Development of a two-part transcription probe to determine the completeness of temporal and spatial compartmentalization of gene expression during bacterial development

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We have developed a two-part test, using the *Bacillus subtilis* *sacB*/SacY transcription antitermination system, to evaluate the completeness of temporal and spatial compartmentalization of gene expression during bacterial cell development. Transcription of *sacY*(1–55) (encoding a constitutively active form of the antiterminator, SacY) is directed by one promoter, whereas transcription of *sacB′-′lacZ* (the target of SacY action) is directed by the same or another promoter. To obtain β-galactosidase activity, SacY(1–55) needs to be present when *sacB′-′lacZ* is being transcribed. We tested the system by analyzing the spatial compartmentalization of the activities of RNA polymerase σ factors, which are tightly regulated during sporulation of *B. subtilis*: σ^F and then σ^G in the prespore, σ^E and then σ^K in the mother cell. We have confirmed that the activities of σ^F and σ^E are spatially compartmentalized. We have demonstrated that there is also sharp temporal compartmentalization, with little or no overlap in the activities of σ^F and σ^G or of σ^E and σ^K. In contrast, we found no compartmentalization of the activity of the main vegetative factor, σ^A, which continued to be active alongside all of the sporulation-specific σ factors. We also found no temporal compartmentalization of expression of loci that are activated during the development of competent cells of *B. subtilis*, a developmental program distinct from spore formation. A possible mechanism to explain the temporal compartmentalization of σ^F and σ^G activities is that the anti-sigma factor SpoIIAB transfers from σ^G to σ^F.

A fundamental problem in biology is to understand how one cell type differentiates into another cell type. An early stage in cell differentiation is commonly an asymmetric division that yields two cells with distinct developmental fates associated with distinct patterns of gene expression (1). Typically, the newly formed cells undergo substantial further change before they acquire their mature differentiated characteristics. Formation of spores by *Bacillus subtilis* has become a paradigm for the study of such cell differentiation in a prokaryote. Development of cells competent for DNA-mediated transformation provides a second distinctive example of *B. subtilis* cell differentiation.

Cell differentiation is associated with substantial changes in the program of gene expression. Spatial compartmentalization of gene expression between different cell types is a major characteristic. This compartmentalization is seen in the activities of the different RNA polymerase σ factors associated with the two cell types involved in formation of spores by *B. subtilis* (2, 3). There is generally also a clear temporal progression in the pattern of gene expression within differentiating cell types. This progression is seen both in sporulation and in the development of competent cells. However, less is known about such temporal progression, and it is not clear whether there is compartmentalization of expression within the temporal progression. Here we describe the development and use of a two-part genetic test, based on the *sacB*/SacY transcription antitermination system (4), to explore the completeness of compartmentalization of gene expression, and, in particular, of temporal compartmentalization. We use the system to analyze both spore formation and competent-cell development in *B. subtilis*.

A critical early stage in spore formation is an asymmetrically located division that yields two different-sized cells, the larger mother cell and the smaller prespore (2, 3). The prespore (also called the forespore) is then engulfed by the mother cell and develops into the mature spore. The mother cell is necessary for this process but ultimately lyses. Distinct genetic programs controlling gene expression in these two compartments (cells) are initiated after the division and are primarily directed by four RNA polymerase sigma factors: σ^F and then σ^G in the prespore, and σ^E and then σ^K in the mother cell (2, 5). Induction of σ^F activity in the prespore follows immediately after septation (6). Activation of σ^G follows engulfment of the prespore by the mother cell (7). Activation of σ^E in the mother cell depends on σ^F activity (8, 9) and also follows rapidly after septum formation (6). Activation of σ^K in the mother cell depends, in turn, on activation of σ^G and thus follows engulfment (5). Although it is clear that σ^G becomes active well after σ^F, it is not known to what extent or how rapidly σ^F activity declines once σ^G becomes active. Likewise, it is not clear whether σ^E activity persists after σ^K becomes active.

The major vegetative σ factor, σ^A, is present in sporulating *B. subtilis*, but the proportion associated with core RNA polymerase in cell extracts declines dramatically during sporulation (10, 11). A small portion of σ^A does remain associated with core RNA polymerase even at late stages of sporulation (11, 12). It seems likely that E-σ^A would be active during sporulation to provide housekeeping functions. However, to our knowledge, such activity has not been demonstrated in either the prespore or the mother cell.

