

Bacillus subtilis SpoIID Protein Binds to Two Sites in the *spoVD* Promoter and Represses Transcription by σ^E RNA Polymerase

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The *Bacillus subtilis* *spoVD* gene encodes a penicillin-binding protein required for spore morphogenesis. SpoIID is a sequence-specific DNA-binding protein that activates or represses the transcription of many different genes. We have defined the *spoVD* promoter region and demonstrated that it is recognized by σ^E RNA polymerase in vitro and that SpoIID represses *spoVD* transcription. Two strong SpoIID-binding sites were mapped in the *spoVD* promoter region, one overlapping the -35 region and the other encompassing the -10 region and the transcriptional start site.

In response to starvation, *Bacillus subtilis* initiates a developmental process involving a series of morphological changes driven by a program of gene expression (6). One of the early morphological changes is the formation of an asymmetric septum that divides the bacterium into two compartments, the mother cell and the forespore. Subsequent migration of the septum engulfs the forespore in a double membrane, pinching it off as a free protoplast within the mother cell. Cell wall-like material known as the cortex is then deposited between the two membranes. Finally, the mother cell synthesizes so-called coat proteins that assemble on the surface of the forespore and the mature spore is released by lysis of the mother cell. The program of gene expression driving these morphological changes involves a cascade of sigma factor activity (9, 18). In the mother cell, the products of genes transcribed by σ^E RNA polymerase ($E\sigma^E$) are primarily responsible for engulfment, cortex formation, and production of σ^K , while the products of genes transcribed by σ^K RNA polymerase ($E\sigma^K$) function mainly in formation of the spore coat, as well as mother cell lysis (6, 9). Another key regulator of mother cell gene expression is SpoIID, a sequence-specific DNA-binding protein that activates or represses many genes in both the σ^E and σ^K regulons (8, 14). Previous genetic studies suggested that the *spoVD* gene is likely to be a member of the σ^E regulon and showed that *spoVD* is overexpressed in *spoIID* mutant cells (3). The product of *spoVD* is a penicillin-binding protein. Penicillin-binding proteins generally carry out the final steps of the synthesis of peptidoglycan, which is the major component of the cortex. Consistent with a proposed role of SpoVD in cortex synthesis, *spoVD* mutant cells are defective in spore cortex development (1, 3). Here we show that $E\sigma^E$ can transcribe *spoVD* in vitro and that SpoIID can repress *spoVD* transcription by binding to the promoter region.

***spoVD* is transcribed by $E\sigma^E$ in vitro, and SpoIID represses this transcription.** Several lines of evidence suggest that *spoVD* is transcribed by $E\sigma^E$ (3). First, expression of a *spoVD-lacZ* fusion was reduced or abolished in strains defective in σ^E production. Second, *spoVD-lacZ* was expressed when transcription of the *spoIIG* operon encoding pro- σ^E and its putative processing enzyme, SpoIIGA, was induced in vegetative

cells. Third, the *spoVD* promoter region shows sequence similarity to other σ^E -dependent promoters. The upstream boundary of the *spoVD* promoter region was defined by deletion analysis with exonuclease III-S1 nuclease digestion and the integrational plasmid vector pSG1301 (3). Plasmids with inserts extending from a range of positions in the vicinity of the transcriptional start site (TSS) to a unique *Hind*III site in the middle of the *spoVD* coding region were integrated into the chromosome of Spo^+ strain SG38 (7). Inserts with their upstream ends located up to and including -28 bp relative to the TSS gave a Spo^- phenotype upon integration, indicating that promoter function was absent, whereas inserts extending to -42 bp or farther upstream were Spo^+ , indicating at least partial promoter activity. The minimal sequences needed for *spoVD* expression thus lie within 42 bp of the TSS. To determine whether $E\sigma^E$ could recognize this promoter in vitro, a plasmid extending well upstream of the promoter (to -211) and to position $+103$ downstream (pSG1362; reference 3) was used as the template in runoff transcription assays. The plasmid was linearized by digestion with restriction enzymes that cleave at different sites downstream of the *spoVD* promoter, generating DNA templates that would produce *spoVD* runoff transcripts of different lengths. In vitro transcription reactions were performed as described previously (8). Runoff transcripts were electrophoresed in a 5% polyacrylamide gel containing 8 M urea and detected by autoradiography. Partially purified $E\sigma^E$ produced runoff transcripts of the expected sizes (Fig. 1, lanes 2 and 3). Some promoters are transcribed by both $E\sigma^E$ and $E\sigma^K$ (8, 16, 24), presumably because both forms of RNA polymerase recognize some similar sequences in promoters (5). To demonstrate that σ^E can direct transcription from the *spoVD* promoter and to test whether σ^K can too, gel-purified σ^E and σ^K were reconstituted with core RNA polymerase as described previously (14) and used to transcribe a *spoVD* template. Only $E\sigma^E$, not $E\sigma^K$, produced a runoff transcript (Fig. 1, lanes 5 and 6). A control experiment showed that the reconstituted $E\sigma^K$ was active because a strong signal was produced from a template containing the σ^K -dependent *cotD* promoter (lane 7). We concluded that *spoVD* is transcribed by $E\sigma^E$. Since $E\sigma^E$ is active only in the mother cell compartment during sporulation (4, 10, 17), expression of *spoVD* is expected to be mother cell specific. This is consistent with the results of a

