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# The *dacF-spoIIA* Operon of *Bacillus subtilis*, Encoding $\sigma^F$, Is Autoregulated

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**The *spoIIA* operon of *Bacillus subtilis* encodes $\sigma^F$ and two proteins that may regulate sigma factor activity. High level induction of the tricistronic *spoIIA* operon occurs early during spore formation. At later times, the locus is cotranscribed with the upstream gene *dacF*, which encodes a putative DD-carboxypeptidase. In this study, the regulation of *dacF-spoIIA* transcription has been analyzed. Expression of a *dacF-lacZ* transcriptional fusion during sporulation required $\sigma^F$ but not the later-expressed sporulation-associated sigma factors. Induction of $\sigma^F$ synthesis during vegetative growth caused expression of *dacF-lacZ* fusions. The *dacF-spoIIA* promoter sequence is similar to sequences of previously identified $\sigma^F$ promoters. It is concluded that *dacF-spoIIA* is transcribed by E$\sigma^F$. We present evidence that *dacF-spoIIA* is also transcribed by E$\sigma^G$, as is the case for the three other promoters known to be transcribed by E$\sigma^F$.**

Vegetative cell division in the gram-positive spore-forming organism *Bacillus subtilis* is marked by the formation of a symmetrically located septum yielding two cells of equal size. However, under starvation conditions, the cell division machinery is altered; an asymmetrically located septum forms creating dissimilar progeny, each with a separate developmental fate (20, 31). The smaller cell, or prespore, is engulfed by the larger, mother cell shortly after septation and differentiates into a distinct cell type called the endospore. The mother cell is largely responsible for the deposition of protective layers around the outer surface of the engulfed prespore (termed the forespore) and lyses upon maturity of the spore.

The process of sporulation requires the activity of five sporulation-associated RNA polymerase sigma subunits, which direct both temporal and spatial control over the transcription of sporulation genes (6, 19). In the latter stages of sporulation, the formation and activity of two of the sigma factors, $\sigma^G$ and $\sigma^K$, is known to be compartmentalized into the forespore and mother cell, respectively (19). This compartmentalization is established, at least in part, by the earlier-acting factors $\sigma^F$ and $\sigma^E$, which are thought to be responsible for the compartment-specific transcription of the genes encoding $\sigma^G$ and $\sigma^K$ (16, 17, 26, 36, 40). Since the transcription of the structural genes for $\sigma^E$ and $\sigma^F$ occurs before spore septum formation (8, 25), it is not compartmentalized; the establishment of compartmentalized gene expression must then involve a mechanism for confining $\sigma^E$ and $\sigma^F$ activity. How this mechanism operates is one of the major unsolved problems of sporulation, but it is thought likely that the activation and/or activity of $\sigma^F$ is crucial for the establishment of compartment-specific gene expression (19).

The $\sigma^F$ protein is encoded by the *spoIIAC* gene (42), which is located within the tricistronic *spoIIA* operon (7, 33). The *spoIIA* operon also encodes two proteins which are involved in the posttranslational regulation of $\sigma^F$ (36) and possibly $\sigma^G$ (3) activity. Low-level transcription of the operon is detected during vegetative growth but is induced at much higher levels about 1 h after the start of sporulation (30, 48). This induction of *spoIIA* requires E$\sigma^H$ and the SpoOA transcription regulator (27, 30, 47).

An additional level of *spoIIA* transcription regulation involves the later, probably postseptation, cotranscription of the entire *spoIIA* operon with an upstream gene, designated *dacF* (48). The *dacF* gene codes for a protein that is similar to DD-carboxypeptidases (48), enzymes involved in cell wall biosynthesis; this protein has not been identified. The *dacF-spoIIA* transcript is induced about 2 h after induction of the earlier, shorter *spoIIA* transcript (35, 48). The role of the *dacF-spoIIA* transcript is unknown, since its disruption has no noticeable effect on the efficiency of spore formation or germination (48). The presence of high levels of β-galactosidase activity in germinating spores isolated from *spo*$^+$ strains bearing transcriptional *dacF-lacZ* fusions indicates that *dacF* is transcribed in the forespore compartment (48). A more extensive analysis of the regulatory mechanisms governing *dacF-spoIIA* expression is reported here. We present evidence that *dacF-spoIIA* transcription requires its own encoded sigma factor, $\sigma^F$.

## MATERIALS AND METHODS

**Bacterial strains.** The *Escherichia coli* strain used was DH5α, F$^-$ *endA1 hsdR17*($r_K^-$ $m_K^+$) *supE44 thi-1* $\lambda^-$ *recA1 gyrA96 relA1* Δ(*lacZYA-argF*)U169 φ80d*lacZ*ΔM15 (Bethesda Research Laboratories). The *B. subtilis* strains used are described in Table 1. Strains containing single-copy promoter-*lacZ* fusions at *amyE* were constructed by transformation with plasmid (SL4834) or chromosomal DNA (all others) as donor. Clones in which the *dacF-lacZ* fusion had integrated at the *amyE* locus by double crossover and those containing three copies of a fusion at *dacF* were identified by Southern blot analysis of appropriately restricted DNA. The insertion of the *spoIIIG*Δ*neo* mutation into strain SL5433 was also confirmed by Southern blotting; in it, the *Pst*I-*Bam*HI region of *spoIIIG* (+453 to +517, relative to its transcription start site) in pMLK101 (kindly provided by Margaret Karow) was replaced by a *Pst*I-*Bam*HI neomycin resistance cassette-bearing fragment from pBEST501 (13).