Competent *B. subtilis* cells develop by a program that is separate from the sporulation program. Only 1–10% of a population becomes competent. The competent cells are nongrowing and are blocked in DNA replication; they show a prolonged lag before they can resume growth in fresh medium (13). Their development involves a distinct program of gene expression that is not characterized by changes in sigma factors. Environmental signals act via a complex signaling pathway involving the products of a number of loci, including *srfA*, to initiate competence development. This pathway leads to transcription of *comK*, which encodes the key transcription factor that activates transcription of late competence loci, such as *comG*. ComK activity is itself subject to complex regulation (13).

The *B. subtilis* SacY protein regulates *sacB* transcription by antitermination (14). In the absence of SacY, the structural gene

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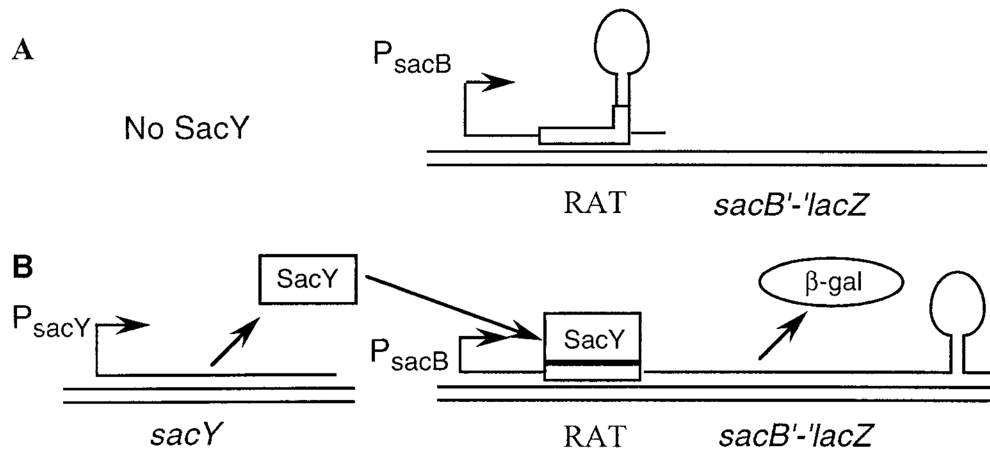

Fig. 1. Schematic representation of the *sacB*/SacY transcription antitermination system. (*A*) In the absence of SacY, transcription of *sacB′*-*′lacZ* terminates upstream of the coding sequence for SacB′-′LacZ, and thus no β-galactosidase is formed. (*B*) In the presence of SacY, SacY binds to the RAT site in the nascent mRNA, causing antitermination so that transcription proceeds through the SacB′-′LacZ coding sequence and β-galactosidase is formed. In the system used here, different promoters were substituted for P_{sacB} and P_{sacY}; *sacY*(1–55), encoding a constitutively active form of SacY, was used in place of *sacY*.

for SacB, levansucrase, is not transcribed. The *sacB* transcription regulation occurs at a site, RAT, which overlaps a rho-independent terminator structure in the leader region of the nascent *sacB* mRNA (Fig. 1; in the present study a *sacB′*-*′lacZ* translational fusion was used). Binding of SacY to this RAT site blocks formation of the terminator structure and thus allows transcription of the *sacB* structural gene (4). The activity of SacY is naturally regulated by sucrose through the phosphotransferase system (PTS). We have used a constitutively active form, SacY(1–55), that is active for antitermination independent of the PTS (15). We have placed *sacY*(1–55) and a *sacB′*-*′lacZ* translational fusion under the control of different combinations of promoters. We have tested the system by analyzing the activities of σ^F and σ^E, which are spatially compartmentalized (2). We find direct evidence of temporal compartmentalization of σ^F and σ^G activities during prespore development, and of σ^E and σ^K activities during mother cell development. We find that activity of the main vegetative σ factor, σ^A, is not compartmentalized with respect to any of the sporulation-associated σ factors. We do not observe temporal compartmentalization during the successive activation of transcription of *srfA*, *comK*, and *comG* during competence development.