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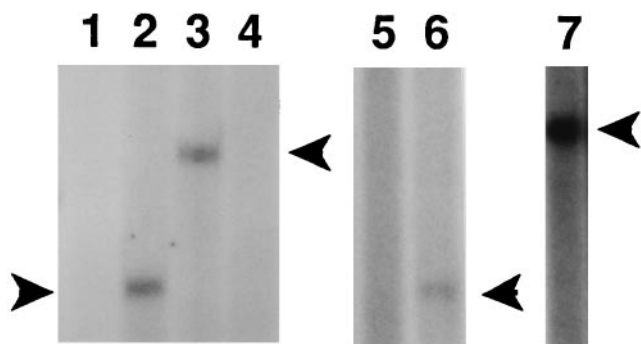


FIG. 1. In vitro transcription of *spoVD* by $E\sigma^E$ and effect of SpoIIID. pSG1362 (2 μ g), containing the *spoVD* promoter (3), was digested with *EcoRI* (lanes 1 and 2, 117-base transcript) or *XhoI* (lanes 3 and 4, 150-base transcript) and transcribed in a total volume of 42 μ l with $E\sigma^E$ (0.2 μ g) alone (lanes 2 and 3) or with 0.1 μ g of gel-purified SpoIIID added (lanes 1 and 4). pSG1362 (2 μ g) was digested with *EcoRI* and transcribed with reconstituted $E\sigma^K$ (lane 5) or with reconstituted $E\sigma^E$ (lane 6). pLRK100 (2 μ g) containing the *cotD* promoter (14) was digested with *HindIII* (225-bp transcript) and transcribed with reconstituted $E\sigma^K$ (lane 7). Arrowheads denote the positions of runoff transcripts of the expected sizes, as judged from the migration of end-labeled DNA fragments of *MspI*-digested pBR322.

genetic experiment showing that expression of *spoVD* only in the mother cell is sufficient to allow sporulation (3).

The ability to transcribe *spoVD* in vitro with $E\sigma^E$ allowed us to test whether SpoIIID can directly affect *spoVD* transcription. The twofold overexpression of *spoVD-lacZ* in *spoIIID* mutant cells suggested that SpoIIID might repress transcrip-

tion of *spoVD* (3). Addition of SpoIIID to the in vitro transcription reaction abolished the *spoVD* signal (Fig. 1, lanes 1 and 4). This was not due to general inhibition of transcription by SpoIIID, because the same preparation of SpoIIID activated transcription of *sigK* by $E\sigma^E$ (data not shown), as reported previously (8). We concluded that SpoIIID can specifically repress *spoVD* transcription by $E\sigma^E$ in vitro.

The *spoIIID* gene is transcribed by $E\sigma^E$ (13, 15, 22, 23), yet SpoIIID represses transcription of *spoVD* (Fig. 1) and some other σ^E -dependent genes. Thus, SpoIIID limits the expression of some genes in its own regulon. Other genes in the σ^E regulon that are repressed by SpoIIID include *bofA*, as demonstrated in vivo (12) and in vitro (8), and probably the *spoIIIA* operon, as implied by in vivo data (11). Another possible SpoIIID-repressed operon in the σ^E regulon is *spoIVF*, based on the observation that a *spoIVF-lacZ* fusion is overexpressed in *spoIIID* mutant cells (2) and the fact that three near-perfect matches to the SpoIIID binding site consensus sequence (8) can be found in the *spoIVF* promoter. Perhaps SpoIIID helps confine the expression of some genes in the σ^E regulon to a physiologically relevant level or time period. Consistent with this notion, in *spoIIID* mutant cells, the products of *spoIVF* overaccumulate and SpoIVFA is mislocalized (20).

