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Disappearance of the σ^E Transcription Factor from the Forespore and the SpoIIE Phosphatase from the Mother Cell Contributes to Establishment of Cell-Specific Gene Expression during Sporulation in *Bacillus subtilis*

KIT POGLIANO,† ANTJE E. M. HOFMEISTER, AND RICHARD LOSICK*

Department of Molecular and Cellular Biology, The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

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We used immunofluorescence microscopy to investigate mechanisms governing the establishment of cell-specific gene transcription during sporulation in the bacterium *Bacillus subtilis*. The transcription factors σ^E and σ^F are synthesized shortly after the start of sporulation but do not become active in directing gene transcription until after polar division, when the activity of σ^E is confined to the mother cell and the activity of σ^F is restricted to the forespore. We show that shortly after septation, σ^E and its proprotein precursor pro-σ^E appear to be absent from the forespore and that a null mutation in *spoIIIE*, a gene known to be required for the translocation of a chromosome into the forespore, allows σ^E and/or pro-σ^E to persist and σ^E to become active in the forespore. These findings suggest that the loss of σ^E/pro-σ^E from the forespore contributes to the compartmentalization of σ^E-directed gene transcription. We also investigated the distribution of SpoIIE, a regulatory phosphatase required for the activation of σ^F which exhibits a bipolar pattern of localization shortly after the start of sporulation. Normally, SpoIIE rapidly disappears from the sporangium, first from the mother-cell pole and then from the forespore pole. Here we show that a null mutation in *spoIIIE* causes the SpoIIE phosphatase to persist at both poles. The persistence of the SpoIIE phosphatase at the mother-cell pole could explain the lack of compartmentalization of σ^F activity observed in a *spoIIIE* null mutant. We conclude that the establishment of cell-specific gene transcription involves the loss of σ^E/pro-σ^E from the forespore and the loss of the SpoIIE phosphatase from the mother-cell pole and that both processes are dependent upon the SpoIIIE protein.

A central issue in developmental biology is how the progeny of a single cell division assume dissimilar fates. An attractive experimental system in which this problem can be addressed is sporulation in *Bacillus subtilis*, in which the formation of an asymmetrically positioned septum partitions the developing cell or sporangium into a small, forespore compartment and a large, mother-cell chamber (27). The dissimilar fates of the forespore and the mother cell are determined by the RNA polymerase sigma factors σ^F and σ^E (recently reviewed in reference 35). Both transcription factors are synthesized in the predivisional sporangium, but σ^F and σ^E do not become active until after polar division, when they differentially switch on gene expression in the forespore and the mother cell, respectively.

The activity of σ^F is governed by a pathway involving the proteins SpoIIAB, SpoIIAA, and SpoIIE (1, 2, 5–8, 10, 19, 24; reviewed in reference 35). SpoIIAB is an inhibitor of σ^F, and SpoIIAA is an inhibitor of SpoIIAB. SpoIIAB is also a protein kinase that inactivates SpoIIAA by phosphorylating it on a serine residue. Thus, SpoIIAB and SpoIIAA are mutually antagonistic. Finally, SpoIIE is a serine phosphatase that activates SpoIIAA-P by dephosphorylation. It is not known how the SpoIIAB-SpoIIAA-SpoIIE pathway restricts the activation of σ^F to one cell, but the subcellular localization of SpoIIE, which is an integral membrane protein, may provide a clue. In the predivisional sporangium, SpoIIE is localized to sites of potential polar septation near both ends of the predivisional sporangium, but shortly after polar division, when σ^F becomes active, the membrane-bound phosphatase is found exclusively at the sporulation septum (3, 4). This septal localization could contribute to cell-specific activation of σ^F by bringing about a relatively higher ratio of phosphatase to kinase in the forespore (6).

Like σ^F, σ^E is present in an inactive state in the predivisional sporangium but in this case as an inactive proprotein called pro-σ^E (16; reviewed in reference 35). Activation of pro-σ^E requires the proteolytic removal of an N-terminal extension of 27 amino acids, which is mediated by the integral membrane protein SpoIIGA (14, 25, 26, 34). Evidence indicates that SpoIIGA is a receptor-protease that catalyzes the conversion of pro-σ^E to mature σ^E in response to a signal protein, SpoIIR, that is produced in the forespore under the control of σ^F (12, 15, 21). It is believed that SpoIIR is secreted from the forespore and interacts with the external domain of SpoIIGA in the mother-cell membrane of the septum, triggering the cleavage of pro-σ^E. Thus, activation of σ^E is governed by an intercellular signal transduction pathway that ties the processing of pro-σ^E to the activation of σ^F, thereby delaying the onset of σ^E-directed gene transcription until after the formation of polar septum. But what is the nature of the mechanism(s) that restricts activation of σ^E to the mother cell?

Because pro-σ^E and SpoIIGA are synthesized prior to septation, both proteins could be expected to be present in the

* Corresponding author. Mailing address: Department of Molecular and Cellular Biology, Harvard University, 16 Divinity Ave., Cambridge, MA 02138. Phone: (617) 495-4905. Fax: (617) 496-4642. E-mail: losick@biosun.harvard.edu.

† Present address: Department of Biology, University of California, San Diego, La Jolla, CA 92093-0322.

forespore as well as in the mother cell after division. If so, then some mechanism must exist for preventing SpoIIR from triggering proteolytic processing of pro-σ^E in the forespore. Two hypotheses as to the nature of this mechanism are (i) that SpoIIR, which is secreted from the forespore, acts directionally, triggering processing only in the mother cell, and (ii) that some other protein produced in the forespore under the control of σ^F prevents pro-σ^E processing or otherwise inhibits σ^E activity in the small sporangial chamber (22). However, a recent experiment by Zhang et al. (42) indicates that the compartmentalization of σ^E activity is independent of σ^F activity. In their experiments, the normal dependence of *spoIIR* expression on σ^F activity was bypassed by fusing the *spoIIR* gene to a promoter active before septation. Thus, the authors were able to investigate whether either compartmentalized synthesis of SpoIIR or σ^F-dependent expression of any other gene was necessary for compartmentalization of σ^E activity. In about half of the sporangia, σ^E apparently became active prior to polar septation, indicating that σ^E activation occurred prematurely if SpoIIR was synthesized prior to septation. However, the remaining half of the engineered sporangia had normally compartmentalized σ^E activity, a finding that indicated the existence of a σ^F-independent mechanism for the compartmentalization of σ^E activity (42).

In the present communication, we report that σ^E and pro-σ^E are apparently absent from the forespore compartment of wild-type sporangia shortly after polar septation. This suggests that one mechanism by which σ^E activity is compartmentalized is the destruction or exclusion of σ^E/pro-σ^E from the forespore. We also report that σ^E/pro-σ^E not only persists but that σ^E is also active in directing gene transcription in the forespore of sporangia bearing a null mutation in the *spoIIIE* gene. This is a surprising finding in that SpoIIIE is required for the transport of chromosomal DNA from the mother cell into the forespore; it has been demonstrated that a complete chromosome is transported into the forespore after the polar septum is formed and that in *spoIIIE* mutant sporangia the forespore receives only about 30% of the origin-proximal region of the chromosome (38, 40).

Null mutations of *spoIIIE* were previously reported to allow the release of σ^F activity in the mother cell (38), an observation reminiscent of our present discovery that SpoIIIE is required for the proper compartmentalization of σ^E activity. This led us to reexamine the localization of the SpoIIE phosphatase in *spoIIIE* mutant sporangia, since elevated levels of SpoIIE phosphatase are known to cause premature activation of σ^F (2, 10). We show that a *spoIIIE* null mutation delays the normal loss of SpoIIE phosphatase from the mother-cell (forespore-distal) pole of the sporangium, a finding that could explain the misactivation of σ^F in the large sporangial chamber. Thus, the *spoIIIE* null mutation apparently disrupts the compartmentalization of σ^F activity by inhibiting the loss of a key regulatory protein (SpoIIE) for σ^F from the mother-cell pole.

In summary, our findings suggest that the compartmentalization of gene expression during sporulation is facilitated, in part, by the loss of the SpoIIE phosphatase from the mother-cell pole, which precludes σ^F from becoming active in the large compartment of the sporangium, and by the loss of σ^E/pro-σ^E from the forespore, which precludes σ^E from becoming active in the small chamber of the sporangium.