Materials and Methods

Strains. *B. subtilis* strains used in this study, apart from BR151, were constructed from SL7643. SL7643 is a derivative of SA501 (15), which was obtained by curing SA501 of the prophage SPβ (and inserts within the prophage) by growth at 50°C. Strain SA501, kindly provided by S. Aymerich (INRA, Thiverval-Grignon, France), has deletions of *sacY*, *sacB*, *licT*, and *sacT*, all of which might interfere with the antitermination system being studied. The *B. subtilis* strains used are available in Table 1, which is published as supporting information on the PNAS web site, www.pnas.org. *Escherichia coli* DH5α (GIBCO/BRL) was used for routine cloning.

Growth Media. Luria–Bertani broth (LB) was routinely used for bacterial growth unless otherwise stated. Modified Schaeffer's sporulation medium (MSSM; refs. 16 and 17) was used for induction of *B. subtilis* sporulation. Competence medium (18) was used for the development of competent cells. Strains were routinely grown at 37°C with aeration. Times are indicated in hours after the end of exponential growth; the end of exponential growth is conventionally taken to be the start of spore formation or competence development.

Plasmids. Plasmids with promoters cloned upstream of *sacY*(1–55) were derivatives of the shuttle plasmid pRB373 (19). The plasmids included a PCR product, amplified from pNDY55 (kindly provided by S. Aymerich), of *sacY*(1–55). The *sacY*(1–55) region was amplified by using primers designed so that a *Bgl*II site was formed upstream of *sacY*(1–55) to facilitate the cloning; the PCR product extended from 22 bp upstream of the *sacY*(1–55) translation start codon to 19 bp downstream of the stop codon. Detailed description of plasmid constructions placing different promoters upstream of *sacY*(1–55) is available in *Supporting Information*, which is published on the PNAS web site.

The P_{trpE}-*sacB′*-*′lacZ* plasmid was constructed by replacing the *Eco*RI-*Bgl*I fragment of pDH32 (20) with the *Eco*RI-*Bgl*I fragment from pIC38 (4). This fragment contains the σ^A-directed *trpE* promoter controlling a *sacB′*-*′lacZ* translational fusion (the *sacB* leader region plus the first 5 codons of *sacB* fused in-frame to the 5′ end of *lacZ*). Derivatives with different promoters controlling *sacB′*-*′lacZ* were constructed in such a way that the 5′ end of the *sacB′*-*′lacZ* transcript would be identical to that of the wild-type *sacB*-mRNA (21). The only exception to this was the P_{spoIIQ}-*sacB′*-*′lacZ* plasmid in which the transcription start site was identical to the +10 site of the artificial *sacB* transcript in pIC38 (4). Detailed description of plasmid construction is provided in *Supporting Information*.

General Methods. Transformation of *B. subtilis* cells with DNA was performed as described (18, 22). DNA manipulations and routine cloning were carried out by using standard procedures (23). β-galactosidase assays were carried out by the procedure described by Nicholson and Setlow (24), using lysozyme treatment to permeabilize cells. Specific β-galactosidase activity is expressed as nM *O*-nitrophenol-β-D-galactopyranoside hydrolyzed per min/mg bacterial dry weight.

Results

Analysis of the Spatial Compartmentalization of σ^F and σ^E Activities by Using the *sacB*/SacY Transcription Antitermination System. Active SacY is required to obtain β-galactosidase expression from a strain in which the *sacB* leader region is followed by a translational *sacB′*-*′lacZ* fusion (4). To determine whether the *sacB*/