**Plasmids.** All plasmids were maintained in *E. coli* DH5α unless otherwise stated. Plasmid pPP324 was constructed by

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TABLE 1. *B. subtilis* strains used

| Strain | Relevant genotype | Source and/or reference[a] |
|---|---|---|
| BD34 | *thr-5 leuA8 metB5* | D. Dubnau |
| BR151 | *trpC2 lys-3 metB10* | F. E. Young |
| MB21 | *leuA8 metC3* | 11 |
| MB24 | *trpC2 metC3* | 32 |
| SL59 | *spoIIIC94 trpC2* | 94U (12, 31)→SL10 (4) |
| SL219 | *spoIIID83 metC3* | 83U (12, 31)→MB21 |
| SL547 | *spoIIE20 trpC2* | N25 (28, 31)→MB24 |
| SL554 | *spoIVCB23 metC3* | E31 (28, 31)→MB21 |
| SL604 | *spoIIIE47 metB5 thr-5* | NG8.17 (28, 31)→BD34 |
| SL608[b] | *spoIIGA49 leuA8* | NG10.2 (28, 31)→MB21 |
| SL609 | *spoIIGA49 metC3* | NG10.2 (28, 31)→MB24 |
| SL639 | *spoIIIA65 metC3* | NG17.17 (28, 31)→MB24 |
| SL670 | *spoIIIJ87 metC3* | 87 (11, 31)→MB21 |
| SL675 | *spoIVF88 metC3* | 88 (6, 11, 31)→MB21 |
| SL3980 | *spoIID66 trpC2* | NG17.22 (28, 31)→MB24 |
| SL4207 | *trpC2 metC3 spoVE*::pPP242 (2 copies of *spoVE-lacZ*) | 9 |
| SL4342 | *spoIIIGΔ1 trpC2 phe-1* $P_{spac}$-*spoIIAC* | RS217 (36) |
| SL4372[c] | *trpC2 metC3* $P_{spac}$-*spoIIGB* | pDG180 (39)→MB24 |
| SL4542 | *trpC2 metC3 dacF*::pPP293 (3 copies of *dacF-lacZ*) | 48 |
| SL4809 | *spoIVF88 metC3 amyE::dacF-lacZ* | SL4834→SL675 |
| SL4810 | *spoIIIA65 metC3 amyE::dacF-lacZ* | SL4834→SL639 |
| SL4812 | *spoIVCB23 metC3 amyE::dacF-lacZ* | SL4834→SL554 |
| SL4813 | *spoIIIC94 trpC2 amyE::dacF-lacZ* | SL4834→SL59 |
| SL4818 | *spoIIIE36 trpC2 amyE::dacF-lacZ* | SL4834→SL14 (4) |
| SL4829 | *spoIVB165 trpC2 amyE::dacF-lacZ* | SL4834→SL765 (2) |
| SL4832 | *spoIIIE47 metB5 thr-5 amyE::dacF-lacZ* | SL4834→SL604 |
| SL4834 | *trpC2 metC3 amyE::dacF-lacZ* | pPP324→MB24 |
| SL4837 | *trpC2 metC3 amyE::lacZ* | pDH32 (38)→MB24 |
| SL4838 | *spoIIID83 metC3 amyE::dacF-lacZ* | SL4834→SL219 |
| SL5008 | *spoIIIGΔ1 trpC2 phe-1* $P_{spac}$-*spoIIAC* SPβ::*cotA-lacZ* | SC34[d]→SL4342 |
| SL5010 | *spoIIIGΔ1 trpC2 phe-1* $P_{spac}$-*spoIIAC dacF*::pPP293 (3 copies of *dacF-lacZ*) | SL4542→SL4342 |
| SL5012 | *spoIIIGΔ1 trpC2 phe-1* $P_{spac}$-*spoIIAC amyE::dacF-lacZ* | SL4834→SL4342 |
| SL5052 | *spoIIIGΔ1 trpC2* $P_{spac}$-*spoIIIG* | pDG298 (42)→SL4007 |
| SL5060 | *spo0H17 trpC2 amyE::dacF-lacZ* | SL4834→SL513 (46) |
| SL5061 | *spoIIIGΔ1 trpC2 amyE::dacF-lacZ* | SL4834→SL4007 (8) |
| SL5106 | *spoIIIGΔ1 trpC2* $P_{spac}$-*spoIIIG dacF*::pPP293 (3 copies of *dacF-lacZ*) | SL4542→SL5052 |
| SL5108 | *spoIIIGΔ1 trpC2* $P_{spac}$-*spoIIIG amyE::dacF-lacZ* | SL4834→SL5052 |
| SL5115 | *spoIIAA69 trpC2 lys-3 amyE::dacF-lacZ* | SL4834→SL1013 (18) |
| SL5117 | *spoIIAC1 trpC2 amyE::dacF-lacZ* | SL4834→SL401 (18) |
| SL5124 | *spoIIIGΔ1 trpC2* $P_{spac}$-*spoIIIG* SPβ::*cotA-lacZ* | SC34[d]→SL5052 |
| SL5132 | *trpC2 metC3* $P_{spac}$-*spoIIGB amyE::dacF-lacZ* | SL4834→SL4372 |
| SL5151 | *spoIIIJ87 metC3 amyE::dacF-lacZ* | SL4834→SL670 |
| SL5228 | *spoIID66 trpC2 amyE::dacF-lacZ* | SL4834→SL3980 |
| SL5230 | *spoIIE20 trpC2 amyE::dacF-lacZ* | SL4834→SL547 |
| SL5433 | *spoIIIGΔneo trpC2 lys-3 metB10* | pMLK101.*neo*→BR151 |
| SL5527 | *spoIIGBΔerm phe-1 trpC2* | B. Beall (EU8701) |
| SL5537 | *spoIIIGΔneo trpC2 metC3 amyE::dacF-lacZ* | SL5433→SL4834 |
| SL5545 | *spoIIGBΔerm phe-1 trpC2 amyE::dacF-lacZ* | SL4834→SL5527 |
| SL5557 | *spoIIGA49 trpC2 amyE::dacF-lacZ* | SL608→SL4834 |
| SL5558 | *spoIIGB55 trpC2 amyE::dacF-lacZ* | SL617 (29)→SL4834 |
| SL5568 | *spoIIGA49 metC3 amyE::dacF-lacZ* | SL4834→SL609 |
| SL5623 | *spoIIGB55 spoIIIGΔneo trpC2 amyE::dacF-lacZ* | SL5433→SL5558 |
| SL5625 | *spoIIGA49 spoIIIGΔneo metC3 amyE::dacF-lacZ* | SL5433→SL5568 |
| SL5678[e] | *trpC2 lys-3 metB10 amyE::spoIIIG-lacZ* | pMLK138→BR151 |
| SL5679 | *spoIIIE36 trpC2 dacF*::pPP293 (3 copies of *dacF-lacZ*) | SL4542→SL14 (4) |
| SL5680 | *spoIIIE47 metB5 thr-5 dacF*::pPP293 (3 copies of *dacF-lacZ*) | SL4542→SL604 |
| SL5709 | *spoIIIGΔ1 trpC2 phe-1* $P_{spac}$-*spoIIAC amyE::spoIIIG-lacZ* | SL5678→SL4342 |
| SL5772 | *trpC2 metC3* $P_{spac}$-*spoIIGB spoVE*::pPP242 (2 copies of *spoVE-lacZ*) | SL4207→SL4372 |
| SL5774 | *spoIIIGΔ1 trpC2* $P_{spac}$-*spoIIIG sspA::sspA-lacZ* | SC262[d]→SL5052 |
| SL5777 | *trpC2 metC3* $P_{spac}$-*spoIIGB dacF*::pPP293 (3 copies of *dacF-lacZ*) | SL4542→SL4372 |

