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The Sigma Factors of *Bacillus subtilis*

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INTRODUCTION

Regulation of gene expression in bacteria occurs primarily at the level of transcription. Although DNA-binding proteins (repressors and activators) can significantly affect the efficiency of transcription, the specificity of the transcription reaction rests on interactions between the transcribing enzyme (RNA polymerase [RNAP]) and the DNA sites (promoters) with which it makes contact. Bacterial RNAPs are routinely isolated in two distinct forms: core RNAP, which catalyzes the polymerization of ribonucleotides into the RNA complement of a DNA template, and RNAP holoenzyme, which contains the subunits of the core molecule (β, β′, and α_2) plus an additional protein (σ factor) that permits the holoenzyme to recognize promoter elements and initiate transcription at these sites. Much of what is known of the interactions between σ, core RNAP, and promoters came originally from experiments with *Escherichia coli* RNAP (reviewed in references 30 and 39). These studies showed that the specificity of the RNAP holoenzyme for its cognate promoter depends on its sigma subunit and that the association of σ with core RNAP is transient. The holoenzyme form initiates transcription; however, shortly after this initiation, the σ subunit is discharged, leaving a processive core enzyme to elongate the initiated transcript.

If cells contained multiple sigma proteins with unique promoter preferences, sigma factor substitution could be a potent vehicle for gene control. The cycling of the σ subunit on and off of core RNAP during the transcription cycle would provide an opportunity for σ factor exchange and a reprogramming of RNAP. The notion that multiple σ factors might exist as bacterial gene regulators was proposed when the properties of σ were first described (31). However, the discovery of alternative sigma factors was not immediately forthcoming. In both *E. coli* and *Bacillus subtilis*, only a single form of RNAP sigma factor was identified to direct the transcription of the available DNA templates (29, 31, 270). Early evidence that RNAP could be reprogrammed by multiple σ factors came from studies of bacteriophage-infected cells. The *B. subtilis* bacteriophages SPO1 and SP82 and the *E. coli* bacteriophage T4 were shown to encode regulatory proteins that were essential for the transcription of portions of their genomes (71, 95, 153, 154, 278, 303). The *B. subtilis* phage system proved to be more amenable than that of *E. coli* to biochemical analysis, and it was from SPO1-infected cells that the first "alternate" sigma factors were purified (71, 95, 228, 303). These proteins fulfilled the requirements of new specificity determinants. They could associate with core RNAP and, in the absence of the known *B. subtilis* σ factor σ^{55} (now designated σ^{A}), direct it to bind and initiate transcription at novel bacteriophage promoters. Details of the discovery and biochemical properties of the *B. subtilis* bacteriophage σ factors have been previously summarized in detail (64, 100). Throughout this review, I will refer to individual RNAP holoenzymes as E-σ^{X}: E represents core RNAP, and σ^{X} represents the particular σ factor that it carries. *B. subtilis* sigma factors were initially designated by a number which corresponded to their apparent molecular masses (e.g., σ^{55}, 55,000 Da; σ^{37}, 37,000 Da). This nomenclature has been abandoned and replaced by letter designations ($\sigma^{55} = \sigma^{A}$, $\sigma^{37} = \sigma^{B}$, etc.), which permits the sigma factor's identity to be incorporated into its genetic designation (e.g., the gene for σ^{A} is *sigA*) (187).

