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# SpoIIAB is an anti-$\sigma$ factor that binds to and inhibits transcription by regulatory protein $\sigma^F$ from *Bacillus subtilis*

(sporulation/cell type/RNA polymerase/gene regulation)

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ABSTRACT **The $\sigma^F$ factor is a regulatory protein that is responsible for directing gene expression in the forespore compartment of developing cells of the spore-forming soil bacterium *Bacillus subtilis*. The $\sigma^F$ factor is encoded by the promoter-distal member of sporulation operon *spoIIA*, which consists of cistrons called *spoIIAA*, *spoIIAB*, and *spoIIAC*. Genetic evidence indicates that the activity of $\sigma^F$ is negatively regulated by the product (SpoIIAB) of the *spoIIAB* cistron. We now report that SpoIIAB is capable of binding to $\sigma^F$ and inhibiting its capacity to direct transcription by core RNA polymerase from the promoter for a forespore-expressed gene. SpoIIAB is an anti-$\sigma$ factor that may be directly involved in the compartmentalization of $\sigma^F$-directed gene expression.**

Spore formation in the Gram-positive soil bacterium *Bacillus subtilis* involves the formation of an asymmetrically positioned septum that partitions the developing cell into two cellular compartments called the forespore and the mother cell (1, 2). Gene expression in the compartments is regulated differentially, with certain genes being expressed in the forespore and others in the mother cell (3, 4). At intermediate to late stages of development, differential gene expression is governed by two compartment-specific RNA polymerase $\sigma$ factors. These are $\sigma^G$, which directs transcription in the forespore, and $\sigma^K$, which directs transcription in the mother cell (5–7). Transcription of the structural genes for $\sigma^G$ (*spoIIIG*) and $\sigma^K$ (*sigK*) is, in turn, governed by two early-acting $\sigma$ factors. These are $\sigma^F$, which directs the transcription of *spoIIIG* (8) and other forespore-expressed genes (9), and $\sigma^E$, which directs the transcription of *sigK* (10) and other mother-cell-expressed genes (11–14). Interestingly, $\sigma^F$ is produced in the predivisional sporangium (ref. 15; S. R. Partridge and J. Errington, personal communication) but does not become active in directing gene expression until after septum formation when its activity is restricted to the forespore (16). These findings indicate that $\sigma^F$ is subject to a regulatory mechanism that restricts its activity to one cellular compartment.

A possible clue to this control mechanism comes from genetic experiments that suggest that the activity of $\sigma^F$ is governed by the sporulation regulatory proteins SpoIIAA and SpoIIAB (16–18). All three proteins are encoded within a three-cistron operon known as *spoIIA* (19–21). The $\sigma^F$ factor is encoded by the promoter-distal member of the operon (5) *spoIIAC*, whereas SpoIIAA and SpoIIAB are the products of the promoter-proximal cistrons *spoIIAA* and *spoIIAB*, respectively. The results of genetic experiments have shown that SpoIIAB is a negative regulator of $\sigma^F$-directed gene expression (16–18, 22) and that SpoIIAA inhibits or otherwise counteracts the inhibitory effect of SpoIIAB on $\sigma^F$ (16, 17). In the present communication, we investigate the nature of the interaction between SpoIIAB and $\sigma^F$ by using proteins purified from cells of *Escherichia coli* that have been engineered to express *spoIIAB* and *spoIIAC* at high levels. Our results indicate that SpoIIAB is an anti-$\sigma$ factor that binds to $\sigma^F$ and blocks its capacity to direct transcription.