MATERIALS AND METHODS

Antibodies and reagents. Mouse monoclonal antibodies directed against β-galactosidase were obtained from Promega and used at a 1:1,500 dilution. Rabbit polyclonal antibodies against β-galactosidase were obtained from 5′-3′ Inc. and used at a 1:1,500 dilution. Antibodies specific for σ^F (rabbit) were prepared by L. Duncan and used at a 1:200,000 dilution. Monoclonal antibodies that bind to both pro-σ^E and σ^E were a gift of W. Haldenwang and were used at a 1:20 dilution. The affinity-purified SpoIIE-specific antibodies used in immunofluorescence experiments were a gift of F. Arigoni and P. Stragier and were used at a 1:7 dilution. The SpoIIE antibodies used in Western blot (immunoblot) experiments were prepared by C. Webb and used at a 1:10,000 dilution. The secondary antibodies (from Jackson Immunolabs) were affinity-purified donkey anti-rabbit or anti-mouse antibodies conjugated either to fluorescein isothiocyanate (FITC) or to indocarbocyanine (Cy3). FITC-conjugated secondary antibodies were used at a 1:100 dilution, while Cy3-conjugated antibodies were used at a 1:200 dilution. 4′,6-Diamidino-2-phenylindole (DAPI) was obtained from Sigma and used at a final concentration of 0.2 μg/ml.

TABLE 1. Strain list

Strain[a]	Genotype
AH48	*amyE*::*spoIID-lacZ spoIIAC1 thrC*::P_{spac}-*spoIIR*
AH110	*spoIVF-lacZ spoIIIE*::*spc*
BZ184	*amyE*::*spoIID-lacZ*
KJP86	*amyE*::*sspE(2G)-lacZ spoIIIGΔ1*
KJP123	*amyE*::*sspE(2G)-lacZ spoIIIGΔ1 spoIIIE*::*spc*
KJP131	*amyE*::*spoIID-lacZ spoIIIE*::*spc*
KJP237	*amyE*::*sspE(2G)-lacZ*
KJP239	*amyE*::*sspE(2G)-lacZ spoIIIE36*
KJP241	*amyE*::*sspE(2G)-lacZ spoIIIE*::*spc*
KJP277	*amyE*::*sspE(2G)-lacZ spoIIIGΔ1 spoIIGB*::*erm*
KJP279	*amyE*::*sspE(2G)-lacZ spoIIIGΔ1 spoIIIE*::*spc spoIIGB*::*erm*
KJP286	*amyE*::*spoIID-lacZ spoIIAC1 thrC*::P_{spac}-*spoIIR spoIIIE*::*spc*
KJP288	*amyE*::*spoIID-lacZ spoIIIE36*
KJP331	*amyE*::*spoIID-lacZ*::*pMLK230* (P_{spoIIE}-*spoIIR*) *spoIIAC*::*kan*
KJP335	*amyE*::*spoIID-lacZ*::*pMLK230* (P_{spoIIE}-*spoIIR*) *spoIIIE36 spoIIAC*::*kan*
KJP337	*amyE*::*spoIID-lacZ*::*pMLK230* (P_{spoIIE}-*spoIIR*) *spoIIIE*::*spc spoIIAC*::*kan*

[a] All strains are derivatives of PY79 (41).

Bacterial strains and growth conditions. The bacterial strains used in these experiments are derivatives of PY79 and are described in Table 1. The strains containing the *amyE*::*spoIID-lacZ*::*pMLK230(PspoIIE-spoIIR)* construct (KJP331, KJP335, and KJP337) were constructed by integrating pMLK230 (*amyE*::*PspoIIE-spoIIR)* (42), a gift of P. Piggot, by a single recombination event into the *amyE*::*spoIID-lacZ* locus. pMLK230 was transformed into BZ184 (*amyE*::*spoIID-lacZ*), and both neomycin resistance (conferred by pMLK230) and chloramphenicol resistance (conferred by *amyE*::*spoIID-lacZ*) were simultaneously selected. Transformants were then screened for linkage between the two drug resistance markers by DNA transformation and for β-galactosidase activity. Chromosomal DNA from one such strain was transformed into strains containing *spoIIAC*::*kan*, with or without *spoIIIE36* and *spoIIIE*::*spc*. Four independent isolates of each strain were tested in immunofluorescence experiments; all four behaved identically.

The *spoIIIE*::*spc* mutation used in this study is an insertion-deletion mutation in which a spectinomycin resistance (*spc*) cassette was used to replace the DNA sequence between nucleotides 258 and 2001 from within the *spoIIIE* coding sequence. It was constructed by P. Levin as follows. Fragments from within the *spoIIIE* coding sequence extending from nucleotides 84 to 258 and from nucleotides 2001 to 2309 were cloned on either side of a spectinomycin resistance cassette. The resulting plasmid was linearized and used to transform strain PY79, selecting for spectinomycin resistance. Southern blot analysis confirmed that the transformants had replaced the chromosomal copy of *spoIIIE* with that from the plasmid. We presume that *spoIIIE*::*spc* is a null mutation, but it could potentially produce the N-terminal 86 amino acids of SpoIIIE (from a total of 787). The *spoIIIE*::*spc* mutation behaved indistinguishably from the *spoIIIE*::*aphA-3* mutation of Wu and Errington (38) with respect to DNA translocation, compartmentalization of σ^F activity, and the persistence of SpoIIE (data not shown).

For the results presented, sporulation was performed at 37°C and induced by the resuspension method (33) in medium supplemented with tryptophan and phenylalanine. Similar results were obtained in control experiments in DS medium (31).

Immunofluorescence methods. Immunofluorescence experiments were performed essentially as described previously (3, 11, 29). Cells were fixed in growth medium for 15 min at room temperature and for 30 min on ice, with final concentrations of 40 mM $NaPO_4$ (pH 7.5), 2.7% paraformaldehyde, and 0.0042 or 0.0065% glutaraldehyde. Samples were incubated overnight at 4°C with the primary antibodies and for several hours at room temperature with the secondary antibodies (with 50 ng of FITC-conjugated wheat germ agglutinin [FITC-WGA]/ml, as appropriate). Images were recorded on Ektachrome 400 slide film, and the images were scanned and processed with Adobe Photoshop.

Staining of the cell wall with FITC-WGA. FITC-WGA (Molecular Probes) was used to stain the cell wall during immunofluorescence studies; a final concentration of 50 ng of FITC-WGA/ml was included with the secondary antibodies. FITC-WGA reliably revealed both vegetative septa and the outline of the cells, although the quality of the staining was dependent upon the extent of lysozyme digestion used to allow antibody permeabilization. Sporulation septa were only occasionally resolved from the cell poles, presumably because the short distance between the septum and the cell pole is close to the maximum resolution of the light microscope. Despite this difficulty, the lectin staining was superior to nucleoid staining for revealing the relative location of the cell pole and, for example, σ^E activity, especially in the *spoIIIE* mutants, which have less DNA in the forespore than the wild type.

Immunostaining proteins in the forespore. During our initial immunofluorescence studies, we noted that many antibodies directed against proteins made before formation of the polar septum appeared to stain the forespore less well than the mother cell, including antibodies directed against some sporulation-specific proteins (SpoIIAB, σ^F, and σ^E), two vegetatively expressed proteins (the β-subunit of the F_1F_0 ATPase and FtsZ), and two non-*Bacillus* proteins (β-galactosidase and β-glucuronidase) (see also reference 19). In the case of σ^F, this effect was manifested as a slightly decreased intensity of staining in the forespore, not the almost complete absence of immunostaining in the forespore as is the case for σ^E. While the reason for the variable staining of proteins in the forespore is unclear, in the experiments presented here, σ^F immunostaining was readily detected in most forespores, whereas σ^E appeared to be absent. This may have been achieved by modifications to our immunofluorescence protocol. First, we now use just enough glutaraldehyde for fixation to keep the cells from lysing (a maximum of 0.0065% versus 0.04%). Second, we now incubate the samples with the primary antibodies overnight at 4°C rather than for just 1 to 2 h at room temperature. Finally, we use a lower concentration of the primary antibodies than that used in our initial studies. It has been suggested that the high concentration of DNA in the forespore contributes to the decreased immunostaining intensity in the forespore compartment (19). While this conclusion is supported by a more uniform staining of the sporangia after DNase treatment, under the conditions of our experiments, a decreased staining intensity of proteins in the forespore did not correlate with the presence of a fully translocated forespore chromosome. For example, in most of the sporangia shown in Fig. 1A to D, our σ^F antibodies stain the forespore as well as the mother cell, despite the presence of a fully condensed forespore chromosome in the sporangia shown.

Scoring criteria. We scored σ^E localization in samples taken 2 h after the start of sporulation in resuspension medium. Generally, σ^E appeared to be almost completely absent from the forespore of wild-type sporangia once chromosome translocation was complete (as judged by nucleoid staining). Occasionally, wild-type sporangia that had faint σ^E immunostaining in the forespore were observed; such sporangia were scored as having σ^E in the forespore. Similarly, the scoring of σ^E immunostaining in the *spoIIIE* mutants was blind to differences in the relative intensity of the σ^E immunostaining between the forespore and the mother cell. The majority of *spoIIIE*::*spc* mutant sporangia exhibited similar levels of σ^E immunostaining in the two sporangial compartments, although occasionally, striking differences were observed. For example, in the *spoIIIE*::*spc* null mutant (but not in the *spoIIIE36* mutant), some sporangia were observed in which the forespore stained more intensely for σ^E than did the mother cell.