[a] *X*→*Y* indicates the donor DNA (*X*) and the recipient strain (*Y*) used in the strain construction.
[b] Contains an additional, uncharacterized mutation.
[c] Constructed by A. Henriques.
[d] Provided by S. Cutting.
[e] Constructed by M. Karow.

using a 597-bp *Eco*RV-*Dra*I fragment (extending from −97 to +500, relative to the *dacF* transcription start site) (48) isolated from pHM2 (18), which was inserted into the *Eco*RI site (blunt end created with mung bean nuclease) of pDH32 (38). The structure of pPP324 was confirmed by the analysis of restriction enzyme digestion patterns and by DNA sequencing of the junction regions. Plasmid pMLK138 contains a *spoIIIG Hin*dIII-*Pst*I fragment, extending from positions −307 to +120 (relative to the *spoIIIG* transcription start site) (15), fused to *lacZ* in pDH32 (38).

**Media.** *E. coli* was maintained on L agar supplemented with ampicillin (50 μg/ml) when required (32). *B. subtilis* was maintained by using Schaeffer's sporulation agar, L agar, or modified Schaeffer's sporulation medium lacking glucose (MSSM) (32), depending on the circumstance. When appropriate, *B. subtilis* was grown in the presence of antibiotics at the following concentrations: chloramphenicol, 5 μg/ml; neomycin, 5 μg/ml on agar and 0.25 μg/ml in broth; and erythromycin, 1 μg/ml.

**Sporulation.** The induction of sporulation was carried out in MSSM as described previously (32); the omission of glucose from the medium resulted in *dacF-lacZ* being expressed about 1 h earlier than described previously (48). Growth was followed by measuring the optical density at 600 nm ($OD_{600}$) and converting this to milligrams (dry weight) of bacteria per milliliter with a standard calibration curve. Time is indicated in hours after the end of exponential growth ($t_1$, 1 h; $t_2$, 2 h; etc.). Sixteen hours after the end of exponential growth, cultures were analyzed by phase-contrast microscopy to determine the extent of sporulation.