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## MATERIALS AND METHODS

**Construction of SpoIIAB and $\sigma^F$ Expression Vectors.** An expression vector capable of driving transcription of *spoIIAC* from a T7 RNA polymerase promoter was constructed by cloning a 1-kb *Bgl* II–*Pst* I fragment containing *spoIIAC* from pPM34 into *Bam*HI/*Pst* I-digested pT713 (BRL), thereby creating pLD4. [pPM34 was derived from pPM24 (17) by replacing the mutant *spoIIAC* allele with a wild-type allele; P. Margolis and R.L., unpublished results.] An expression vector bearing *spoIIAB* downstream of a T7 RNA polymerase promoter was constructed by cloning an 820-bp *Dra* I–*Sal* I insert from pHM2 (23) into *Sma* I/*Sal* I-digested pT713 to create pLD5. To increase SpoIIAB production, the *spoIIAB* ribosome binding site was replaced with a stronger ribosome binding site by using the Kunkel method (24) of site-directed mutagenesis and the oligonucleotide 5′-CGATTTGAACAA-TCTGAGCTCAGGAGGTAAAAATCATGAAAAATG-3′ to generate plasmid pLD8. This plasmid was used to produce [$^{35}$S]methionine-labeled SpoIIAB for use in *in vitro* cross-linking studies. When unlabeled SpoIIAB was produced, however, we used pSP72IIAB-SD1 (C. Moran, personal communication), which is similar to pLD8 except that the oligonucleotide used to replace the *spoIIAB* ribosome binding site was 5′-CTGCAGAGGAGGTAAAACATTAT-GAAAAA-3′, and the resulting gene was cloned into the *Pst* I site of the phage T7 RNA polymerase expression vector pSP72 (Promega).

pLD4 and pLD8 were transformed into an *E. coli* T7 RNA polymerase expression strain, BL21(DE3)/pLysS (Novagen, Madison, WI), to create strains LDE7 and LDE15, respectively, whereas pSP72IIAB-SD1 was transformed into *E. coli* BL21(DE3) (Novagen) to generate strain EUD72AB. Control strains called LDE6 and LDE8 were produced by transforming the parental plasmid pT713 into BL21(DE3) and BL21(DE3)/pLysS, respectively.

**[$^{35}$S]Methionine Labeling of SpoIIAB and $\sigma^F$.** LDE15 and LDE7 were grown at 37°C in 20 ml of M9 minimal medium supplemented with 0.2% glucose, vitamin $B_1$ (1 $\mu$g/ml), ampicillin (75 $\mu$g/ml), and chloramphenicol (25 $\mu$g/ml) until $OD_{595}$ was >0.2 at which time T7 RNA polymerase synthesis was induced by the addition of isopropyl $\beta$-D-thiogalactoside to 1 mM. After 30 min, rifampicin (Sigma) in methanol was added to 200 $\mu$g/ml (final concentration) to inhibit *E. coli* RNA polymerase. After host mRNA species were allowed to decay for 1 h, 1-ml aliquots of cells were labeled with 20 $\mu$Ci

Abbreviation: DSS, disuccinimidyl suberate.

of [$^{35}$S]methionine (NEN; 1 Ci = 37 GBq) for 5 min. After the addition of unlabeled excess methionine, the reaction was allowed to proceed 5 min. The labeled cells were then collected by centrifugation, washed, and frozen at −70°C. Cell pellets containing 1 ml of [$^{35}$S]methionine-labeled cells were resuspended in 500 μl of cell lysis buffer (50 mM Tris·HCl, pH 8/2 mM EDTA/100 mM NaCl/0.1 mM dithiothreitol/0.5% Triton X-100). Cells were lysed by repeated freeze–thaw steps, and particulate matter was removed by centrifugation.

**Production of Unlabeled SpoIIAB and $\sigma^F$.** LDE6 and EUD72AB were grown in 1000 ml of Luria broth containing ampicillin (75 μg/ml) at 37°C until the culture reached a density of $OD_{595}$ >0.8. Production of T7 RNA polymerase was induced by addition of isopropyl β-D-thiogalactoside to 1 mM. After 30 min, rifampicin was added to 200 μg/ml (final concentration), and cells were harvested after overnight incubation with rifampicin and stored at −70°C. LDE7 and LDE8 were induced in a similar manner except that they were grown in Luria broth containing ampicillin (75 μg/ml) and chloramphenicol (25 μg/ml; to maintain the pLysS plasmid), and cells were harvested 2–4 h after the addition of rifampicin.

**Preparation of Crude Lysates from EUD72AB and LDE6 Cells.** EUD72AB and LDE6 cell pellets were suspended in TEG buffer [20 mM Tris·HCl, pH 8/10% (vol/vol) glycerol/1 mM EDTA/10 mM $MgCl_2$/1 mM dithiothreitol/phenylmethylsulfonyl fluoride (0.1 mg/ml)] and lysed by sonication. After removing cell debris by centrifugation at 10,000 rpm for 30 min in a Sorvall SS34 rotor, $(NH_4)_2SO_4$ was added to the supernatant to 45% (wt/vol) and mixed 30 min on ice. The precipitates were collected by centrifugation at 10,000 rpm for 30 min in an SS34 rotor and suspended in and dialyzed against TEG buffer. The extracts were next loaded onto a small heparin-agarose (Sigma) column equilibrated in TEG buffer. Most of the SpoIIAB did not bind to the column matrix under the conditions used, and the flow-through produced a crude *E. coli* extract slightly enriched for SpoIIAB. The column eluates from the EUD72AB and LDE6 cells were used as a crude preparation of SpoIIAB and of control proteins, respectively.