Western blot sample preparation. We prepared our Western blot samples by the method described by Arigoni et al. (2) or as follows. At the appropriate time after the initiation of sporulation, 1 ml of cells was added to 110 μl of 50% trichloroacetic acid on ice. After 30 min on ice, the bacteria and precipitated proteins were harvested by centrifugation at 16,000 × *g* in a microcentrifuge for 5 min, washed once with 0.75 ml of 1 M Tris (pH 8.0), and resuspended in 60 μl of Tris-sucrose-EDTA (33 mM Tris [pH 8.0], 40% sucrose, 1 mM EDTA) to which phenylmethylsulfonyl fluoride had just been added to a final concentration of 300 μg/ml. Lysozyme was added to a final concentration at 16,000 × *g* of 1 mg/ml, and the samples were incubated at 37°C for 15 min. Two microliters of Nonidet P-40 was added; the addition of 60 μl of 2× sodium dodecyl sulfate loading buffer followed. Samples were stored at −70°C prior to running the gel.

RESULTS

Loss of σ^E/pro-σ^E from the forespore. To investigate the mechanisms of compartmentalization of σ^F and σ^E activities, we examined the distribution of σ^F and σ^E proteins by immunofluorescence microscopy. We were able to simultaneously detect σ^F and σ^E by using σ^F-specific rabbit polyclonal antibodies and σ^E-directed mouse monoclonal antibodies. The mouse monoclonal antibodies bind to both mature σ^E and pro-σ^E (36), which we therefore could not distinguish in these studies; therefore, σ^E/pro-σ^E refers to both mature σ^E and its inactive precursor pro-σ^E. We used a mutation in the gene (*spoIIIG*) for σ^G, which is closely related to σ^F, to eliminate a potential immunofluorescence signal resulting from the weak recognition of σ^G by our σ^F-directed antibodies. We included the DNA-specific dye DAPI in our fluorescence analysis to distinguish between sporangia just before and shortly after the stage of septation and to identify the forespore compartment (32). Immediately prior to septum formation, the sporangial nucleoid assumes an elongated structure known as the axial filament (30, 27). After translocation of the chromosome into the forespore (40), sporangia are readily identified by the brightly staining, condensed forespore nucleoid and the more diffuse mother-cell nucleoid (32). Later, after the stage of engulfment, the forespore chromosome becomes more diffuse and more closely resembles the mother-cell chromosome, making it harder to distinguish between the chromosomes until later, when the forespore nucleoid assumes its toroidal structure upon association with the α/β-type SASP (29).

Sporulation was initiated by the resuspension method, and 2 h after the start of sporulation, sporangia were rapidly fixed and processed for immunofluorescence (3, 29). Sporangia with an axial filament chromosome morphology showed bright and uniform σ^F staining and somewhat less intense σ^E/pro-σ^E staining (data not shown). Among sporangia with a condensed forespore nucleoid (Fig. 1D, arrows), σ^E/pro-σ^E stained more brightly but was generally (~90% of the cases) present only in the mother cell (Fig. 1B), whereas σ^F was present in both the forespore and the mother cell (Fig. 1A and C). In some sporangia, σ^F appeared to be present at reduced levels in the forespore relative to the levels in the mother cell, suggesting that either its levels decrease in the forespore or that it was less well detected in the forespore under these conditions (this issue is more fully discussed in Materials and Methods) (see also reference 19). In any case, it is clear that under these conditions, σ^E/pro-σ^E protein appeared to be substantially absent from the forespore whereas σ^F appeared to be present throughout the sporangium. We cannot be precise about the timing, but we infer that σ^E/pro-σ^E disappeared shortly after polar septum formation because σ^E/pro-σ^E was deficient or absent from almost all sporangia that exhibited a condensed forespore nucleoid (as judged by DNA staining).

A *spoIIIE* null mutation causes σ^E/pro-σ^E protein to persist and become active in the forespore. We previously noted that several proteins appear by immunofluorescence microscopy to be present at reduced levels in the forespore compartment (see Materials and Methods) and that such proteins show a more uniform distribution in sporangia bearing a null mutation (*spoIIIE*::*spc*) in *spoIIIE*, a gene needed for DNA translocation into the forespore (38, 40). We therefore localized σ^E/pro-σ^E together with β-galactosidase synthesized under the control of a σ^E-dependent promoter in wild-type and *spoIIIE*::*spc* mutant sporangia. The *lacZ* fusion used in these experiments (*spoIID-lacZ*) was present at a site (*amyE*) within the origin-proximal region of the chromosome and hence was present in the forespore in the presence or absence of the DNA translocation function of *spoIIIE* (see the introduction). Sporangia were harvested 2 h after the start of sporulation, and σ^E/pro-σ^E protein (red) and β-galactosidase produced from the *amyE*::*spoIID-lacZ* fusion (green) were localized by immunofluoresence microscopy. We found that most (91%) wild-type sporangia had both σ^E/pro-σ^E protein and σ^E activity confined to the mother cell (Fig. 1E to H; Table 2). In contrast, most (89%) *spoIIIE*::*spc* null mutant sporangia had σ^E/pro-σ^E protein in both the forespore and the mother cell (Fig. 1M to P, short arrows; Table 2), and a few sporangia appeared to have more σ^E/pro-σ^E protein in the forespore than in the mother cell. Furthermore, σ^E was active in directing gene transcription in the forespores of many of the *spoIIIE*::*spc* mutant sporangia (Fig. 1M and O, long arrows; Table 2); 2 h after the start of