**Induction of the $P_{spac}$ promoter by IPTG.** Overnight cultures were diluted 100-fold into 50 ml of prewarmed MSSM and incubated at 37°C with aeration. At an $OD_{600}$ of 0.3, cultures were divided in two; to one of the split cultures, 1 mM isopropyl-β-D-thiogalactoside (IPTG) was added. Immediately after IPTG addition and at 30-min intervals thereafter, the $OD_{600}$ was determined for each culture (with and without IPTG) and 1.0-ml samples were removed for β-galactosidase assays. Bacteria were harvested by centrifugation and stored at −20°C for later analysis of β-galactosidase activity.

**β-Galactosidase activity.** Samples were assayed with *o*-nitrophenyl-β-D-galactopyranoside (ONPG) as a substrate as described by Nicholson and Setlow (23). Specific β-galactosidase activity is expressed as nanomoles of ONPG hydrolyzed per minute per milligram (dry weight) of bacteria.

**Other methods.** Methods used for transformation and for chromosomal and plasmid DNA isolation have been described previously (46).

## RESULTS

**Dependence of *dacF-lacZ* expression on the genes encoding the five known sporulation-associated sigma factors.** Chromosomal DNA isolated from the $spo^+$ strain SL4834 was used to transfer the 597-bp *dacF-lacZ* fusion into a set of sporulation mutants carrying lesions in the coding regions of the five sporulation-associated sigma factors. Strains carrying the fusion were then induced to sporulate. *dacF-lacZ* expression was unimpaired by mutations in either *spoIIIC* or *spoIVCB* (Fig. 1A), both of which encode the mother cell-specific sigma factor $\sigma^K$ (6). Mutations in *spoIIGB*, which encodes $\sigma^E$ (44), did not impair *dacF-lacZ* expression; indeed, the fusion was induced at higher levels than in the corresponding $spo^+$ strain (Fig. 1B).

Mutations in *spo0H*, encoding $\sigma^H$ (5), and *spoIIAC*, encoding $\sigma^F$ (42), blocked expression of *dacF-lacZ* (Fig. 1C). The amount of fusion-directed activity in these two backgrounds

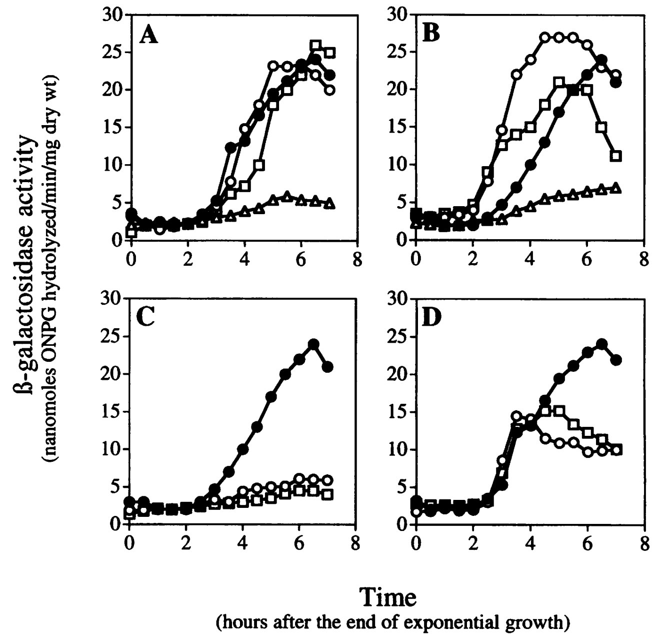


FIG. 1. Effect on *dacF-lacZ* expression of mutations in structural genes for sigma factors. Strains were cultured in MSSM with or without chloramphenicol (depending on strain requirements) and sampled for the determination of β-galactosidase activity at the indicated times after the end of exponential growth. In panels C and D, the values for background are not shown but are similar to the values shown in panels A and B for strains MB24 and SL4837, respectively. (A) △, MB24; ●, SL4834 (MB24 *amyE::dacF-lacZ*); ○, SL4813 (*spoIIIC94 amyE::dacF-lacZ*); □, SL4812 (*spoIVCB23 amyE::dacF-lacZ*). (B) △, SL4837 (MB24 *amyE::lacZ*); ●, SL4834 (MB24 *amyE::dacF-lacZ*); ○, SL5558 (*spoIIGB55 amyE::dacF-lacZ*); □, SL5545 (*spoIIGBΔerm amyE::dacF-lacZ*). (C) ●, SL4834 (MB24 *amyE::dacF-lacZ*); ○, SL5117 (*spoIIAC1 amyE::dacF-lacZ*); □, SL5060 (*spo0H17 amyE::dacF-lacZ*). (D) ●, SL4834 (MB24 *amyE::dacF-lacZ*); ○, SL5061 (*spoIIIGΔ1 amyE::dacF-lacZ*); □, SL5537 (*spoIIIGΔneo amyE::dacF-lacZ*). The results represent the average of two independent experiments with each strain.

was similar to the levels of activity detected in the strain bearing no fusion (Fig. 1A) and in the strain bearing a promoterless *lacZ* (Fig. 1B), indicating an absolute requirement for the products of *spo0H* and *spoIIA* in *dacF-lacZ* expression. *dacF-lacZ* expression was also blocked in a *spoIIAC37* background (data not shown). Because *spoIIA* transcription requires $\sigma^H$ (47) and *spo0H* transcription does not require $\sigma^F$ (45), these findings are consistent with transcription of *dacF-spoIIA* by RNA polymerase containing $\sigma^F$.