**Purification of SpoIIAB and $\sigma^F$ by Preparative SDS/Polyacrylamide Gel Electrophoresis.** SpoIIAB was purified by separating proteins present in a crude EUD72AB extract containing SpoIIAB either after the heparin-agarose column stage or the 45% $(NH_4)_2SO_4$ stage (see above) on preparative 1.5-mm high-resolution SDS/18% polyacrylamide gels (25). Sodium thioglycolate (0.1 mM) was added to the SDS running buffer as a protective agent. These gels were stained with Coomassie brilliant blue R-250 in 50% (vol/vol) methanol/10% (vol/vol) acetic acid and destained until SpoIIAB was clearly discernible from neighboring proteins. Slices from these preparative gels containing SpoIIAB were diced and SpoIIAB was eluted, precipitated, and renatured as described by Hager and Burgess (26). As a control, protein (referred to as control protein) was eluted and renatured as above from a gel slice corresponding to the position of SpoIIAB from a gel in which proteins from an extract of induced LDE6 cells (which lack the *spoIIAB* gene; see above) had been subjected to electrophoresis. $\sigma^F$ was purified and renatured similarly, except that induced cell pellets of LDE7 were lysed by addition of SDS/PAGE loading dye and boiled for 5 min before electrophoresis on preparative gels.

**Chemical Cross-Linking Reactions.** Chemical cross-linking was carried out in 50-μl reaction mixtures containing disuccinimidyl suberate (DSS; Pierce; 1 mg/ml, final concentration) dissolved in dimethyl sulfoxide and 5–10 μg of *E. coli* extract containing either $^{35}$S-labeled SpoIIAB or $^{35}$S-labeled $\sigma^F$. The reaction mixtures were supplemented, as indicated, with one of the following: no added protein, 0.4 μg of purified SpoIIAB, 0.4 μg of purified $\sigma^F$, 300 μg of the heparin flow-through fraction from SpoIIAB-producing cells (see above), or 300 μg of the heparin flow-through fraction from control cells not producing SpoIIAB (see above). The cross-linking reactions were allowed to proceed for 1–3 h on ice and were terminated by the addition of SDS loading dye and 20 mM lysine (final concentration). Samples were boiled for 5 min and subjected to electrophoresis in high-resolution SDS/18% polyacrylamide slab gels (25). Gels were stained with Coomassie brilliant blue R-250, impregnated with a scintillation fluor (Entensify, DuPont), dried, and analyzed by autoradiography.

**Construction of a Plasmid Containing the *spoIIIG* Promoter.** A 660-bp *Kpn* I–*Sph* I fragment containing the *spoIIIG* promoter region was isolated from pDG180 (a gift of P. Stragier, Institute de Biologie Physico-Chimique, Paris) and cloned into *Kpn* I/*Sph* I-digested M13mp18 to generate φVO.5 (V. Oke and R.L., unpublished results). This same fragment was then isolated from the replicative form of φVO.5, digested with *Eco*RI and *Pst* I, and cloned into *Eco*RI/*Pst* I-digested pUC19 to generate pLD9. pLD9 was cut with *Pst* I or *Sph* I and the 3′ sticky ends were removed with T4 DNA polymerase (27) yielding a linearized plasmid capable of producing a 121-nt or 127-nt run-off transcript from the *spoIIIG* promoter, respectively.