The discovery of multiple sigma factors in bacteriophage-infected cells established a precedent for the reprogramming of RNAP by a change in sigma factor composition, but direct evidence for alternative σ factors in uninfected bacteria was still lacking. It was possible that the bacteriophage-encoded sigma factors were merely specialized devices for usurping the host cell's biosynthetic machinery to viral ends rather than an indication that multiple σ factors existed as a general mode of gene control to be used by uninfected bacteria. Circumstantial evidence that multiple forms of RNAP were present in uninfected bacteria came from studies of sporulating *B. subtilis*. Sporulation is a starvation response by which *B. subtilis* converts itself from an actively growing vegetative cell into a dormant spore. It represents a series of morphological and physiological events that occur by the sequential activation and silencing of blocks of genes (a recent review of sporulation can be found in reference 76). It was noted more than 20 years ago that during sporulation, changes occur in the template specificity of the sporulating cell's RNAP. Virulent bacteriophages, which normally multiply in and lyse vegetatively growing *B. subtilis*, fail to replicate in bacteria that are sporulating. The phage genome enters the developing cell but becomes trapped and unable to direct the synthesis of progeny phage (276, 332). The failure of the viral genomes to be expressed in sporulating *B. subtilis* parallels a progressive inability of RNAP extracted from these cells to transcribe bacteriophage templates in vitro. During the first 2 h after the onset of sporulation, there is a sharp decrease in both the ability of the cells to support a productive bacteriophage infection and the activity of the extractable RNAP to transcribe bacteriophage but not synthetic [i.e., poly(dAT)] DNAs in vitro (25, 26, 185). The loss in bacteriophage-specific transcription correlates with a loss of σ^{A} (σ^{55}) from the isolated RNAP (25, 185) and prompted Losick and Sonenshein to suggest that changes in the template specificity of RNAP during sporulation are responsible for the failure of virus replication (185). Following this finding, workers in the Losick laboratory (107, 108, 181) and others (98, 129, 219) purified RNAP from sporulating *B. subtilis* and isolated several different sporulation forms of this enzyme. Each of the enzymes had distinct subunit structures and chromatographic properties. They contained the same RNAP core component but had additional sporulation-specific polypeptides that ranged in size from 20,000 to 95,000 Da, copurifying with the core molecule (98, 107, 108, 129, 181, 216, 219).

Although it was widely believed that these RNAP-associated proteins could be contributing to the altered specificity of the sporulating cell's RNAP, a lack of cloned sporulation-specific promoters prevented a direct test of their significance. A breakthrough occurred when a sporulation-specific gene was identified and its coding sequence cloned on an *E. coli* plasmid (265). This cloned sporulation gene (*0.4Kb*, now known as *spoVG*) was the first *B. subtilis* gene to be cloned. By using in vitro transcription of *spoVG* as an assay, a novel form of RNA polymerase which initiated transcription at the *spoVG* promoter but not at promoters that are recognized by the known *B. subtilis* RNAP holoenzyme E-σ^{A} was isolated from sporulating *B. subtilis* (113, 114). The novel RNAP had the subunit composition of core RNAP plus an additional polypeptide with an apparent molecular mass of 37,000 Da. Reconstitution experiments demonstrated that the promoter specificity of the RNA polymerase was a function of this protein and that, in the absence of σ^{A}, it conferred on core RNAP the ability to bind and initiate transcription at a unique promoter element (114). From these criteria, this protein was designated a novel σ factor (σ^{37}, renamed σ^{B}). At about the same time that σ^{B} was discovered, a third unique RNAP holoenzyme (E-σ^{28}, now E-σ^{D}) was detected and purified from vegetative *B. subtilis* on the basis of novel in vitro transcription from an *E. coli* bacteriophage T7 DNA template (143, 324). Since then, seven additional σ proteins have been identified in *B. subtilis*. Although multiple sigma factors have now been documented in several

different bacterial species and are likely to be a common feature in most, if not all, bacteria, *B. subtilis* remains the bacterium with the largest number of characterized sigma proteins. This review will describe these interesting proteins and their discovery, likely functions, and unexpectedly complex regulation.

COMPARISON BETWEEN RNAPs OF *E. COLI* AND *B. SUBTILIS*

Although it is assumed that much of the RNAP biochemistry learned in *E. coli* is directly applicable to the *B. subtilis* enzyme, the RNAPs from these bacteria are not identical. In *E. coli*, the largest polypeptide of core RNAP is β′, a subunit that can bind DNA (99, 267), while the second largest subunit, β, is the target for the initiation inhibitors rifampin and streptolydigin (141, 243). In *B. subtilis*, rifampin resistance mutations occur in β′, the largest subunit (115). Furthermore, mutations that confer streptolydigin resistance do not map to this subunit but are found in the gene for the other large subunit, β (116). The significance of these differences in antibiotic targeting is unknown.