Mutations in *spoIIIG*, encoding $\sigma^G$ (15, 42), reduced but did not abolish *dacF-lacZ* expression (Fig. 1D). Expression appeared to increase until $t_4$ to $t_5$ at the same rate as in the $spo^+$ strain. Thereafter, β-galactosidase activity continued to increase in the $spo^+$ strain but declined in the two different *spoIIIG* mutants tested. This is consistent with a change in the regulation of *dacF-spoIIA* transcription during the latter stages of sporulation to a mechanism requiring $\sigma^G$.

**An extended analysis of the effects of *spo* mutations on the expression of *dacF-lacZ* supports *dacF-spoIIA* transcription by $E\sigma^F$ and by $E\sigma^G$.** The results of an extended analysis of the effects of *spo* mutations on *dacF-lacZ* expression were most similar to those obtained for *spoIIIG* and *gpr* expression (6, 15, 25, 37, 43), which is known to involve both $E\sigma^F$ and $E\sigma^G$ (40,

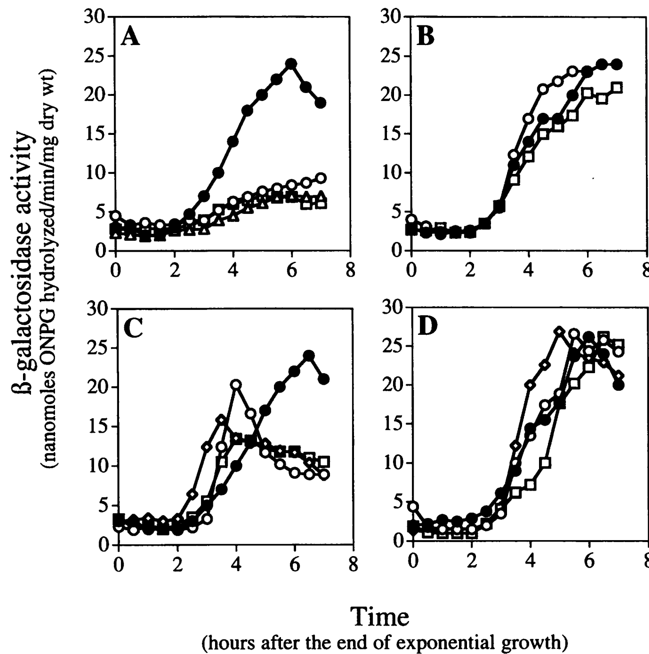


FIG. 2. *dacF-lacZ* expression in the presence of *spo* mutations that affect sigma factor activity. The results were obtained in the same manner as those shown in Fig. 1. Values for background activity in panels B to D are not shown but are similar to the value shown in panel A for SL4837. (A) △, SL4837 (MB24 *amyE::lacZ*); ●, SL4834 (MB24 *amyE::dacF-lacZ*); ○, SL5230 (*spoIIE20 amyE::dacF-lacZ*); □, SL5115 (*spoIIAA69 amyE::dacF-lacZ*). (B) ●, SL4834 (MB24 *amyE::dacF-lacZ*); ○, SL5568 (*spoIIGA49 amyE::dacF-lacZ*); □, SL5557 (*spoII GA49 amyE::dacF-lacZ*). (C) ●, SL4834 (MB24 *amyE::dacF-lacZ*); ○, SL4810 (*spoIIIA65 amyE::dacF-lacZ*); □, SL5228 (*spoIID66 amyE::dacF-lacZ*); ◇, SL5151 (*spoIIIJ87 amyE::dacF-lacZ*). (D) ●, SL4834 (MB24 *amyE::dacF-lacZ*); ○, SL4838 (*spoIIID83 amyE::dacF-lacZ*); □, SL4809 (*spoIVF88 amyE::dacF-lacZ*); ◇, SL4829 (*spoIVB165 amyE::dacF-lacZ*). In the course of this work, we noticed that some *spoIIGA* mutant strains had apparently accumulated second-site mutations that prevented *dacF-lacZ* expression.

41, 43). Expression was blocked in a strain containing a *spoIIE20* mutation (Fig. 2A) or a *spoIIE48* mutation (data not shown). Mutations in *spoIIE* prevent E$\sigma^F$- and E$\sigma^E$-dependent transcription (14, 21). A *spoIIAA69* mutation, which blocks $\sigma^F$ activity (36), also prevented *dacF-lacZ* expression (Fig. 2A). Since *dacF-lacZ* is expressed in a *spoIIGB* mutant (Fig. 1B) lacking $\sigma^E$, it is the absence of $\sigma^F$ activity that appears to prevent *dacF* expression in the *spoIIE* and *spoIIAA* mutants. Consistent with this interpretation, mutation in *spoIIGA*, which is required for $\sigma^E$ activity but not $\sigma^F$ activity (6, 19), did not block *dacF-lacZ* expression (Fig. 2B).