**Purification of Core RNA Polymerase.** Core RNA polymerase was isolated from a *B. subtilis* strain, LD10 (*sigB*::*cat*, $P_{spac}$-*spoOH*), constructed to be deficient in the production of both $\sigma^B$ and sporulation-specific $\sigma$ factors. LD10 was constructed by transforming ML6 (*sigB*Δ*Hin*dIII–*Eco*RV::*cat*; ref. 28) with chromosomal DNA isolated from RS169 (R. Schmidt and R.L., unpublished data), which contains $P_{spac}$–*spoOH* linked to *erm*, and selecting for erythromycin and lincomycin resistance. RS169 was derived from BH19 (29) by replacing *cat* with *erm*. LD10 used to produce RNA polymerase was grown at 37°C in 2× YT medium in the presence of chloramphenicol (5 μg/ml), lincomycin (25 μg/ml), and erythromycin (1 μg/ml); cells were harvested by centrifugation when the culture had reached an $OD_{595}$ of 1 and frozen at −70°C. RNA polymerase was isolated from 40 g of LD10 cells as described by Cummings and Haldenwang (30). RNA polymerase activity was detected using the poly(dAT) assay as described by Moran (31), and active fractions were pooled, dialyzed against homogenization buffer (30) supplemented with 50% glycerol/50 mM Tris·HCl, pH 8, and stored at −20°C.

***In Vitro* Transcription Reactions.** RNA polymerase run-off transcription assays were carried out in 50 μl or 100 μl reaction mixtures containing 40 mM Tris·HCl (pH 8), 50 mM KCl, 10 mM $MgCl_2$, 0.4 mM dithiothreitol, 0.2 mM CTP, 0.2 mM GTP, 0.2 mM UTP, 0.2 μM unlabeled ATP, 2.5 μCi of [α-$^{32}$P]ATP (10 μCi/μl, 3.3 μM; NEN), 0.7 μg of purified $\sigma^F$, and 2 μg of pLD9 linearized with *Pst* I or *Sph* I. For the inhibition experiments, $\sigma^F$ was preincubated with the indicated amounts of gel-purified SpoIIAB, with control protein, or with protein dilution buffer (26) for 30 min at room temperature. The reaction was initiated at 37°C by the sequential addition of template, core RNA polymerase (0.5 μg), and nucleotides. Heparin (500 μg/ml, final concentration) was added after 2 min to prevent reinitiation. Incubation with heparin for 3 min was followed by a 2-min chase with 200 μM unlabeled ATP (final concentration). In some experiments, unincorporated nucleotides were removed by centrifugation through Sephadex columns as described (27), except that Sephadex G-25 was used. The samples were ethanol-precipitated in the presence of 10 μg of yeast carrier RNA, air-dried, dissolved in formamide loading dye, heated to 90°C for 3 min, and electrophoresed on an 8% sequencing gel. Gels were dried and analyzed by autoradiography. Transcripts of known sizes generated by run-off transcription of plasmids

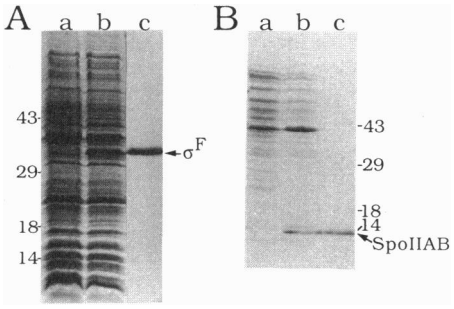


FIG. 1. Purification of $\sigma^F$ and SpoIIAB. The figure displays Coomassie-stained proteins that had been subjected to electrophoresis in SDS/18% polyacrylamide slab gels (25). The proteins were from *E. coli* cells that had been engineered to express the *spoIIAC* or *spoIIAB* gene at high levels. (*A*) Lanes: a, proteins in a crude lysate from the control strain LDE8; b, proteins in a crude lysate from the $\sigma^F$-producing strain LDE7; c, purified $\sigma^F$. (*B*) Lanes: a, proteins in the heparin flow-through fraction from the control strain LDE6; b, proteins in the heparin flow-through fraction from the SpoIIAB-producing strain EUD72AB; c, purified SpoIIAB. Numbers at the side of the gels indicate the position of protein size markers in kDa.

bearing a T7 or SP6 promoter were used to determine the length of transcripts produced by $\sigma^F$.