More striking than the potential distinctiveness of the β and β′ subunits of *E. coli* and *B. subtilis* is the presence of a unique RNAP subunit (δ) in *B. subtilis*. Delta peptide was discovered as a host-encoded 21,000-Da protein that was associated with the RNAP extracted from SPO1-infected cells and subsequently found to be a normal component of RNAP from uninfected bacteria (228). In vitro, the addition of δ protein to transcription reactions reduces nonspecific initiation by RNAP containing either σ^A or bacteriophage-encoded σ factors (2, 3, 62, 277, 302). Although there is a report that the binding of either σ or δ to core RNAP is mutually exclusive (325), others have found that δ can bind to core RNAP in either the presence or absence of σ (131). The idea that δ functions prior to the initiation of transcription was suggested by a study in which the subunit composition of *B. subtilis* RNAP was determined under conditions which allowed the formation of stable enzyme-DNA complexes but which either blocked the initiation of transcription or permitted transcription to occur (279). Delta peptide was shown to be released from RNAP upon formation of enzyme-DNA complexes, whereas the sigma subunit was not released until transcription was ongoing (279).

Methylation protection and DNA footprinting experiments demonstrated that the presence or absence of δ has little or no effect on the region of promoters bound by E-σ (1). This result has been taken as evidence that δ enhances promoter selectivity by limiting the number of possible interactions that E-σ can make with DNA rather than by contributing to promoter recognition. In *E. coli*, σ^{70} has been attributed with both the properties of promoter recognition and the inhibition of nonspecific complex formation. It has been hypothesized that these functions may be allocated to separate proteins in *B. subtilis* (1, 120). Demonstrating a role for δ in vivo has been problematic. By using a synthetic oligonucleotide as a probe, the structural gene for δ (*rpoE*) was cloned and sequenced (173). *rpoE* has no significant homology to any other protein, including σ factors and the known bacterial RNAP subunits, and its disruption confers no obvious phenotype on *B. subtilis*. Viability, sporulation, and bacteriophage growth are unimpaired in an *rpoE* mutant strain (173). It has been suggested that if δ plays an important role in gene expression in vivo, there must be other gene products that can substitute for it in the *rpoE* mutant (173).

Although differences in antibiotic targeting to separate RNAP core subunits and the presence of the δ protein in the *B. subtilis* enzyme imply that the *B. subtilis* and *E. coli* RNAPs have unique qualities, there do not appear to be substantial differences between the core enzymes in the way that they interact with and use σ factors. Transcription by either *E. coli* or *B. subtilis* core RNAP can be stimulated in vitro by the other's sigma factor (322). In addition, *B. subtilis* σ^A protein has been shown to direct *E. coli* core RNAP to σ^A-dependent promoters in vitro (58), and synthesis of the *B. subtilis* σ^D protein in *E. coli* has been reported to substitute for its *E. coli* counterpart (σ^F) in activating flagellar gene expression (43).

B. SUBTILIS σ FACTORS

The first alternate sigma factors were discovered in *B. subtilis* on the basis of their biochemical qualities, with their structural genes revealed in later studies. More recently, homology searches of protein sequence databases have reversed the process and established several cloned regulatory genes as the coding sequences for putative σ factors. The structural genes for σ^E, σ^F, σ^G, and σ^L were identified in this manner (60, 78, 152, 201, 283, 285).

There are at present 10 known σ factors in *B. subtilis* (Table 1). Nine of these have been cloned, sequenced, and mapped to sites on the *B. subtilis* chromosome. These sites, along with the relative map positions of the other RNAP subunits (β and β′ [*rpoBC*], α [*rpoA*], and δ [*rpoE*]), are illustrated in Fig. 1. Only two of the known sigma factor genes (*sigE* and *sigG*) are linked. The remaining sigma factor genes are scattered along the *B. subtilis* chromosome with no obvious rationale for their location. The current and historical designations of the 10 sigma factors, along with their putative functions and target promoter sequences, are included in Table 1. The sigma factors that were discovered on the basis of biochemical activity (e.g., σ^A) were initially designated on the basis of their apparent mass when electrophoresed on sodium dodecyl sulfate (SDS)-polyacrylamide gels (e.g., $\sigma^A = \sigma^{55}$). Invariably, these apparent molecular masses were overestimates. When the structural genes for the sigma factors were cloned and sequenced, the predicted masses of their products were found to be approximately 20% less than the size estimated by their mobilities on SDS-polyacrylamide gel electrophoresis (PAGE). Hence, σ^{55} became σ^{43} (314). The confusion caused by the changing estimates of σ factor size was lessened with the introduction of letter designations for the sigma factors ($\sigma^{55} = \sigma^{43} = \sigma^A$). The letter designations for the sigma factors provide a simple way of carrying their identity over to the names for their structural genes (e.g., the gene for σ^A is *sigA*) (187); however, historical influences have complicated the naming of sigma factor genes. For example, the target promoter sequence and function of σ^A are similar to those of the principal sigma factor of *E. coli* (σ^{70}), which led to the σ^A structural gene's being given the genetic designation used for its *E. coli* counterpart (i.e., *rpoD*) (239). In addition, several of the sigma factors were found to be the products of genes that were originally identified as sporulation-essential loci and designated on the basis of this feature (e.g., σ^H is encoded by *spo0H*) (70). These designations are included in Table 1.