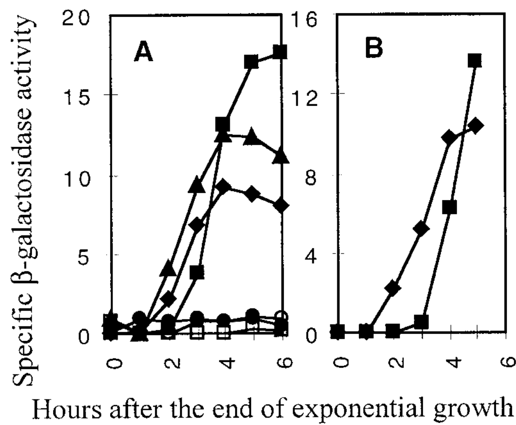

Fig. 2. Compartmentalized expression of β-galactosidase activity during sporulation as indicated by strains containing *sacB′-′lacZ* and *sacY*(1–55) under the control of different combinations of promoters directed by σ^F or σ^E. Culture growth and β-galactosidase activity are as described (28). (*A*) ▲, σ^F/σ^F; □, σ^F/σ^E; ◆, (P_{katX})σ^F/σ^F; ●, (P_{katX})σ^F/σ^E; ■, σ^E/σ^E; ○, σ^E/σ^F. (*B*) ◆, *spoIIIE::spc*, σ^F/σ^E; ■, *spoIIIE::spc*, σ^E/σ^F. Background β-galactosidase activities from strains containing the corresponding *sacB′-′lacZ* fusion, but no copy of *sacY*(1–55), have been subtracted to clarify presentation. Results are the average of values from two independent experiments. The σ^F promoter is P_{spoIIQ} except where indicated.

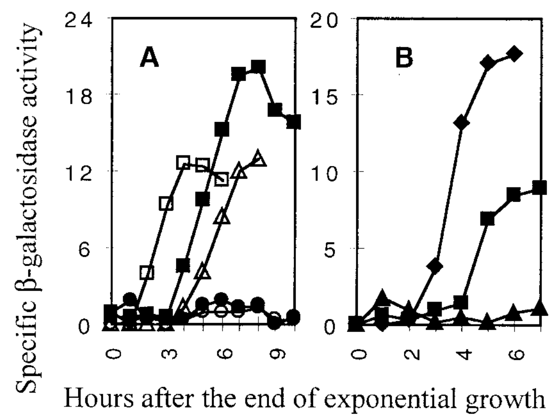

Fig. 3. Temporal progression of σ^F and σ^G (*A*) and of σ^E and σ^K (*B*) activities during sporulation as indicated by strains containing *sacB′-′lacZ* and *sacY*(1–55) under the control of different combinations of promoters. (*A*) ■, σ^G/σ^G; ○, σ^F/σ^G; ●, (P_{katX})σ^F/σ^G; □, σ^F/σ^F; △, σ^G/σ^F. (*B*) ■, σ^K/σ^K; ▲, σ^E/σ^K; ◆, σ^E/σ^E. Background β-galactosidase activities from strains containing the corresponding *sacB′-′lacZ* fusion, but no copy of *sacY*(1–55), have been subtracted to clarify presentation. Results are the average of values from two independent experiments. The σ^F promoter is P_{spoIIQ}, except where indicated, and the σ^G promoter is P_{sspA}.

SacY antitermination system could be used to evaluate compartmentalized gene expression, promoters recognized by E-σ^F or E-σ^E were tested. Fusions of *sacB′-′lacZ* to promoters of interest were introduced into the parent strain, SL7643, by double crossover at the *amyE* locus (20). Autonomously replicating plasmids were used to introduce *sacY*(1–55) under the control of the desired promoter.

A series of strains was constructed in which transcription of *sacB′-′lacZ* and *sacY*(1–55) was directed by σ^F/σ^F, σ^F/σ^E, σ^E/σ^F, or σ^E/σ^E [here, and in the sections that follow, the σ factor for *sacB′-′lacZ* is indicated first and the σ factor for *sacY*(1–55) is indicated second]. The p1 promoter of *cotE* was used as a promoter that is recognized by E-σ^E (25). Two strong well characterized E-σ^F-transcribed genes are known that are not also transcribed by E-σ^G during sporulation, *spoIIQ* and *katX* (26, 27), and their promoters were used in the present study.

Strains were induced to sporulate in modified Schaeffer's sporulation medium (MSSM). There was substantial induction of β-galactosidase during sporulation in strains with the σ^F/σ^F and the σ^E/σ^E configuration (Fig. 2*A*). However, there was little or no induction of β-galactosidase in strains with the σ^F/σ^E or σ^E/σ^F configurations. Analysis by immunofluorescence microscopy (6) indicated that β-galactosidase was confined to the prespore for σ^F/σ^F strains and to the mother cell for σ^E/σ^E strains (Table 2, which is published as supporting information on the PNAS web site). Compartmentalization σ^F and σ^E activities is disrupted in strains containing a *spoIIIE*-null mutation (29, 30). Consistent with these observations, β-galactosidase was detected in σ^F/σ^E and σ^E/σ^F strains that had a *spoIIIE::spc* mutation (31) introduced into them (Fig. 2*B*). These results reinforce our conclusion that the two-part *sacB*/SacY antitermination system (4, 15) provides an accurate test for the compartmentalization of gene expression. We have not investigated the significance of the difference in timing of β-galactosidase expression in the two strains.