## RESULTS AND DISCUSSION

**Complexes of SpoIIAB and $\sigma^F$.** To investigate the possible interaction of SpoIIAB with $\sigma^F$, we prepared the proteins in radioactive and nonradioactive forms by engineering the expression of their structural genes in *E. coli*. Fig. 1*A* shows an SDS/polyacrylamide gel of purified $\sigma^F$ (lane c) and Fig. 2*A* (lane a) displays $^{35}$S-labeled SpoIIAB. Our preparation of $^{35}$S-labeled SpoIIAB additionally contained small amounts of a radioactive polypeptide of 12 kDa, which is likely to be a fragment of the full-length *spoIIAB* gene product (14 kDa). (Results similar to those presented below have been obtained with other preparations of $^{35}$S-labeled SpoIIAB that contained much less of the contaminating species; L.D., data not shown.) Incubation of radioactive SpoIIAB with DSS generated a 29-kDa species (Fig. 2*A*, lane b). DSS is a chemical cross-linking reagent that covalently joins lysine residues that are located about 11 Å apart. This probably indicates that SpoIIAB exists as a dimer, but we cannot exclude the possibility that SpoIIAB binds to an unlabeled *E. coli* protein of similar size present in our preparation. Incubation of radioactive SpoIIAB with the cross-linker in the presence of purified unlabeled $\sigma^F$ generated a cluster of higher molecular mass species ranging from 41 to 95 kDa in apparent size (Fig. 2*A*, lane c). We interpret these higher molecular mass species to be complexes of SpoIIAB and $\sigma^F$. As an indication of the specificity of complex formation, the formation of the high molecular mass species was unaffected by the presence (Fig. 2*A*) or absence (data not shown) of bovine serum albumin at a 60-fold molar excess relative to $\sigma^F$. Also, radioactive SpoIIAB did not form high molecular mass complexes with any of the unlabeled *E. coli* proteins present in our preparation of the $^{35}$S-labeled polypeptide; that is, the complexes were not observed when cross-linking was carried out in the absence of added $\sigma^F$ (Fig. 2*A*, lane b). Because cross-linked proteins sometimes migrate aberrantly with respect to the combined molecular mass of their components (32), we cannot deduce the stoichiometry of SpoIIAB and $\sigma^F$ in the complexes with confidence, but the simplest interpretation of our results is that the highest molecular mass complex (apparent molecular mass, 95 kDa) contains two molecules of SpoIIAB and two molecules of $\sigma^F$ [$\sigma^F$ has a molecular mass of 29 kDa (21) but migrates with an apparent size of 35 kDa] and that the lower molecular mass complexes represent partially cross-linked components of the putative tetramer ($SpoIIAB_2\sigma^F_2$).

To further investigate the binding of SpoIIAB with $\sigma^F$, we carried out the cross-linking experiments with a reverse protocol in which we used $^{35}$S-labeled $\sigma^F$ (Fig. 2*A*, lane f) and purified unlabeled SpoIIAB. Fig. 1*B* displays the purified SpoIIAB (lane c) and crude protein preparations from *E. coli* cells producing (lane b) or not producing (lane a) SpoIIAB. Incubation of radioactive $\sigma^F$ alone with the cross-linking reagent generated no higher molecular mass complexes but did cause the appearance of a 27-kDa species labeled X in Fig. 2*A*, lane e. We do not understand the basis for the appearance of this fragment, but we presume that DSS generates an intramolecular cross-link that increases the electrophoretic mobility of $\sigma^F$ or that reaction of $\sigma^F$ with DSS renders the protein susceptible to cleavage by a proteolytic activity present in our preparation of $\sigma^F$. In any event, incubation of radioactive $\sigma^F$ with cross-linker in the presence of purified SpoIIAB generated a cluster of higher molecular mass species (Fig. 2*A*, lane d) that were indistinguishable in their electrophoretic mobility from those observed in the experiment of Fig. 2*A*, lane c. As a further demonstration of specificity, the same set of complexes was generated with radioactive $\sigma^F$ and a crude preparation of proteins from *E.*