Table 1 also lists the generally accepted functions of the regulons that each sigma factor directs. It should be noted, however, that this list of functions is almost certainly incomplete because of limited representation of candidate genes for some of the sigma factors (e.g., σ^B and σ^C). The small number of available "target genes" for some of the sigma factors also compromises the list of consensus sequences. Although most consensus sequences are based on homologies shared by many promoters and verified by mutational studies (e.g., σ^A and σ^E),

TABLE 1. *B. subtilis* σ factors[a]

Sigma factor (alternative designation)	Gene(s)	Function	Promoter sequence[b]			Reference
			−35	Spacer (bp)	−10	
Vegetative-cell factors						
σ^A (σ^{43}, σ^{55})	*sigA*, *rpoD*	Housekeeping/early sporulation	TTGACA	17	TATAAT	210
σ^B (σ^{37})	*sigB*	General stress response	RGGXTTRA	14	GGGTAT	24
σ^C (σ^{32})	Unknown	Postexponential gene expression	AAATC	15	TAXTGYTTZTA	145
σ^D (σ^{28})	*sigD*, *flaB*	Chemotaxis/autolysin/flagellar gene expression	TAAA	15	GCCGATAT	119
σ^H (σ^{30})	*sigH*, *spoOH*	Postexponential gene expression; competence and early sporulation genes	RWAGGAXXT	14	HGAAT	237
σ^L	*sigL*	Degradative enzyme gene expression	TGGCAC	5	TTGCANNN	59
Sporulation-specific factors						
σ^E (σ^{29})	*sigE*, *spoIIGB*	Early mother cell gene expression	ZHATAXX	14	CATACAHT	252
σ^F ($\sigma^{spoIIAC}$)	*sigF*, *spoIIAC*	Early forespore gene expression	GCATR	15	GGHRARHTX	291
σ^G	*sigG*, *spoIIIG*	Late forespore gene expression	GHATR	18	CATXHTA	217
σ^K (σ^{27})	*sigK*, *spoIVCB*:*spoIIIC*	Late mother cell gene expression	AC	17	CATANNNTA	338

[a] The designations for the sigma proteins and their structural genes as well as likely functions of their regulons are listed. References for each item can be found in the text. The probable consensus sequences for the holoenzyme forms are aligned at their −10 positions (underlined). The spacer region represents the number of bases between the upstreammost −10 region base that is given and the downstreammost base of the −35 region. The reference for each consensus sequence is listed to its right.

[b] H, A or C; N, A, G, C, or T; R, A or G; W, A, G, or C; X, A or T; Y, C or T; Z, T or G.

others (e.g., σ^C) are inferred from as few as two promoters. A detailed description of each of the sigma factors follows.