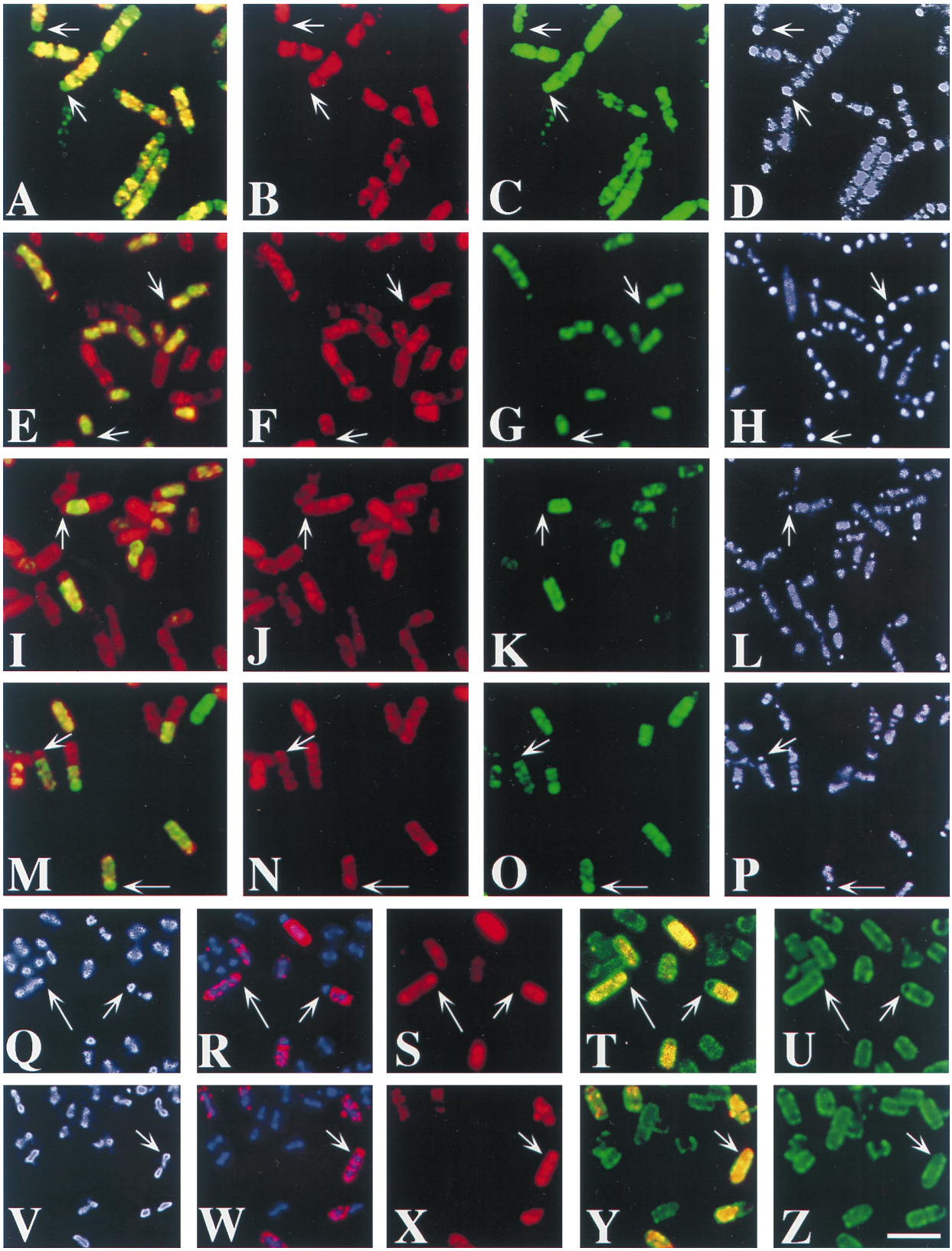

FIG. 1. Immunolocalization of σ^F, σ^E/pro-σ^E, and σ^E activity in wild-type and *spoIIIE* mutant sporangia. The sporangia were harvested 2 h after the start of sporulation and processed for immunofluorescence microscopy. Arrows point to the forespore compartment and are oriented perpendicularly to the long axis of the sporangia. The activity of σ^E was monitored by immunostaining β-galactosidase produced from a *spoIID-lacZ* fusion that had been inserted at the *amyE* locus; the DNA was stained with DAPI (blue) (D, H, L, P, Q, R, V, and W). (A to D) Immunolocalization of σ^F and σ^E/pro-σ^E in sporangia from a *spoIIIG* mutant (strain KJP86). Two sporangia displaying σ^E/pro-σ^E immunostaining (red) in the mother cell (B) and σ^F immunostaining (green) in both the mother cell and the forespore (C) are

sporulation, 38% of the mutant sporangia scored as having σ^E/pro-σ^E protein in both sporangial compartments exhibited σ^E-directed β-galactosidase synthesis in both the forespore and the mother cell. Diagrams summarizing these results are presented in Fig. 2A.

Sporangia from a strain carrying a *spoIIIE* missense mutation (*spoIIIE36*), which confers a DNA translocation defect similar to that of the null mutation (38), also frequently had σ^E/pro-σ^E protein in the forespore as well as the mother cell. Among such sporangia, many exhibited compartmentalized σ^E activity (Fig. 1I to L, arrows; Table 2). A low but significant population of missense mutant sporangia that had σ^E activity in the forespore as well as the mother cell was observed (Table 2). Thus, the missense mutation exerted a milder effect than the null mutation in breaking the compartmentalization of σ^E activity. Nonetheless, in toto, our results are consistent with the idea that the depletion of σ^E/pro-σ^E from the forespore contributes to the compartmentalization of σ^E activity and that the elimination of σ^E/pro-σ^E from the forespore depends, directly or indirectly, on SpoIIIE. At the same time, the existence of sporangia with σ^E/pro-σ^E in both compartments but exhibiting σ^E activity only in the mother cell could indicate the existence of an additional mechanism impeding σ^E-directed gene expression in the forespore (e.g., a mechanism operating at the level of pro-σ^E processing in the forespore).

A *spoIIIE* null mutation abolishes compartmentalization of σ^E activity when SpoIIR is synthesized before septation. Zhang et al. (42) reported that among cells engineered to synthesize SpoIIR prior to septation and independently of σ^F, sporangia are frequently observed that reach the stage of polar septation and exhibit compartmentalized σ^E activity. Their experiments indicated the existence of a σ^F-independent mechanism to confine σ^E activity to the mother cell (42). We therefore wondered whether the compartmentalization of σ^E-directed gene expression observed in sporangia in which SpoIIR synthesis had been uncoupled from σ^F could be attributed to the SpoIIIE-dependent loss of σ^E from the forespore.

To carry out these experiments, we constructed a strain (AH48) harboring *spoIIR* fused to the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible *spac* promoter (P*spac*) (21), *lacZ* fused to a gene under σ^E control (*amyE*::*spoIID-lacZ*), and a mutation in the gene (*spoIIAC*) coding for σ^F. In addition, we constructed a derivative (KJP286) of AH48 that contained the *spoIIIE*::*spc* null mutation. As the forespores of *spoIIIE* mutant sporangia are often difficult to visualize due to the decreased DNA content, we simultaneously immunolocalized β-galactosidase and stained the cell wall with FITC-WGA, a lectin that binds to *N*-acetylglucosamine. While the lectin did not reliably detect the sporulation septum, it did allow the boundaries of the sporangium to be more clearly visualized. Approximately 31% of *spoIIIE*$^+$ sporangia exhibited compartmentalized σ^E activity after induction of *Pspac-spoIIR* (Fig. 1Q to U, short arrows; Table 3), whereas the remaining 69% appeared to have activated σ^E prior to septation (Fig. 1Q to U, long arrows). The σ^E/pro-σ^E protein was absent from the forespores of the sporangia that exhibited compartmentalized σ^E activity, demonstrating that the loss of σ^E/pro-σ^E protein was not dependent upon σ^F activity (data not shown). In sporangia from the *spoIIIE*::*spc* null mutant (Fig. 1V to Z), both σ^E activity (Fig. 1W to Y) and σ^E/pro-σ^E protein (data not shown) were present throughout the sporangium. The forespore nucleoid was especially difficult to visualize in the *spoIIIE*::*spc* mutant sporangia after synthesis of SpoIIR before septation. However, in those few mutant sporangia in which a clear forespore nucleoid was visualized, σ^E was both present and active in the forespore (data not shown). In a second set of experiments, similar results were obtained with the strains of Zhang et al. (42), in which *spoIIR* was fused to a sporulation promoter (P*spoIIE*) that is induced just prior to polar septation (data not shown).

We draw two conclusions from these results. First, in confirmation of the findings of Zhang et al. (42), the lack of σ^E activity in the forespore can be attributed to a mechanism that does not depend on σ^F-directed gene expression. Second, the compartmentalization of σ^E activity that is observed when SpoIIR is produced prior to septation is due, at least in part, to the loss of σ^E/pro-σ^E from the forespore in a manner that appears to be dependent upon SpoIIIE.

***spoIIIE* mutations cause the SpoIIE phosphatase to persist.** The observation that *spoIIIE* null mutant sporangia have σ^E activity in the forespore as well as the mother cell was reminiscent of an earlier, complementary observation that a *spoIIIE* null mutant has σ^F activity in the mother cell as well as the forespore (38). This led us to reexamine the effect of *spoIIIE* mutations on the localization of the SpoIIE phosphatase that is responsible for the activation of σ^F (2, 6, 10). The SpoIIE phosphatase initially localizes to the sites of potential polar septation near both ends of the predivisional sporangium and, after division, disappears first from the mother-cell pole and then from the forespore pole (3). It was previously observed that the bipolar localization of SpoIIE is more evident in *spoIIIE* null mutant sporangia than in wild-type sporangia. These observations suggested that the increased levels or persistence of the SpoIIE phosphatase in the mother cell of a *spoIIIE* null mutant may be responsible for the lack of compartmentalization of σ^F-directed gene expression in the mutant sporangia. As a test of this hypothesis, we asked whether SpoIIE persists at the mother-cell pole in the *spoIIIE* missense mutant *spoIIIE36*, in which misactivation of σ^F in the mother cell does not occur.

indicated by arrows. Where the red and green fluorophores overlap, as in the doubly exposed image shown in panel A (and also in panels E, I, M, T, and Y), a yellow to orange color is visible. (E to P) Immunolocalization of σ^E/pro-σ^E and σ^E-directed β-galactosidase synthesis in wild-type, *spoIIIE36* mutant, and *spoIIIE*::*spc* mutant sporangia. (E to H) Wild-type sporangia (strain BZ184) lack both σ^E/pro-σ^E (red) (E and F) and σ^E-directed β-galactosidase synthesis (green) (E and G) in the forespore (arrows). (I to L) *spoIIIE36* mutant sporangia (strain KJP288) show the presence of σ^E/pro-σ^E (red) (I and J) but not σ^E-directed β-galactosidase synthesis (green) (I and K) in most forespores (arrows) (although in some forespores σ^E activity can be detected [data not shown; see Table 2]). (M to P) Two classes of *spoIIIE*::*spc* mutant sporangia (strain KJP131) were observed: one class had σ^E/pro-σ^E (red) (M and N) but not σ^E-directed β-galactosidase synthesis (green) (M and O) in the forespore (short arrows), whereas the other had both σ^E/pro-σ^E and σ^E-directed β-galactosidase synthesis in the forespore (long arrows). (Q to Z) Immunostaining of σ^E-directed β-galactosidase synthesis following induction of *Pspac-spoIIR* before formation of the polar septum in *spoIIIE*$^+$ and *spoIIIE*::*spc* sporangia. The cell wall was stained with FITC-WGA (green) (T, U, Y, and Z), and the DNA was stained with DAPI (blue) (Q, R, V, and W). Where the blue and red fluorophores overlap, as in the doubly exposed images in panels R and W, a pink color is visible. (Q to U) Examples of the two classes of *spoIIIE*$^+$ sporangia (strain AH48) displaying σ^E-directed β-galactosidase synthesis (red) are shown (R, S, and T). One class has σ^E activity (red) (R and S) confined to the mother cell and appears to have a forespore compartment as judged by the presence of a fully condensed forespore nucleoid (R and S, short arrows). The second class has σ^E-directed β-galactosidase synthesis throughout the sporangium and lacks an obvious forespore nucleoid (R and S, long arrows). (V to Z) In *spoIIIE*::*spc* mutant sporangia (strain KJP286), all the sporangia displaying σ^E-directed β-galactosidase synthesis (red) have σ^E activity throughout the sporangia (W, X, and Y, arrows). Because of the DNA translocation defect caused by the *spoIIIE* mutation, the forespore nucleoid was difficult to visualize in these experiments. Bar (Z), 5 μm. Magnifications are identical for all panels.

