegetative cells engineered to produce both proteins have so far been unsuccessful (Camp and Losick, unpublished results). A similar experiment successfully demonstrated CsfB-mediated inhibition of σ^G (19, 29). Conceivably, Fin may require one or more factors or conditions present only during sporulation to interact with and inhibit σ^F. We also cannot exclude a model in which Fin does not bind to σ^F but instead inhibits it indirectly.

In conclusion, our identification and characterization of *fin* represent significant steps forward in our understanding of the switch from σ^F to σ^G during *B. subtilis* spore formation. Moreover, the sporulation defect associated with *fin* mutation underscores the importance of properly regulated transitions in developmental gene expression during sigma factor cascades, as predicted decades ago (23, 34), and raises the possibility that the full list of sporulation genes is not yet complete.

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