Temporal Compartmentalization of σ^F and σ^G Activities in the Prespore. Two σ factors become active in the prespore during sporulation, σ^F immediately after spore septum formation and σ^G immediately after engulfment (2, 3). It remains unclear whether σ^F continues to be active after σ^G has become activated or whether σ^F activity rapidly declines. We have used the two-part *sacB*/SacY antitermination system to investigate this question. The promoters for *spoIIQ* and for *katX* were again used as σ^F-directed promoters (26, 27). The promoters for *sspA* and *sspE* were used as σ^G-directed promoters (32). Strains with the σ^G/σ^G configuration expressed substantial levels of β-galactosidase (Fig. 3*A*). By using the same σ^G-directed promoters for *sacY*(1–55) and with *sacB′-′lacZ* fusion under the control of either of the σ^F-directed promoters (the σ^F/σ^G configuration), very little β-galactosidase was detected (Fig. 3*A*), indicating that the activities of the two prespore σ factors are temporally compartmentalized. For comparative purposes, the data for σ^F/σ^F from Fig. 2*A* are incorporated into Fig. 3*A*; σ^F/σ^F was expressed 1–2 h before σ^G/σ^G, consistent with the known order of expression of σ^F and σ^G. (The data for the P_{sspA} promoter are shown; similar results were obtained with P_{sspE}.)

The two components that interact in the test are the SacY(1–55) protein and the RAT sequence of the nascent *sacB′-′lacZ* mRNA. They are not equal. The protein is relatively stable, whereas the nascent mRNA is unstable and is destined for transcription termination upstream of the structural gene encoding β-galactosidase unless SacY(1–55) is present. This distinction has clear consequences in the analysis of temporal progression, which becomes apparent in the analysis of σ^F and σ^G. By using the configuration, σ^G/σ^F, substantial β-galactosidase activity was detected, which was consistent with the SacY(1–55) protein persisting for some time after it ceased to be synthesized (Fig. 3*A*).

Temporal Compartmentalization of σ^E and σ^K Activities in the Mother Cell. σ^E is formed before spore septum formation as an inactive precursor, pro-σ^E (33). Its activation requires the E-σ^F-directed transcription of *spoIIR* and thus occurs after septation (8, 9). σ^K is also formed from an inactive precursor, pro-σ^K (34). Pro-σ^K is formed before engulfment after E-σ^E-directed transcription of its structural gene, *sigK* (35). Activation of pro-σ^K requires the E-σ^G-directed transcription of *spoIVB* and thus occurs after engulfment (36). Despite the parallels between the two mother–cell activation pathways, the mechanisms are unrelated (3). To test for temporal separation of the activities of σ^E and σ^K, we

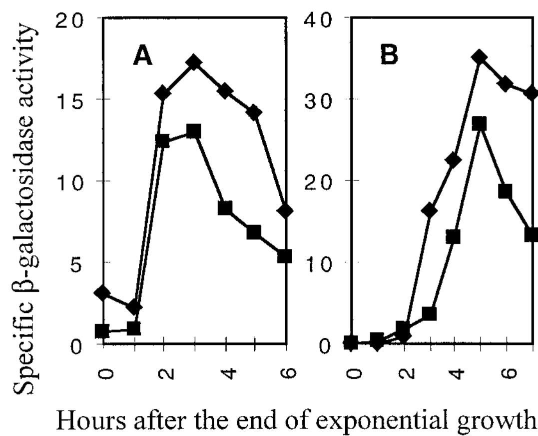

Fig. 4. Activity of σ^A during sporulation after septation (*A*) and engulfment (*B*), as indicated by strains containing *sacB′-′lacZ* and *sacY*(1–55) under the control of different combinations of promoters. (*A*) ◆, σ^A/σ^F; ■, σ^A/σ^E. (*B*) ◆, σ^A/σ^G; ■, σ^A/σ^K. Background β-galactosidase activities from SL7721 (P_{trpE}-*sacB′-′lacZ*) were subtracted from the values for the corresponding strains that contained *sacY*(1–55).