TABLE 2. *spoIIIE* mutants have σ^E/pro-σ^E protein and σ^E activity in the forespore and the mother cell[a]

Strain	Relevant genotype	Sporangia with σ^E/pro-σ^E protein and σ^E activity in MC only (%)	Sporangia with σ^E/pro-σ^E protein in whole sporangium (%)[b]	
			σ^E activity in MC only	σ^E activity in whole sporangia[c]
BZ184	Wild type	91	9	0
KJP288	*spoIIIE36*	18	80	2
KJP131	*spoIIIE*::*spc*	11	55	34

[a] Sporangia were collected 2 h after the initiation of sporulation. A total of 509 wild-type, 345 *spoIIIE36*, and 327 *spoIIIE*::*spc* sporangia were scored. σ^E activity was monitored by immunofluorescence microscopy localizing β-galactosidase produced from the *amyE*::*spoIID-lacZ* fusion. σ^E/pro-σ^E protein was monitored by immunolocalization. MC, mother cell.

[b] In most of these sporangia, a clear forespore nucleoid could be detected, suggesting that the sporulation septum had formed.

[c] When three additional experiments were scored for the compartmentalization of σ^E activity and the results were averaged with those of this experiment, 0.1% of wild-type sporangia, 9% of the *spoIIIE36* mutant sporangia, and 44% of the *spoIIIE*::*spc* sporangia had noncompartmentalized σ^E activity.

To do this, we immunolocalized the SpoIIE phosphatase (Fig. 3, green stain) and β-galactosidase synthesized from the σ^F-dependent *sspE(2G)-lacZ* fusion (Fig. 3, red stain) in wild-type sporangia, *spoIIIE36* mutant sporangia, and *spoIIIE*::*spc* null mutant sporangia. In all three strains, sporangia lacking σ^F activity but having the axial filament chromosome morphology characteristic of early-stage sporangia showed bipolar SpoIIE localization (Fig. 3A to D, unfilled arrowheads). However, in sporangia with low levels of σ^F activity, both the wild-type (Fig. 3A to D, long arrows) and *spoIIIE36* mutant (Fig. 3E to H, arrows) sporangia exhibited SpoIIE immunostaining predominantly or exclusively at the forespore-proximal end of the sporangia. In contrast, many *spoIIIE*::*spc* null mutant sporangia with detectable σ^F activity had SpoIIE immunostaining at both the forespore and mother-cell poles (Fig. 3I to L, arrows). Thus, there was a correlation between a delay in the loss of the SpoIIE phosphatase from the mother-cell pole and σ^F activity in the mother cell. Given the apparently identical effects of *spoIIIE36* and *spoIIIE*::*spc* on DNA translocation (38), these results, which are summarized in the diagrams of Fig. 2B, suggest that some unique consequence of the *spoIIIE*::*spc* null mutation leads to the persistence of the SpoIIE phosphatase in the mother cell.

TABLE 3. SpoIIIE is required for compartmentalization of σ^E activity in P_{spac}-*spoIIR*

Strain	Relevant genotype	% Sporangia with σ^E activity present in[a]:		% Staining[d]
		Mother cell[b]	Whole sporangium[c]	
AH48	*spoIIAC*Δ*1*	30.5	69.5	39
KJP286	*spoIIAC*Δ*1* *spoIIIE*::*spc*	1.2	98.8	33

[a] Sporangia were collected 3 h after the initiation of sporulation. SpoIIR expression was induced either at the time of resuspension or one doubling prior to resuspension, with identical results.

[b] Percent of immunostaining sporangia in which β-galactosidase was confined to the mother cell.

[c] Percent of immunostaining sporangia in which β-galactosidase was present either in both the mother cell and forespore or in the predivisional sporangium. Although these two classes were readily discerned in the wild type, the reduced amount of DNA in the forespores of *spoIIIE* mutant sporangia made such a distinction difficult.

[d] A total of 1,264 wild-type sporangia and 1,030 *spoIIIE*::*spc* mutant sporangia were scored from two independent experiments.

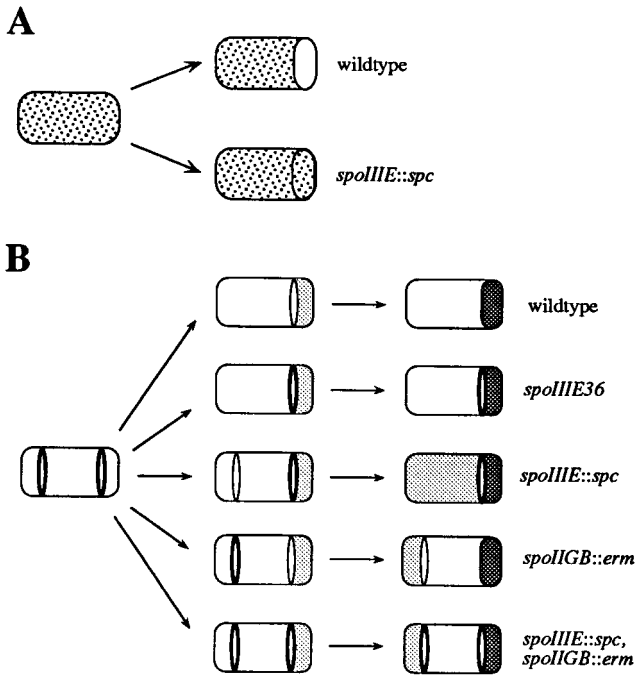

FIG. 2. Summary of the distribution of σ^E/pro-σ^E and the SpoIIE phosphatase in wild-type and mutant sporangia. (A) The results of immunofluorescence staining of wild-type and *spoIIIE* null mutant sporangia with σ^E-directed antibodies. The σ^E/pro-σ^E protein could be detected in the predivisional sporangia of both the mutant and the wild type. Among postdivisional sporangia, σ^E/pro-σ^E was absent or deficient in the forespore of the wild type but was present in both compartments of the mutant. (B) The distribution of the SpoIIE phosphatase is indicated by heavy and light rings, and the pattern of σ^F activity (σ^F-directed β-galactosidase synthesis) is indicated by light and dark shading. In predivisional sporangia, SpoIIE initially exhibited a strong bipolar pattern of distribution, as represented by the single cell on the left. Shortly after polar septation (middle column) all of the forespores (at the right ends of the sporangia) had low levels of σ^F activity (indicated by the light shading). Sporangia that had progressed further (right column) had higher levels of σ^F activity (indicated by heavier shading). Each of the *spoIIGB*::*erm* mutant sporangia (bottom two rows), which lacked σ^E activity, formed a second forespore compartment and thus had both the original forespore (at the right end of the sporangium), which had high levels of σ^F activity, and a more recently formed forespore with lower levels of σ^F activity (at the left end). In wild-type sporangia, as soon as σ^F activity could be detected, the SpoIIE phosphatase was absent from the mother-cell pole; subsequently, SpoIIE disappeared from the forespore pole as well. In *spoIIIE36* mutant sporangia, SpoIIE was rapidly lost from the mother-cell pole but persisted at the forespore pole. In *spoIIIE*::*spc* sporangia, the bipolar staining pattern persisted until after σ^F activity could be detected, although ultimately it was lost from the mother-cell pole, remaining only at the forespore pole. In *spoIIGB*::*erm* mutants (which lack σ^E activity), the SpoIIE phosphatase disappeared normally from the forespore pole but persisted at the mother-cell pole until the formation of the second forespore. Mutants lacking σ^F activity behaved identically to those that lack σ^E activity, suggesting both that σ^F is not required for the loss of SpoIIE from the forespore pole and that σ^E is required for its loss from the mother-cell pole. In doubly mutant *spoIIIE*::*spc spoIIGB*::*erm* sporangia, SpoIIE phosphatase persisted at both sites.