SpoIIID binds to two sites in the *spoVD* promoter region. Gel retardation assays indicated that SpoIIID binds to DNA fragments containing the *spoVD* promoter region (data not shown). To localize the binding sites of SpoIIID in this promoter more precisely, DNase I protection experiments were performed as described previously (8). Radioactive DNA probes, labeled separately on the nontranscribed or tran-

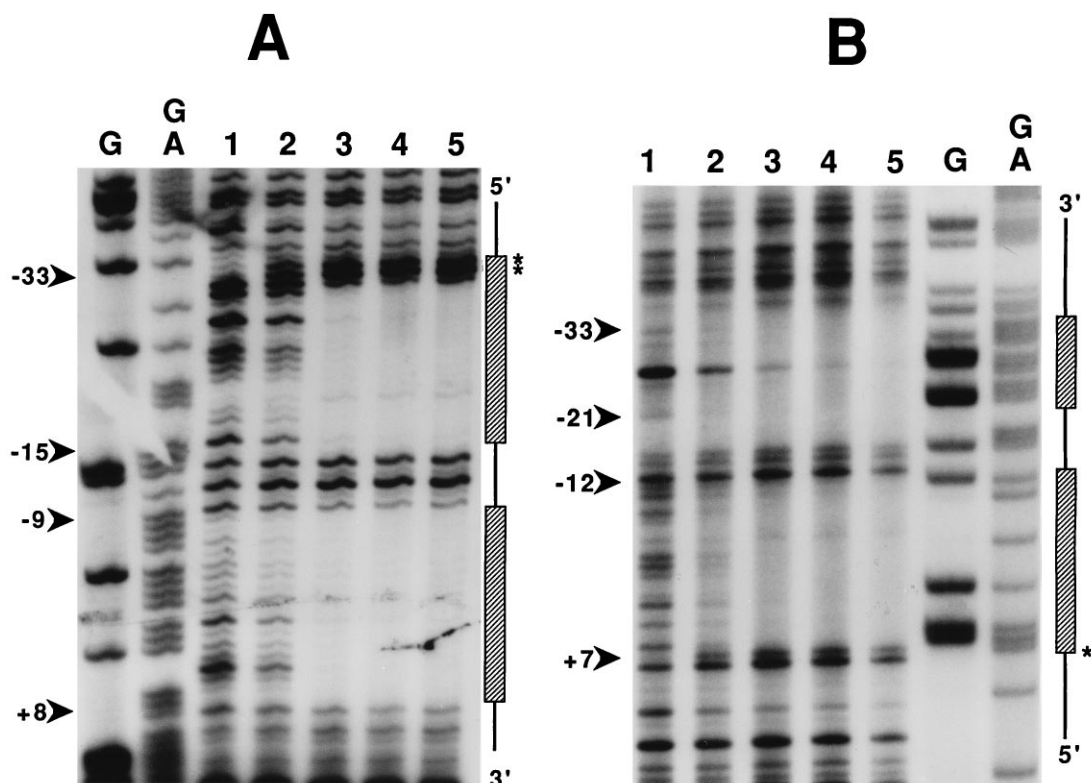


FIG. 2. SpoIIID footprints in the *spoVD* promoter region. Radioactive DNA fragments separately end labeled on the nontranscribed (A) or transcribed (B) strand were incubated in separate reactions with a carrier protein (bovine serum albumin, 21 μ g) only (lane 1) or with 0.025 (lane 2), 0.05 (lane 3), 0.1 (lane 4), or 0.2 (lane 5) μ g of gel-purified SpoIIID in addition to the carrier protein and then subjected to DNase I footprinting in a total volume of 42 μ l. Arrowheads denote the boundaries of protection by SpoIIID, and numbers to the left refer to positions relative to the TSS (3). Stars indicate enhanced cleavage by DNase I upon SpoIIID binding.

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