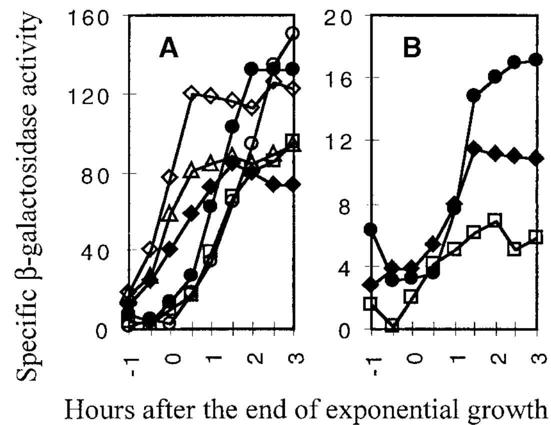

Fig. 5. Activity of different promoters during the development of competent cells as indicated by strains containing sacB′-′lacZ and sacY(1–55) under the control of different combinations of promoters. (*A*) ◆, P_{srfA}/P_{srfA}; ◇, P_{srfA}/P_{comK}; △, P_{srfA}/P_{comG}; □, P_{comG}/P_{srfA}; ○, P_{comG}/P_{comK}; ●, P_{comG}/P_{comG}. (*B*) ◆, P_{comK}/P_{srfA}; □, P_{comK}/P_{comK}; ●, P_{comK}/P_{comG}. Background β-galactosidase activities from strains containing the corresponding *sacB′-′lacZ* fusion, but no copy of *sacY*(1–55), have been subtracted to clarify presentation.

used the P1 promoter of *cotE* for σ^E (25) and the *gerE* promoter for σ^K (37). A strain with the σ^K/σ^K configuration expressed β-galactosidase activity during sporulation 1–2 h after a strain with the σ^E/σ^E configuration, consistent with the known progression in their activities (Fig. 3*B*). In contrast, very little β-galactosidase activity was detected for a strain with the σ^E/σ^K configuration, indicating that the activities of the two mother–cell σ factors are temporally compartmentalized (Fig. 3*B*).

Activity of σ^A During Sporulation. To test E-σ^A activity during sporulation, we assayed the expression of the *sacB′-′lacZ* fusion under the control of the *trpE* promoter, which is recognized by E-σ^A (38). Expression of P_{trpE}-*sacB′-′lacZ* is tightly regulated by SacY(1–55) (ref. 15 and unpublished results). Induction of β-galactosidase during sporulation was observed in strains with the σ^A/σ^F, σ^A/σ^E, σ^A/σ^G, and σ^A/σ^K configurations (Fig. 4). The results indicate that there is sufficient RNA polymerase containing σ^A to drive P_{trpE}-*sacB′-′lacZ* expression in the prespore and in the mother cell both before and after engulfment.

Transformation. The promoters of three competence-associated loci were tested. The loci, *srfA* (39), *comK* (40), and *comG* (41), are associated with the early, middle, and late phases of competent-cell development, respectively. Initiation of transcription of *comK* depends on *srfA* expression, and initiation of *comG* transcription depends on *comK* expression. There is temporal progression in the expression of the three loci (13). Our analysis with the *sacB*/SacY system confirms the temporal progression and makes it clear that transcription of the three loci is not temporally compartmentalized (Fig. 5).

Discussion

In this study we have developed a method based on the *sacB*/SacY antitermination system to analyze the temporal and spatial compartmentalization of gene expression during cell differentiation of *B. subtilis*. The essence of this two-part test is to have transcription of the *sacY*(1–55) gene (encoding a constitutively active truncated form of the antitermination protein SacY) directed by one promoter, while transcription of *sacB′-′lacZ* (the target of SacY action) is directed by the same or another promoter. In the absence of SacY(1–55), transcription of *sacB′-′lacZ* terminates before reaching the structural gene for β-galactosidase (14, 15). Consequently, SacY(1–55) needs to be present when transcription of the *sacB′-′lacZ* region is initiated to obtain β-galactosidase. Thus, the method provides a sensitive indicator for the presence of SacY during the short time when the *sacB′-′lacZ* leader region is being transcribed. The system was tested with promoters directed by σ^F or σ^E, whose activities during sporulation are compartmentalized into the prespore and the mother cell, respectively (2). The results confirmed the validity of using the *sacB*/SacY two-part system for studying the compartmentalization of gene expression. The system provides a radically different way to study spatial compartmentalization from fluorescence microscopy, which is presently the method of choice. To our knowledge, no comparably sensitive method is available for studying temporal compartmentalization of gene expression where the conclusions are applicable to individual cells. The two-part test should have general applicability to the analysis of compartmentalization of gene expression.