We noted one further difference between wild-type and *spoIIIE* mutant sporangia. In wild-type sporangia with intermediate to high levels of σ^F activity, the SpoIIE phosphatase was absent from the forespore pole as well as from the mother-cell pole (Fig. 3A to D, short arrows). However, in both *spoIIIE* mutants, the SpoIIE phosphatase persisted at the forespore pole, even when σ^F activity reached high levels (Fig. 3E to L). This indicates that both classes of *spoIIIE* mutants are defective in the loss of the SpoIIE phosphatase from the forespore pole. The similar effects of these two classes of mutations on

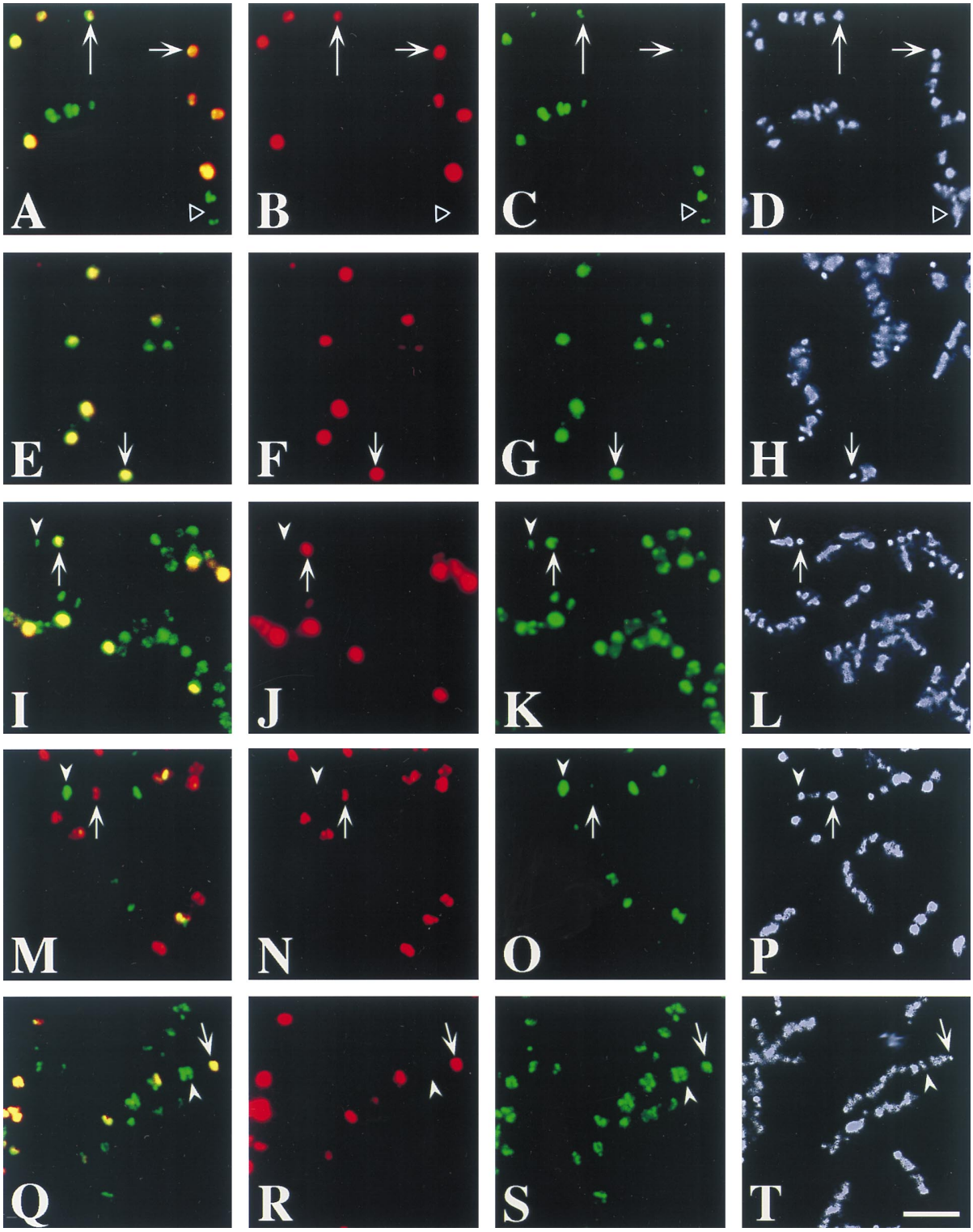

FIG. 3. Immunolocalization of the SpoIIE phosphatase and σ^F-directed β-galactosidase synthesis in *spoIIIE* and *spoIIG* mutant sporangia. Sporangia were harvested 2 h after the start of sporulation and immunostained for the SpoIIE phosphatase (green) (A, C, E, G, I, K, M, O, Q, and S) and for σ^F-directed β-galactosidase synthesis from a *sspE(2G)-lacZ* fusion inserted at the *amyE* locus (red) (A, B, E, F, I, J, M, N, Q, and R). DAPI was used to stain the DNA (blue) (D, H, L, P, and T). The arrows point to the forespore and are oriented perpendicularly to the long axis of the sporangia. (A to D) In wild-type sporangia, once σ^F activity could be detected (red) (A and B), the SpoIIE phosphatase (green) (A and C) was almost entirely limited to the forespore pole (long arrows). In sporangia with higher levels of σ^F activity, the SpoIIE phosphatase was absent from the forespore as well as the mother-cell poles (short arrows). Predivisional sporangia, which lack σ^F activity, displayed bipolar SpoIIE staining (unfilled arrowheads). (E to H) *spoIIIE36* mutant sporangia with both high and low levels of σ^F activity had SpoIIE phosphatase only at the forespore pole (arrows). (I to L) *spoIIIE*::*spc* mutant sporangia had bipolar SpoIIE phosphatase immunostaining in sporangia with low levels of σ^F activity (arrows) (the SpoIIE immunostaining at the mother-cell pole is indicated with arrowheads), whereas sporangia with high levels of σ^F activity had SpoIIE only at the forespore pole (data not shown). (M to P) In *spoIIGB*::*erm* sporangia, which lack σ^E activity, SpoIIE phosphatase persisted at the mother-cell pole (arrowheads), until the formation of a second septum at this site. (Q to T) In doubly mutant *spoIIIE*::*spc spoIIGB*::*erm* sporangia, the SpoIIE phosphatase evenly stained both the forespore pole (arrows) and the mother-cell pole (arrowheads), even when both forespore compartments had σ^F activity. Bar (T), 5 μm. Magnifications are identical for all panels. The panels in the far left column are double exposures, the yellow color resulting from the coincidence of red and green fluorescence.

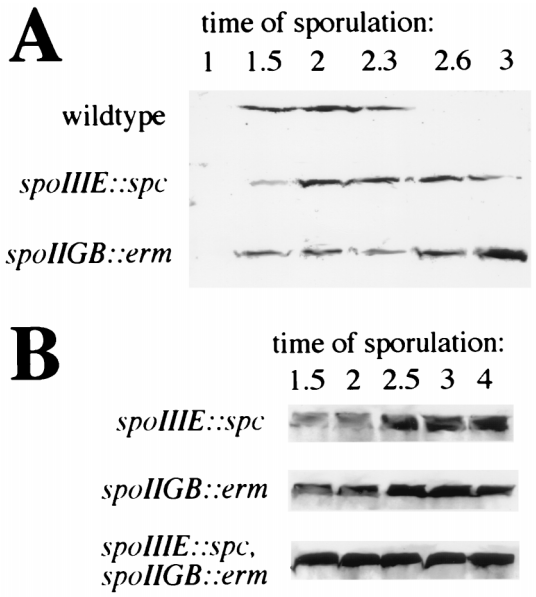

FIG. 4. Western blot analysis of SpoIIE phosphatase in wild-type and mutant cultures during sporulation. (A) Western blot analysis of SpoIIE in wild-type (KJP86), *spoIIIE*::*spc* (KJP123) mutant, and *spoIIGB*::*erm* (KJP277) mutant strains. Samples were taken 1, 1.5, 2, 2.3, 2.6, or 3 h after the start of sporulation and processed as described previously (2). (B) Western blot analysis of *spoIIIE*::*spc* (KJP123), *spoIIGB*::*erm* (KJP277), and *spoIIIE*::*spc spoIIGB*::*erm* (KJP279) mutant strains. Samples were harvested 1.5, 2, 2.5, 3, and 4 h after the start of sporulation and processed as described in Materials and Methods.

the loss of SpoIIE phosphatase from the forespore pole suggests that this phenotype could be a consequence of their shared DNA translocation defect.

The rapid loss of SpoIIE phosphatase during sporulation of wild-type sporangia could also be detected by Western blot analysis, which revealed that the SpoIIE phosphatase reached maximal levels about 2 h after the start of sporulation and was undetectable 2.6 h after the start of sporulation (Fig. 4A). However, in *spoIIIE*::*spc* mutant sporangia, the SpoIIE phosphatase persisted at elevated levels until at least 4 h after the start of sporulation (Fig. 4). This demonstrates that the SpoIIE phosphatase is normally only transiently present during sporulation and that it persists longer in the *spoIIIE* mutant than in the wild type, in accordance with our immunofluorescence studies.