VEGETATIVE-CELL SIGMA FACTORS

Sigma Factor σ^A

Isolation and characterization. σ^A is the principal sigma factor present in vegetatively growing *B. subtilis*. E-σ^A was the first holoenzyme isolated from *B. subtilis*, being readily purified by the standard chromatographic techniques that had been used for the isolation of the *E. coli* enzyme (8, 185, 190, 234, 270, 271). σ^A, like its *E. coli* counterpart σ^{70}, is separable from core RNAP by chromatography on phosphocellulose (190, 271). The σ^A protein has an apparent molecular mass of 55,000 Da when electrophoresed through SDS-polyacrylamide gels (8, 270, 271). The addition of purified σ^A to core RNA polymerase stimulates in vitro transcription from several *B. subtilis* bacteriophage DNAs as well as *E. coli* phage T4 and synthetic [poly(dA-dT)] DNAs (41, 190, 271, 302). The specificity of E-σ^A appears to be very similar to that of *E. coli* E-σ^{70}. Early transcription studies revealed that *E. coli* RNAP and E-σ^A bound the same restriction endonuclease-generated fragments of *B. subtilis* SPO1 DNA and initiated transcription at similar sites on SPO1 (100, 176) and ϕ29 (57, 58) DNA. More recently, genetic experiments demonstrated that homologous substitutions in σ^A and σ^{70} have similar consequences for promoter recognition (160). Although the transcription specificities of the *B. subtilis* and *E. coli* holoenzymes are similar, they are not identical. *E. coli* RNAP can initiate transcription at additional sites in ϕ29 template that are not used by E-σ^A (58). In addition, E-σ^A transcribes T4 DNA relatively poorly (271) and interacts more weakly with T7 promoter sites (323) than does the *E. coli* enzyme. In one study, E-σ^A transcribed an SPO1 promoter 30 times more effectively than the *lacUV5* promoter, while E-σ^{70} was only 50% more active on the SPO1 promoter (176). When σ^A-dependent promoters were cloned and sequenced, it was found that their common sequences, at the regions known to be important in promoter recognition by σ^{70} (i.e., bp −10 and −35), were identical to their counterparts in σ^{70}-dependent promoters (176, 210, 214). To account for the difference in specificity between the holoenzymes in light of identical −10 and −35 recognition regions, additional sequence elements, either between these regions or upstream of the −35 region, were proposed to be important for efficient use of promoters by E-σ^A (176, 210, 214). One study attempted to identify such elements by isolating mutant *lacUV5* promoters that displayed increased activity in *B. subtilis* (123). Mutations conferring the desired phenotype were found to be clustered between bp −18 and −14 and near the transcription initiation site. Most of the mutations had little effect on promoter activity in *E. coli*, but they increased *lacUV5* activity as much as 28-fold in *B. subtilis*. It was concluded that although the sequences in the highly conserved −35 and −10 regions of the promoter are

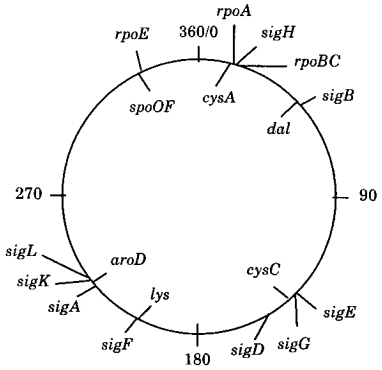

FIG. 1. Chromosomal locations of *B. subtilis* RNAP subunits. The map is based on the maps of Henner and Hoch (124) and Losick et al. (187). RNAP subunits are represented on the outside of the map, with "linked" landmark loci drawn within the circle. Min 360/0, 90, 180, and 270 are indicated.

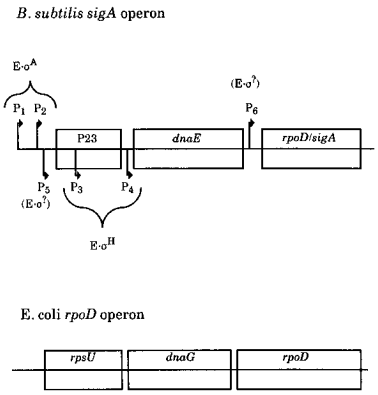

FIG. 2. *B. subtilis sigA* operon. The *sigA* operon, based on an overview by Qi et al. (242), is drawn with the *E. coli rpoD* operon (32) provided for comparison. The *dnaE*/*dnaG* and *sigA*/*rpoD* genes encode homologous proteins. The six promoters of *sigA* (P_1 to P_6) and the approximate start sites of their mRNAs (arrows) are illustrated. The form of RNAP that recognizes each promoter is indicated, if known.

important, additional sequences in the promoter region contribute to gene expression in *B. subtilis* (123).