We have used the system to investigate the temporal compartmentalization of σ^F and σ^G activity in the prespore. The σ^G/σ^G combination showed induction of β-galactosidase activity during sporulation (Fig. 3*A*), which was confined to the postengulfment prespore as indicated by immunofluorescence microscopy (*Supporting Information*). The very low level of β-galactosidase that was detected for the σ^F/σ^G strains is entirely consistent with temporal separation of transcription from the pairs of promoters being tested. It strongly suggests that the action of σ^F ceased at the time of, or shortly after, activation of σ^G, as a very similar result was obtained with two distinct σ^F-directed promoters, P_{spoIIQ} and P_{katX}. It remains possible that the result indicates not a curtailment of σ^F action, but rather curtailment of the action of an activator, or activation of a repressor, which regulates both promoter regions. However, no such activator or repressor is known, and further, there is no motif shared by the two promoter regions that might suggest the target of action of such a hypothetical regulator. Thus, we think it is likely that the result indeed indicates curtailment of σ^F action as soon as, or very soon after, σ^G becomes active, i.e., soon after engulfment. Thus, there is essentially complete temporal compartmentalization of σ factor activity in the prespore associated with a major morphological change, completion of engulfment (Fig. 6*A*). Our results also indicate that there is comparable compartmentalization in the mother cell of the activities of σ^E and σ^K (Fig. 6*B*).

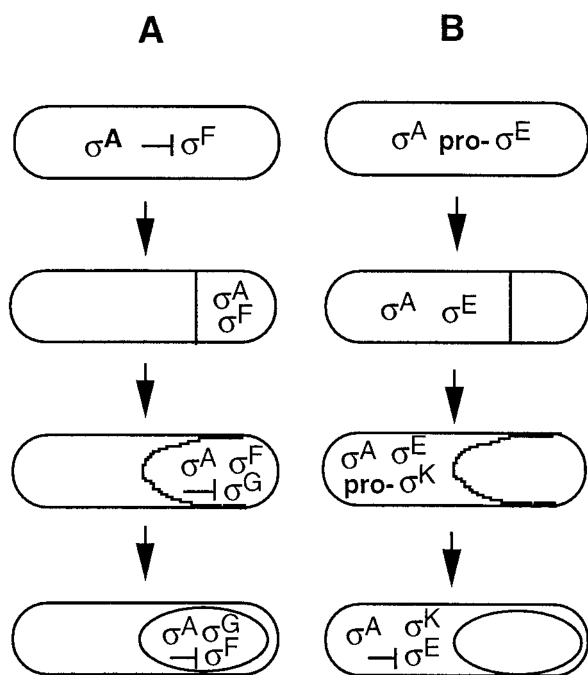

Fig. 6. Schematic representation of stages of spore formation showing σ factors associated with the prespore (*A*) and mother cell (*B*) pathways of development. For clarity, the paths are shown separately.

Temporal progression in the activation of successive transcription factors is a characteristic feature of cell differentiation, as illustrated by the successive activation of σ^F and then σ^G in the prespore, and σ^E and σ^K in the mother cell (3). However, it has not been clear how rapidly the earlier factors cease activity as the later factors become active. Techniques such as Northern blot provide a sensitive indicator of the cessation of transcription, but are limited to studies of the population as a whole. Because of population heterogeneity, they do not give a clear indication whether curtailment of the activity of an early factor occurs before or after activation of a later factor. The two-part test described here provides just such information, in essence showing that, in individual cells, σ^F has effectively ceased to be active in the prespore by the time σ^G becomes active; likewise, σ^E has effectively ceased to be active in the mother cell by the time σ^K becomes active. There is also temporal progression in the induction of transcription of loci involved in the development of competent cells of *B. subtilis*, but our results indicate that there is no temporal compartmentalization of transcription (Fig. 5). Nor do we observe temporal compartmentalization of the activity of the main vegetative σ factor, σ^A, during sporulation, in contrast to that observed for the sporulation-associated σ factors; σ^A continues to be active alongside all of the sporulation-associated σ factors (Figs. 4 and 6).