The loci *spoIID*, *spoIIIA*, and *spoIIIJ* are required for activation of $\sigma^G$ (6). Consistent with E$\sigma^G$ being required for late *dacF-spoIIA* transcription, mutations in each of these loci curtailed the expression of *dacF-lacZ* after $t_4$ (Fig. 2C), a result similar to that obtained with the *spoIIIG* mutants (Fig. 1D). Mutations in *spoIIID*, *spoIVF*, and *spoIVB* that do not prevent $\sigma^F$, $\sigma^E$, or $\sigma^G$ activity but affect the activity of $\sigma^K$ (6, 19) had little or no effect on the expression of the *dacF-lacZ* fusion (Fig. 2D).

**Effects of sigma factor induction on the activity of a *dacF-lacZ* fusion.** To examine further the role of $\sigma^F$ in *dacF-spoIIA* transcription, we made use of a fusion of *spoIIAC* to the IPTG-inducible promoter $P_{spac}$ (36, 49), so that $\sigma^F$ synthesis

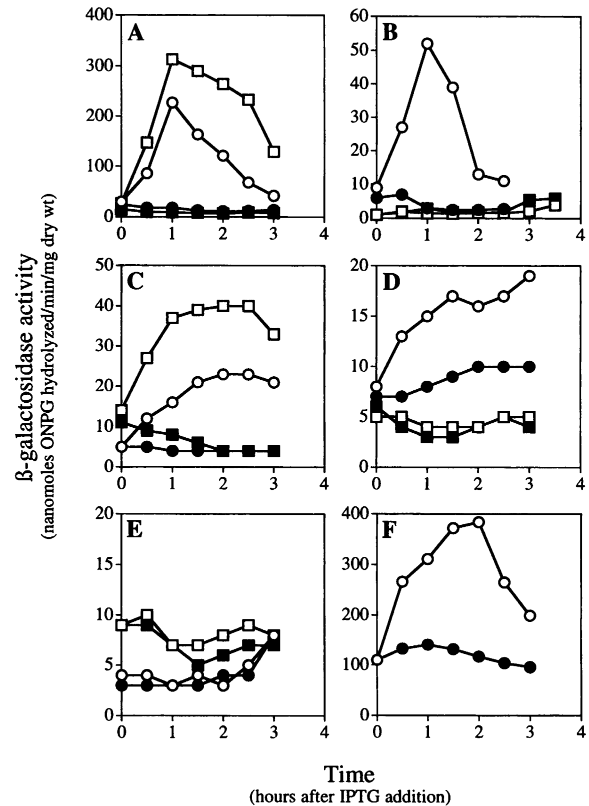


FIG. 3. Effect on *dacF-lacZ* expression of induction of sigma factors whose structural genes have been placed under inducible control by IPTG. Strains that carried a $P_{spac}$-*spoIIAC* fusion were used in panels A and B. Strains that contained an integrated copy of pDG298 ($P_{spac}$-*spoIIIG*) were used in panels C and D, whereas strains with an integrated copy of pDG180 ($P_{spac}$-*spoIIGB*) were used in panels E and F. Values are shown for cultures with (□, ○) and without (■, ●) IPTG added. (A) □, ■, SL5010 (*dacF*::pPP293 [three copies of *dacF-lacZ*]); ○, ●, SL5012 (*amyE::dacF-lacZ*). (B) □, ■, SL5008 (SPβ::*cotA-lacZ*); ○, ● SL5709 (*amyE::spoIIIG-lacZ*). (C) □, ■, SL5106 (*dacF*::pPP293 [three copies of *dacF-lacZ*]); ○, ●, SL5108 (*amyE::dacF-lacZ*). (D) □, ■, SL5124 (SPβ::*cotA-lacZ*); ○, ●, 5774 (*sspA::sspA-lacZ*). (E) □, ■, SL5777 (*dacF*::pPP293 [three copies of *dacF-lacZ*]); ○, ●, SL5132 (*amyE::dacF-lacZ*). (F) ○, ●, 5772 (*spoVE*::pPP242 [two copies of *spoVE-lacZ*]). In each case, the results have been repeated at least twice in separate experiments.

was induced by IPTG addition. High levels of β-galactosidase activity were detected in the $P_{spac}$-*spoIIAC* strain with a *dacF-lacZ* fusion at either *dacF* or *amyE*, within 30 min after addition of IPTG (Fig. 3A). These strains contained a *spoIIIGΔ1* mutation, so that $\sigma^G$ was not produced. Expression of a *spoIIIG-lacZ* fusion was also induced after IPTG addition, whereas expression of a $\sigma^K$-controlled *cotA-lacZ* fusion (34) was not (Fig. 3B). Thus, $\sigma^F$, induced during vegetative growth, is capable of directing *dacF-lacZ* expression.