Effect of σ^E on loss of SpoIIE phosphatase. Finally, we consider the contribution of σ^E to the loss of the SpoIIE phosphatase. Mutants lacking σ^E produce aberrant, disporic sporangia that form septa at both polar sites (27, 35). Earlier work showed that in the absence of σ^E, the SpoIIE phosphatase persists at the mother-cell pole but disappears normally from the first forespore to be formed and, later, from the second forespore formed at the opposite end of the sporangium (3) (see also Fig. 3M to P). We now report that the increased persistence of SpoIIE in a mutant (*spoIIGB*::*erm*) lacking σ^E could also be detected by Western blot analysis, which revealed that the phosphatase persisted until at least 4 h after the start of sporulation (Fig. 4). Taken together, our results suggest that both σ^E and SpoIIIE contribute to the loss of the SpoIIE phosphatase from the mother-cell pole. If this is correct, then sporangia lacking both SpoIIIE and σ^E activity should have stably bipolar SpoIIE. This was indeed the case, since doubly mutant sporangia showed uniformly bipolar immunostaining of SpoIIE phosphatase, even at a time when both polar forespore compartments had σ^F activity (Fig. 3Q to T; summarized in Fig. 2B). A similar effect was observed by Western blot analysis, which revealed that in doubly mutant cells, the SpoIIE phosphatase reached its maximal levels earlier than in either wild-type or singly mutant cells and maintained these high levels until at least 4 h after the start of sporulation (Fig. 4B).

DISCUSSION

A σ^F-independent mechanism for excluding or depleting σ^E/pro-σ^E from the forespore. A principal contribution of this investigation is the discovery that although pro-σ^E is produced prior to the formation of the sporulation septum, little or no immunoreactive form of the protein (that is, either pro-σ^E or mature σ^E) can be detected in the forespore shortly after polar division. Thus, some mechanism evidently exists for excluding or depleting σ^E/pro-σ^E from the forespore. Mutations that block σ^F activity had no effect on the absence of σ^E/pro-σ^E from the forespore, but a *spoIIIE* null mutation prevented the elimination of σ^E/pro-σ^E from the small chamber of the sporangium. Furthermore, among sporulating cells of the *spoIIIE* mutant, sporangia that exhibited σ^E activity in the forespore as well as in the mother cell were frequently observed. Regardless of the mechanism by which a null mutation in the *spoIIIE* gene allows σ^E/pro-σ^E protein to persist in the forespore (see below), the observation that σ^E can become active in the forespore of such mutant sporangia suggests that exclusion or depletion of σ^E/pro-σ^E protein from the forespore is part of the normal mechanism by which σ^E-directed gene expression is restricted to the mother cell.

Activation of σ^E normally requires σ^F activity due to the dependence of pro-σ^E processing on the SpoIIR protein, whose synthesis depends on σ^F (15, 21). This has raised the possibility that another σ^F-transcribed gene may serve as a forespore-specific inhibitor of pro-σ^E processing or σ^E activity or that SpoIIR may act directionally, only allowing pro-σ^E processing in the mother cell (22). However, our present results, in confirmation and extension of those of Zhang et al. (42), indicate that when expression of *spoIIR* is engineered to occur prior to septation, many sporangia that exhibit compartmentalized σ^E protein and activity even in the absence of σ^F can be observed; the remaining sporangia that exhibit σ^E activity seem to lack a forespore, suggesting that in these sporangia, σ^E has become active before formation of the sporulation septum. Two inferences can be drawn from these observations. First, the σ^F-dependent expression of *spoIIR* is a timing device that ensures that activation of σ^E occurs only after septation (42). Second, a separate, σ^F-independent mechanism(s) exists to eliminate σ^E/pro-σ^E protein and to block σ^E-directed gene expression in the forespore.

How might σ^E/pro-σ^E be depleted from the forespore? One attractive possibility is that the depletion of σ^E/pro-σ^E protein from the forespore is mediated by a protease that is either more active or more concentrated in the forespore than in the mother cell. A similar hypothesis has been proposed for the degradation of the methyl-accepting chemotaxis receptor (McpA) of *Caulobacter crescentus*, which occurs specifically in just one of the two daughter cells of the *Caulobacter* cell cycle (13). However, the forespore-specific instability of σ^E/pro-σ^E protein may not be necessary for the depletion of σ^E/pro-σ^E from the forespore. Depletion of σ^E/pro-σ^E protein could also be achieved if synthesis of pro-σ^E is specifically inhibited in the forespore. In this respect, it is interesting to note that the *spoIIG* operon, which encodes both pro-σ^E and SpoIIGA (the putative pro-σ^E protease), is located in the origin-distal region of the chromosome and therefore would be among the last

genes to enter the forespore during the 10 to 15 min required to translocate a chromosome into the forespore (9, 28). Thus, the *spoIIG* operon cannot be transcribed in the forespore until 10 to 15 min after septation. If, during this time, the preexisting pro-σ^E protein and its mRNA were degraded (perhaps in a SpoIIIE-dependent manner) and some mechanism to prevent *spoIIG* transcription were implemented, depletion of σ^E/pro-σ^E from the forespore could be achieved.

Depletion of the SpoIIE phosphatase from the mother-cell pole. In contrast to σ^E/pro-σ^E, σ^F appears to be present in the mother cell as well as the forespore, and thus the compartmentalization of σ^F activity does not seem to rely on the loss of σ^F from the mother cell. Instead, our results suggest that the loss of the SpoIIE phosphatase from the mother cell pole contributes to the compartmentalization of σ^F activity. The SpoIIE phosphatase is synthesized before the sporulation septum is formed and initially localizes to potential division sites near each pole of the sporangium (3). After septation, SpoIIE rapidly becomes sequestered to the sporulation septum, prior to disappearing entirely from the sporangium (3). Our present results indicate a close correlation between this disappearance of SpoIIE from the mother-cell pole and the compartmentalization of σ^F activity. In a *spoIIIE* null mutant, in which σ^F becomes active in the mother cell (38), the SpoIIE phosphatase is observed to persist at the mother-cell pole. In contrast, in a *spoIIIE* missense mutant (*spoIIIE36*), in which σ^F activity is strictly compartmentalized, SpoIIE disappears normally from the mother-cell pole. Thus, the rapid disappearance of SpoIIE from the mother-cell pole correlates with, and evidently contributes to, the compartmentalization of σ^F activity.

An important unanswered question is whether the disappearance of SpoIIE occurs only at the mother-cell pole of the sporangium or from the mother-cell face of the septum as well. If SpoIIE is entirely eliminated from the mother cell, then the phosphatase would be present only in the forespore, thereby ensuring that σ^F is activated exclusively in one cell. Even if SpoIIE is eliminated only from the mother-cell pole of the sporangium, however, and hence persists on both faces of the septum, the septal localization of SpoIIE could nonetheless contribute to the cell-specific activation of σ^F: because of the small size of the forespore, the ratio of the SpoIIE phosphatase to the SpoIIAB kinase will be relatively higher in the small chamber of the sporangium, thereby contributing to the selective activation of σ^F in the compartment (6).

The elimination of the SpoIIE phosphatase from the mother-cell pole requires the concerted action of two proteins, σ^E and SpoIIIE. A null mutation in the *spoIIIE* gene causes a delay in the loss of the SpoIIE phosphatase from the mother-cell pole and also strongly inhibits the loss of SpoIIE from the forespore pole. In contrast, a mutation in the gene (*spoIIGB*) coding for σ^E has no effect on the loss of SpoIIE from the septum but strongly inhibits loss of SpoIIE from the potential division site within the mother cell distal to the forespore (3). Mutants lacking σ^E activity ultimately divide at this second site, forming a disporic sporangium with two chromosome-containing forespore compartments and a central compartment that lacks a chromosome (27, 32, 20). After the formation of the second forespore compartment in disporic mutants, the SpoIIE phosphatase is rapidly lost from the newly formed forespore, representing the original mother-cell pole (3). These results suggest that SpoIIIE is involved in the loss of the SpoIIE phosphatase from both poles of the sporangium, being required for the loss of the SpoIIE phosphatase from the forespore pole of the sporangium and acting together with σ^E to mediate the loss of SpoIIE from the mother-cell pole. Consistent with the hypothesis that both proteins contribute to the loss of the SpoIIE phosphatase, a doubly mutant strain lacking both σ^E and SpoIIIE showed stably bipolar SpoIIE immunostaining.