The two prespore-specific sigma factors, σ^F and σ^G, have overlapping promoter specificities (42). Some promoters, such as those for *spoIIQ*, *spoIIR*, and *katX* (8, 9, 26, 27), are recognized only by E-σ^F; some, such as *dacF* and *spoIIIG*, are recognized by E-σ^F and E-σ^G (28, 43); and some, such as *sspA* and *sspE*, are recognized only by E-σ^G (32). The rapid reduction of σ^F activity concomitant with engulfment would cause a rapid reduction in transcription of genes transcribed only by E-σ^F and provide a clear distinction between those promoters, the σ^F/σ^G promoters and the σ^G promoters. A comparable argument can be made for promoters in the mother cell recognized by E-σ^E, by both E-σ^E and E-σ^K, and by E-σ^K (3). The σ^A activity that we detected during sporulation would fulfill a need for housekeeping gene expression both before and after engulfment. It is consistent with some σ^A remaining associated with core RNA polymerase in extracts harvested late into sporulation (11, 12). Although σ^F may compete with σ^A (44), our results indicate that the two coexist *in vivo*.

An ongoing question about sporulation has been how σ^G is held inactive while σ^F is active, as both are regulated by the same anti-sigma factor, SpoIIAB (45, 46). Relief of σ^F from SpoIIAB inhibition is itself subject to complex regulation (47–49). It remains unclear how SpoIIAB apparently inhibits σ^G in conditions where σ^F is active. Our present studies raise a second question that is the reverse of the first, namely, how σ^F becomes inactive when σ^G is activated. Transcription of *spoIIAC*, the structural gene for σ^F, continues from the upstream promoter P_{dacF}, which is recognized by both E-σ^F and E-σ^G (28). Consequently, cessation of σ^F activity is not caused by cessation of transcription of its structural gene (the function of σ^G-directed *dacF-spoIIA* transcription is unclear). The apparent rapidity of the switch from σ^F to σ^G makes it unlikely that an E-σ^G-transcribed gene mediates the curtailment of σ^F activity. The rapid switch from σ^F activity to σ^G activity could be a consequence of inhibition of σ^F. A possible mechanism for σ^F inhibition is that SpoIIAB shuttles back from σ^G to σ^F, thus switching σ^F off as σ^G becomes active. The gradual loss of SpoIIAB starting at about the time σ^G becomes active (45, 50) seems to occur too slowly to invalidate this model. Loss of σ^F (50) may subsequently render permanent the switch to σ^G. Consistent with a role for degradation and loss of some protein, β-galactosidase was formed in both σ^F/σ^G and σ^G/σ^F strains in which the gene for the LonA protease (51) had been insertionally inactivated (data not shown). It may well be that both inhibition and degradation ensure the temporal compartmentalization of the activities of σ^F and σ^G.

It is formally possible that σ^G has a greater affinity for core RNA polymerase than does σ^F, and thus displaces σ^F once activated. However, σ^A and σ^F compete for core polymerase (44), and yet both are active in the prespore (Fig. 4). Likewise, σ^A and σ^G are both active in the postengulfment prespore. Thus, it is difficult to explain the temporal σ^F/σ^G compartmentalization by competition for core polymerase. Further, σ^F and σ^G activities coexist in the *lonA* mutant, so that in that background competition for core polymerase is not a problem. We think that the sudden activation of an inhibitor of σ^F, such as SpoIIAB, is a likelier explanation for the sharp separation of the activities of σ^F and σ^G.

It is also possible that an inhibitor may account for the loss of σ^E activity in the mother cell when σ^K becomes active. Activation of σ^K is by processing of the inactive precursor, pro-σ^K, and not by removal of an anti-sigma factor, so that there is no anti-sigma factor comparable to SpoIIAB. It has been shown that the appearance of σ^K accelerates the disappearance of σ^E, and multiple signals are thought to mediate this change (52, 53). σ^E becomes displaced from core RNA polymerase as σ^K appears (11). This observation is consistent with σ displacement explaining the temporal compartmentalization of σ^E and σ^K activities. However, comparable σ^E displacement was not found with a mutant σ^K that retained core binding but had lost its ability to direct transcription (11). Moreover, an excess of σ^K did not prevent E-σ^E-mediated transcription *in vitro* (12). As with σ^F and σ^G, we think that factors other than simply σ competition for core RNA polymerase are involved in the temporal compartmentalization of σ^E and σ^K activities. The factors remain to be elucidated.

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