Induction of $\sigma^G$ from the $P_{spac}$ promoter also induced *dacF-lacZ* expression (Fig. 3C). The induction of $\sigma^G$ resulted in the expression of an *sspA-lacZ* fusion (which has a $\sigma^G$-controlled promoter) (24); however, a *cotA-lacZ* fusion was silent (Fig. 3D). The levels of enzyme activity for the *dacF-lacZ* fusions were much lower after $\sigma^G$ induction than after $\sigma^F$ induction (Fig. 3A), as was the case for a *gpr-lacZ* fusion (as referred to in reference 43). Induction of $\sigma^E$ from the $P_{spac}$ promoter led to no observable activity from the *dacF-lacZ*

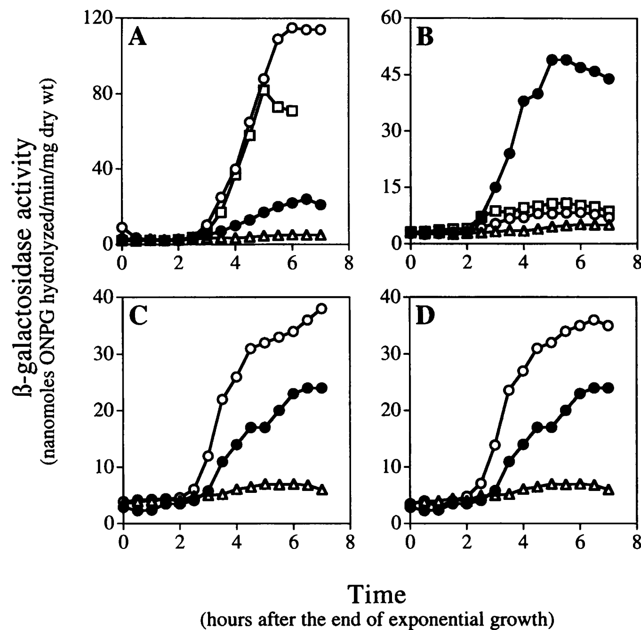


FIG. 4. Expression of *dacF-lacZ* in *spoIIIE* and in *spoIIG spoIIIG* backgrounds. (A) △, MB24; ●, SL4834 (MB24 *amyE*::*dacF-lacZ*); ○, SL4818 (*spoIIIE36 amyE*::*dacF-lacZ*); □, SL4832 (*spoIIIE47 amyE*::*dacF-lacZ*). (B) △, MB24; ●, SL4542 (MB24 *dacF*::pPP293 [three copies of *dacF-lacZ*]); ○, SL5679 (*spoIIIE36 dacF*::pPP293 [three copies of *dacF-lacZ*]); □, SL5680 (*spoIIIE47 dacF*::pPP293 [three copies of *dacF-lacZ*]). (C) △, MB24; ●, SL4834 (MB24 (*amyE*::*dacF-lacZ*); ○, SL5623 (*spoIIGB55 spoIIIG*Δ*neo amyE*::*dacF-lacZ*). (D) △, MB24; ●, SL4834 (MB24 *amyE*::*dacF-lacZ*); ○, SL5625 (*spoIIGA49 spoIIIG*Δ*neo amyE*::*dacF-lacZ*).

fusions (Fig. 3E). Induction did result in the expression of a *spoVE-lacZ* fusion (which is transcribed by E$\sigma^E$) (10, 22) (Fig. 3F).

**Analysis of *dacF-lacZ* expression in *spoIIIE* and *spoIIG spoIIIG* backgrounds.** Sun et al. have shown that chromosomal position determines the effect of a *spoIIIE* mutation on the expression of E$\sigma^F$-transcribed genes (40, 41). The *gpr-lacZ*, *spoIIIG-lacZ*, or *gerA-lacZ* fusions integrated at *gpr*, *spoIIIG*, or *gerA*, respectively, in a *spoIIIE* background, were not expressed; however, when these fusions were integrated at the *amyE* locus, they were expressed at (the *spoIIIG-lacZ* fusion) or substantially above (the *gpr-lacZ* and the *gerA-lacZ* fusions) levels obtained with *spo*$^+$ strains (41). This chromosomal position effect in the *spoIIIE* background was also observed with *dacF-lacZ* fusions (Fig. 4A and B). In the cases of both *spoIIIE* mutants analyzed, the *dacF-lacZ* fusion at *amyE* was greatly overexpressed, whereas its expression at *dacF* was greatly reduced. This effect has been observed only with the $\sigma^F$-controlled genes (41).

It has been reported that *spoIIIG* expression (and, therefore, $\sigma^G$ activity) is blocked in *spoIIG* mutants (15, 25). Nevertheless, to exclude the possibility that residual $\sigma^G$ activity might account for the enhanced expression of *dacF-lacZ* in the *spoIIG* mutant (Fig. 1B), we tested *dacF-lacZ* expression in *spoIIG spoIIIG* double mutants. Similar high levels of expression were observed in *spoIIG spoIIIG* double mutants (strains SL5623 and SL5625; Fig. 4C and D). The elevated levels of expression (above that in the *spo*$^+$ strain) may be the result of extension of the first period of $\sigma^F$ synthesis, a loss of competition from $\sigma^E$ for core RNA polymerase, or E$\sigma^F$-directed