How might these proteins act to promote the timely disappearance of the SpoIIE phosphatase from the sporangium? One model is that they directly or indirectly increase the rate of proteolysis of the SpoIIE phosphatase in either the mother cell or the forespore. For example, since both SpoIIE and SpoIIIE are known to be located in the sporulation septum (3, 39), it is conceivable that the proteins are in contact with each other in the septum, allowing SpoIIIE to directly enhance the instability of SpoIIE. Alternatively, the SpoIIE phosphatase may be equally unstable at all stages of sporulation and in both mutants, and the two proteins (σ^E and SpoIIIE) may prevent the continued synthesis of SpoIIE as sporulation proceeds. A third possibility is suggested by recent findings demonstrating a connection between the SpoIIE phosphatase and the FtsZ protein, an essential and evolutionarily conserved cell division protein (23, 37). Like SpoIIE, FtsZ initially localizes to the two potential sites of septation in the early sporangium but is rapidly lost from the second potential division site after septation (18). The loss of FtsZ from the second division site depends on σ^E activity, perhaps explaining why mutants lacking σ^E activity are disporic. Intriguingly, localization of SpoIIE to potential division sites requires FtsZ (17), suggesting that the σ^E-dependent loss of FtsZ from the second division site may be sufficient to allow the loss of the SpoIIE phosphatase from this site.

Role of SpoIIIE in compartmentalizing the activities of σ^E and σ^F. The most surprising result we have presented is that the same *spoIIIE* null mutation that allows σ^E/pro-σ^E protein to persist and become active in the forespore also allows the SpoIIE phosphatase to persist at the mother-cell pole and thus for σ^F to become active in the mother cell. How might a protein whose primary role is to promote the translocation of the chromosome into the forespore compartment cause defects in the compartmentalization of the activities of both σ^F and σ^E? One obvious possibility is that these compartmentalization defects are a secondary consequence of the DNA translocation defect, which could prevent some key gene from entering and being expressed in the forespore. However, *spoIIIE36* mutant sporangia have a DNA translocation defect evidently similar to that of *spoIIIE* null mutant sporangia (38) yet have a less severe compartmentalization defect. Although it remains possible that the two mutations may have subtle differences in their DNA translocation defects, this suggests that some other function of SpoIIIE may be differentially affected by these two classes of mutations.

Another possibility is that septa formed by *spoIIIE* null mutant sporangia are leaky due to the presence of an incompletely translocated chromosome traversing the polar septum, allowing the diffusion of proteins between the two cells. Three observations argue against this possibility, however. First, when a *lacZ* gene fusion whose synthesis depends on σ^E (*spoIVF-lacZ*) was located in the origin-distal portion of the chromosome and was thereby trapped in the mother cell of *spoIIIE* mutant sporangia, 94% of *spoIIIE*::*spc* mutant sporangia had β-galactosidase confined to the mother cell, indicating that β-galactosidase produced in the mother cell does not leak into the forespore compartment (data not shown). Second, in *spoIIIE*::*spc* sporangia, in which σ^F becomes active in the mother cell (38) (see below), β-galactosidase produced from a σ^F-dependent *lacZ* fusion (*spoIIIG-lacZ*) trapped in the mother cell (by virtue of its location on the origin-distal region of the chromosome) was present only in the mother cell (data not shown). Thus, even those *spoIIIE* sporangia in which compartmentalization of σ^F activity is disrupted have a septum that prevents

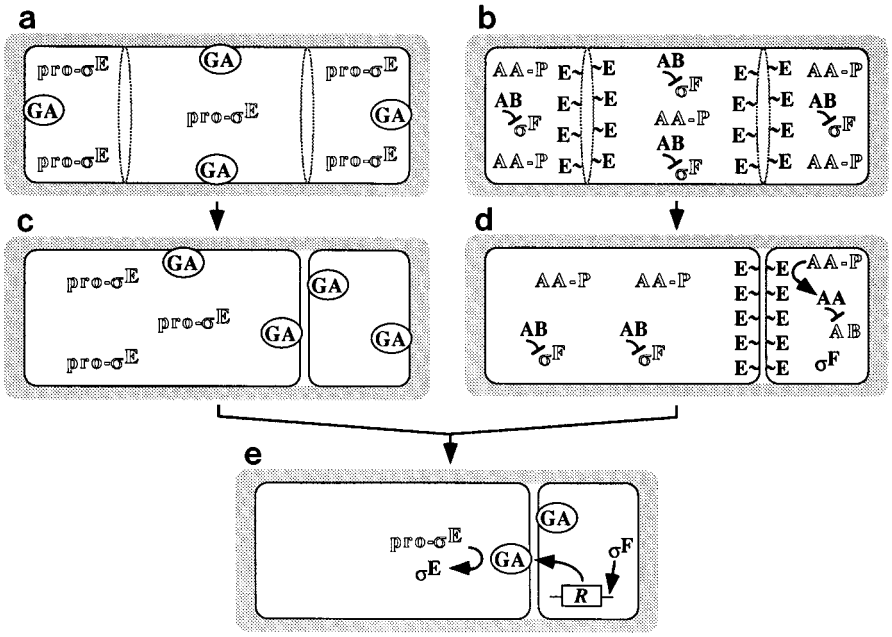

FIG. 5. Model for the establishment of cell-specific gene expression. Separate diagrams of sporangia are used to depict events leading to the activation of σ^F in the forespore (b and d) and the elimination of σ^E/pro-σ^E from the forespore (a and c). In the predivisional sporangium (b), the SpoIIE phosphatase is associated with two rings of FtsZ located at sites of potential polar division. In the postdivisional sporangium (d), SpoIIE is eliminated from the mother-cell pole as depicted and possibly from the mother-cell face of the septum as well (not shown). Preferential dephosphorylation of SpoIIAA-P in the forespore leads to activation of σ^F. Meanwhile, in the predivisional sporangium (a), pro-σ^E and the SpoIIGA protease are present throughout the cell. In the postdivisional sporangium (c), σ^E/pro-σ^E is eliminated from the forespore and SpoIIGA becomes associated with the septum. It is possible that SpoIIGA is also eliminated from the forespore. Finally (e), σ^F-directed transcription of *spoIIR* in the forespore leads to SpoIIGA-mediated activation of pro-σ^E processing in the mother cell. AA, SpoIIAA; AA-P, SpoIIAA-P; AB, SpoIIAB; E, SpoIIE; GA, SpoIIGA; R, *spoIIR*.

diffusion of β-galactosidase from the mother cell to the forespore. Finally, some *spoIIIE*::*spc* mutant sporangia have normal compartmentalization of both σ^F and σ^E activities, suggesting that at least in these *spoIIIE* mutant sporangia, neither β-galactosidase, nor active σ^F or σ^E, nor any small molecule effectors of their activity leak either from the forespore to the mother cell or from the mother cell to the forespore. It therefore seems unlikely that diffusion of proteins or molecules through the septum explains the defective compartmentalization of σ^E or σ^F activities in *spoIIIE* mutants. We therefore favor the hypothesis, at least provisionally, that the two classes of *spoIIIE* mutations differentially affect some other unknown function of SpoIIIE that contributes, directly or indirectly, to the loss of σ^E/pro-σ^E from the forespore and of the SpoIIE phosphatase from the mother-cell pole.

A model for the establishment of cell type. In summary, our present findings in extension of previous reports (for a review, see reference 35) point to the following updated model for the establishment of cell type (Fig. 5). The forespore transcription factor σ^F is present in the predivisional sporangium and in both compartments of the postdivisional sporangium. Only in the forespore does σ^F escape the inhibitory effect of the SpoIIAB anti-sigma factor through a pathway involving the regulatory phosphatase SpoIIE. We have only a partial understanding of how this happens, but our present results indicate that loss of the SpoIIE phosphatase from the mother cell or at least from the mother-cell pole contributes to the forespore-specific activation of σ^F. Moreover, our present results show that disappearance of the SpoIIE phosphatase from the mother-cell pole depends in some unknown fashion upon the action of SpoIIIE. Meanwhile, the mother-cell transcription factor σ^E, which is present in the predivisional sporangium as the inactive proprotein, pro-σ^E, comes to be excluded from or destroyed in the small chamber of the postdivisional sporangium. Once again, and in an unknown fashion, the elimination of σ^E/pro-σ^E from the forespore depends on the action of SpoIIIE. Finally, once σ^F has become active in the forespore and σ^E/pro-σ^E has been eliminated from the small sporangial chamber, an intercompartmental signal transduction pathway that is dependent upon σ^F-directed synthesis of the secreted signal protein SpoIIR triggers the conversion of pro-σ^E to mature and active σ^E in the mother cell. In this model, SpoIIIE-dependent elimination of σ^E/pro-σ^E from the forespore contributes to cell-specific gene expression, whereas the σ^F-dependent signal transduction system serves as a timing device to ensure that pro-σ^E is not activated until after polar division.

An important feature of this model is that the SpoIIIE protein contributes to the proper spatial regulation of both σ^F and σ^E activities. Just how a protein that is required for the transport of a chromosome into the forespore could be involved in cell-specific activation of σ^F and σ^E raises intriguing new questions about the nature of SpoIIIE and the underlying basis for compartmentalized gene expression.

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