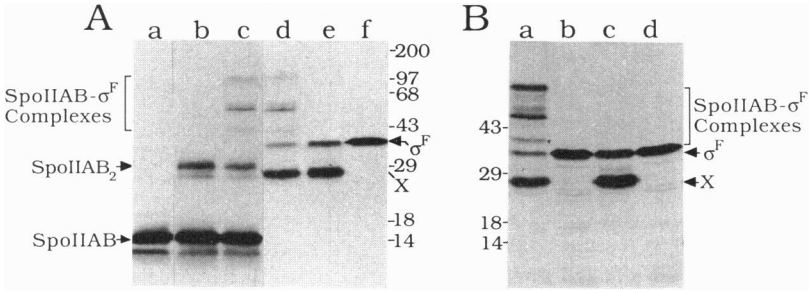


FIG. 2. Specific interaction between SpoIIAB and $\sigma^F$. The figure is an autoradiograph of the products of cross-linking reactions between SpoIIAB and $\sigma^F$ that had been subjected to SDS/polyacrylamide gel electrophoresis. (*A*) Lanes: a, $^{35}$S-labeled SpoIIAB incubated in the absence of DSS; b, $^{35}$S-labeled SpoIIAB incubated in the presence of DSS; c, mixture of $^{35}$S-labeled SpoIIAB and unlabeled $\sigma^F$ incubated in the presence of DSS; d, mixture of $^{35}$S-labeled $\sigma^F$ and unlabeled SpoIIAB incubated in the presence of DSS; e, $^{35}$S-labeled $\sigma^F$ incubated in the presence of DSS; f, $^{35}$S-labeled $\sigma^F$ incubated in the absence of DSS. (*B*) Lanes: a, mixture of $^{35}$S-labeled $\sigma^F$ and unlabeled heparin flow-through proteins containing SpoIIAB incubated in the presence of DSS; b, mixture of $^{35}$S-labeled $\sigma^F$ and unlabeled heparin flow-through proteins containing SpoIIAB incubated in the absence of DSS; c, mixture of $^{35}$S-labeled $\sigma^F$ and unlabeled heparin flow-through proteins lacking SpoIIAB incubated in the presence of DSS; d, mixture of $^{35}$S-labeled $\sigma^F$ and unlabeled heparin flow-through proteins lacking SpoIIAB incubated in the absence of DSS. All reaction mixtures in the experiments of *A* were supplemented with bovine serum albumin (Sigma, fraction V; 1 $\mu$g/$\mu$l, final concentration). Band X is an altered form of $\sigma^F$ induced by DSS treatment (see the text). Numbers at the side of the gels indicate the position of protein size markers in kDa.

*coli* in which SpoIIAB was a minor component (Fig. 2*B*, lane a) but not with a similar preparation of *E. coli* proteins lacking SpoIIAB (Fig. 2*B*, lane c).

**Inhibition of $\sigma^F$-Directed Transcription by Purified SpoIIAB.** To investigate whether SpoIIAB was also capable of inhibiting $\sigma^F$-directed transcription, we used as templates DNA fragments containing the *spoIIIG* promoter from *B. subtilis*, which is known to be under $\sigma^F$ control *in vivo* and to be used by $\sigma^F$-containing RNA polymerase *in vitro* (8, 17, 18). Core RNA polymerase failed to transcribe from the *spoIIIG* promoter (Fig. 3, lanes a and b) but core enzyme supplemented with purified $\sigma^F$ generated run-off transcripts of the expected sizes (Fig. 3, lanes c and d) from templates that had been cut at 121 and 127 bp downstream from the known *spoIIIG* start site (8). This transcription was prevented by the addition of renatured SpoIIAB that had been purified by elution from an SDS gel slice (Fig. 3, lanes f and h) but not by the addition of protein dilution buffer (lane e) or control protein that had been eluted and renatured from an SDS/polyacrylamide gel of proteins from cells lacking the *spoIIAB* gene (Fig. 3, lane g). The ratio of SpoIIAB to $\sigma^F$ in these experiments was approximately 20:1 but a significant level of inhibition was observed at a molar ratio of as little as 2:1 (data not shown). Independent experiments by K.-T. Min, C. M. Hilditch, J. Errington, and M. D. Yudkin (personal communication) show that purified SpoIIAB inhibits transcription by $\sigma^F$-supplemented RNA polymerase.

Thus, SpoIIAB both binds to $\sigma^F$ and blocks its capacity to direct transcription from a cognate promoter. Confirming and extending the results of previous genetic experiments, the present results indicate that SpoIIAB inhibits $\sigma^F$ by interacting with it directly. SpoIIAB can therefore be considered to be an anti-$\sigma$ factor. The work of Ohnishi *et al.* (33) has shown that the product of the *Salmonella typhimurium flgM* gene is similarly an anti-$\sigma$ factor that binds to and inhibits the action of the *S. typhimurium* $\sigma$ factor (coincidentally also called $\sigma^F$) that governs the transcription of certain genes involved in motility and chemotaxis. SpoIIAB and FlgM exhibit little similarity in their amino acid sequences (L.D., analysis not shown), and it is unlikely that they are homologous, although they evidently act in a similar fashion. On the other hand, SpoIIAB is highly similar to the *B. subtilis* RsbW protein (34), which has been shown on the basis of genetic experiments to be a negative regulator of the nonsporulation $\sigma$ factor $\sigma^B$ (35, 36). Indeed, experiments by Benson and Haldenwang (37) demonstrate that RsbW binds to $\sigma^B$ and blocks its capacity to direct transcription from a cognate promoter.