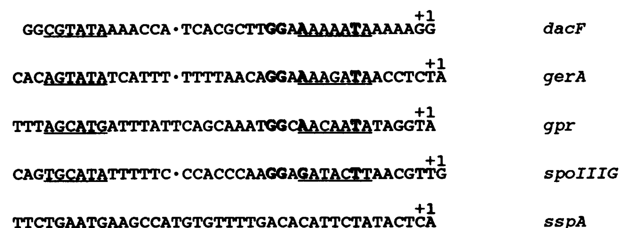


FIG. 5. Comparison of other promoters with that of *dacF*. The promoter regions of *gerA*, *spoIIIG*, and *gpr* ($\sigma^F$-controlled genes) and of *sspA* (a $\sigma^G$-controlled gene) were taken from Sun et al. (41). The +1 symbols lie immediately above the base deduced to be the start site of transcription for the indicated gene. The underlined regions denote the −10 and −35 sequences. The boldface bases represent those residues required for efficient E$\sigma^F$-directed transcription (41). To line up the various promoter sequences with that of *gpr*, gaps have been introduced and are indicated by dots.

expression of *dacF-lacZ* in both compartments of the abortively disporic *spoIIG* mutant, as suggested for similar results with *gpr* (6).

## DISCUSSION

In this report, we present three lines of evidence that *dacF-spoIIA* is transcribed by E$\sigma^F$. (i) Expression of *dacF-lacZ* required the intact structural gene for $\sigma^F$, *spoIIAC*, and that for $\sigma^H$, *spo0H*, which is required to transcribe *spoIIAC*. It did not require the intact structural genes for other sporulation-associated sigma factors (Fig. 1). (ii) Expression of *dacF-lacZ* required the correct expression of *spoIIE* and of *spoIIAA*, which are required for E$\sigma^F$ activity. It did not require the expression of a series of *spo* loci that are not required for $\sigma^F$ activity but are required for the functioning of other sporulation-associated $\sigma$ factors (Fig. 2). (iii) In a strain containing a $P_{spac}$-*spoIIAC* fusion, in which $\sigma^F$ synthesis was initiated by IPTG addition, *dacF-lacZ* expression was rapidly induced by the addition of IPTG (Fig. 3). The same three types of evidence indicate that *dacF-spoIIA* is transcribed by E$\sigma^G$ at later times during sporulation. Perhaps, as the forespore develops, the ratio of active $\sigma^F$ to active $\sigma^G$ decreases and $\sigma^G$ maintains the expression of $\sigma^F$-controlled genes such as *dacF*.

The *gpr* and *spoIIIG* loci have previously been shown to be transcribed by E$\sigma^F$ (26, 36, 40, 41, 43), and there is evidence that the *gerA* locus also belongs to the $\sigma^F$ regulon (41). *dacF-spoIIA* thus becomes the fourth member of this regulon. The *dacF-spoIIA* promoter has significant similarity to the promoters of the other three loci (41) (Fig. 5), although this was not apparent when we first described *dacF* (48). The $\sigma^F$ regulon has been subdivided on the basis of the effect of *spoIIG* mutations: expression of *spoIIIG* is greatly reduced by them, but expression of *gpr* is not (15, 25, 37). By this criterion, *dacF-spoIIA* falls into the *gpr* subdivision. This subdivision is supported by the observation that *dacF-spoIIA* and *gpr* are both overexpressed when located at *amyE* in a *spoIIIE* mutant background as compared with a *spo*$^+$ background (Fig. 4A and B) (41), whereas *spoIIIG* is expressed at a level similar to that in a *spo*$^+$ background (41).

Cotranscription of *dacF* and *spoIIA* by E$\sigma^F$ indicates that the products of *spoIIA* are capable of directing their own transcription. The system governing *spoIIA* expression seems to allow for the production of *spoIIA* transcripts, not only before septation but also after septation (as a result of autoregula-

tion), with expression becoming localized in the prespore compartment (1). Transcription of *dacF-spoIIA* by $E\sigma^G$ may serve to continue its expression within the forespore after engulfment. The expression of genes encoding other sporulation sigma factors is known to be, at least in part, autoregulated. *spoIIIG*, while it is transcribed initially by $E\sigma^F$ in the prespore, is transcribed from the same promoter by $E\sigma^G$ (40). The structural gene for $\sigma^K$ is initially transcribed by $E\sigma^E$; however, the majority of its transcription from the same promoter is accounted for by $E\sigma^K$ (16, 17). While the autoregulation of *dacF-spoIIA* is not unique, the mechanism of transcription regulation is unusual in that it involves two different promoters, the $\sigma^H$ promoter at *spoIIA* and the $\sigma^F/\sigma^G$ promoter at *dacF*, which are thought to be transcribed in different cell types (the predivisional cell and the developing prespore/forespore). The purpose of the autoregulation is unclear; it may serve to coordinate $\sigma^F$ regulatory activity with a protein, DacF, that may be involved in affecting morphological change, and/or it may serve to maintain $\sigma^F$ expression after septation. However, in the absence of a clear phenotype for strains in which the cotranscript is disrupted (48), its true significance is unknown.

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