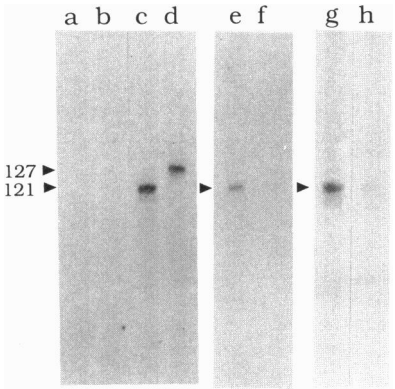


FIG. 3. SpoIIAB inhibits $\sigma^F$-directed transcription *in vitro*. The templates for transcription were the *spoIIIG*-containing plasmid pLD9 that had been cut with *Pst* I (lanes a, c, and e–h) or *Sph* I (lanes b and d). The transcription reaction mixtures contained template, core RNA polymerase, and the following components. Lanes: a and b, no additions; c and d, $\sigma^F$; e, $\sigma^F$ that had been preincubated with protein dilution buffer; f, $\sigma^F$ that had been preincubated with 7.5 $\mu$g of gel-purified SpoIIAB; g, $\sigma^F$ that had been preincubated with control protein (representing an equivalent volume of gel eluate to that containing 5 $\mu$g of SpoIIAB); h, $\sigma^F$ that had been preincubated with 5 $\mu$g of gel-purified SpoIIAB. The experiments of lanes a–d, e and f, and g and h were carried out separately. The products of transcription were subjected to electrophoresis in 8% polyacrylamide sequencing gels and visualized by autoradiography.

Inhibition of $\sigma^F$ by SpoIIAB is counteracted during sporulation by the action of SpoIIAA (16, 17), the product of the promoter-proximal member of the *spoIIA* operon (19). SpoIIAA does not prevent the transcription of the *spoIIAB* cistron or evidently the translation of its mRNA (L.D., unpublished results). Rather, it seems likely that SpoIIAA interacts directly with SpoIIAB, and indeed immunoprecipitation experiments by K.-T. Min, C. M. Hilditch, J. Errington, and M. D. Yudkin (personal communication) indicate the existence of complexes between SpoIIAA and SpoIIAB in cells undergoing sporulation. Parallel experiments (35, 36) indicate that inhibition of $\sigma^B$ by RsbW is reversed by the action of RsbV, a homolog of SpoIIAA (34), which, as for SpoIIAA and SpoIIAB, is capable of forming a complex with RsbW (37). In light of the evidence that SpoIIAB and RsbW are anti-$\sigma$ factors, it is perhaps appropriate to designate SpoIIAA and RsbV as anti-anti-$\sigma$ factors.

A crucial unsolved problem is the issue of how the activity of $\sigma^F$ is confined to the forespore (16) even though the transcription factor is synthesized prior to septation (ref. 15; S. R. Partridge and J. Errington, personal communication). The discovery that $\sigma^F$ is subject to regulation by SpoIIAA and SpoIIAB has led to the view that these proteins are directly involved in compartmentalizing the activity of $\sigma^F$ (4). SpoIIAB is believed to inhibit $\sigma^F$ in the predivisional sporangium and in the mother cell (16). Some unidentified feature of the forespore could provoke an interaction between SpoIIAA and SpoIIAB, thereby inhibiting SpoIIAB and unleashing $\sigma^F$-directed gene expression in the small compartment of the sporangium. It is not known how this happens [although genetic evidence implicates sporulation protein SpoIIE (16)], but it may be relevant that SpoIIAB is significantly similar [as judged by a BLAST search (38) of the GenBank database (May 1991); L.D. and P. Margolis, data not shown] to the histidine protein kinase KinA (SpoIIJ) from *B. subtilis* (39, 40), although SpoIIAB lacks similarity to the kinase in the region in which KinA undergoes autophosphorylation.

**Note Added in Proof.** Orsini *et al.* (41) have recently reported that a bacteriophage T4 gene called *asiA* encodes a 10-kDa polypeptide that corresponds to an antagonist of *E. coli* $\sigma^{70}$ (42). Our analysis indicates that the *asiA* gene product is not significantly similar to SpoIIAB, RsbW, or FlgM.

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