Structure of the σ^A operon. The structural gene for σ^A was isolated by using polyclonal anti-σ^A antibody to probe a *B. subtilis* genomic library (239). The nucleotide sequence of the cloned DNA revealed two genes preceding *sigA* (314). The three genes are organized into an operon which is similar to the operon that encodes σ^{70} of *E. coli* (Fig. 2). The *E. coli* operon specifies three products: the ribosomal protein S21 (*rpsU*), DNA primase (*dnaG*), and σ^{70} (*rpoD*) (32). The three genes forming the *B. subtilis* σ^A operon are P^{23}, *dnaE*, and *rpoD* (*sigA*) (314). Both *dnaE* and *sigA* resemble the *E. coli* genes. *dnaE* encodes a 68,428-Da protein with significant similarity (31%) to *E. coli* DNA primase (318). The *sigA*/*rpoD* product (42,828 Da) is more than 50% homologous with σ^{70} (105, 314).

The similarities that are found in the two promoter-distal genes of the *E. coli* and *B. subtilis* operons do not extend to the first gene of the operons. P23 is more than twice the size of S21 and shares no homology with it (314). The sequence of *P23* contains three potential translation initiation sites that, if used, would form proteins of 23,000 (P23), 19,000 (P19), and 9,000 (P9) Da with the same carboxy terminus. Fusion protein studies, using *E. coli lacZ* as a reporter gene, have shown that all three translation initiation sites are used in vivo and that their expression is developmentally regulated (316). During vegetative growth, P23 and P19 but not P9 are synthesized; however, when the stationary phase is reached, promoter switching occurs within the operon (see below), resulting in P9's becoming the primary translation product (316). The *P23* function is unknown. Mutagenesis studies in which *P23* was inactivated by inserting a chloramphenicol acetyltransferase (CAT) gene into its interior revealed no obvious detrimental effect on growth or sporulation (343). One mutant in which *P23* was deleted sporulated earlier than the wild-type strain; however, this deletion also removed one of the operon's σ^H-dependent promoters, thus making the cause of the altered sporulation phenotype unclear (343).

Regulation of the *sigA* operon. Two of the three genes encoded by the *sigA* operon are essential for growth. It is therefore not unreasonable to expect that the cell would provide multiple means by which these gene products could be expressed in response to a number of different growth conditions. The observed transcription of the *sigA* operon seems to bear out this notion. Six promoters of the *sigA* operon have been identified (Fig. 2) and characterized. The P1 and P2 promoters are active at a significant level only during vegetative growth, with P2 being used to a greater extent then P1 (317). P1 and P2 were found to contain sequences typical of promoters recognized by σ^A, with the −35 region of P2 overlapping the −10 region of P1 (317). Why two similar overlapping promoters direct vegetative expression of *sigA* has not been determined. A third promoter, P3, differs from the preceding two in both sequence and time of expression. P3 is not active during vegetative growth but is used after *B. subtilis* begins sporulation (317). The sequence of P3 as well as its time of expression suggested that it might be transcribed by an alternate form of RNAP. E-σ^B was initially proposed to be the transcribing enzyme (317); however, studies of *sigA* expression in *B. subtilis* mutants that lacked σ^B demonstrated that σ^B is not needed for expression from P3 (38). Instead, E-σ^H appears to be the enzyme that recognizes P3. E-σ^H efficiently and accurately uses P3 in vitro, and the P3 transcript is not synthesized in cells which lack σ^H (38). σ^H is believed to direct post-exponential-phase gene expression (see below), and so the identification of an E-σ^H promoter for the *sigA* operon can be viewed as an indication of the need for continued *sigA* expression after the end of vegetative growth (38). A second σ^H-dependent promoter (P4) was subsequently demonstrated (241). Both σ^H-dependent promoters are employed at low levels during vegetative growth and are activated during early stationary phase (241). Expression from the upstream σ^H-dependent promoter (P3) allows transcription of a portion of the P23 gene (316) as well as the *dnaE* and *sigA* genes (317); however, expression from P4, which is located in the ribosome-binding site (RBS) of *dnaE*, results in the expression of only the *sigA* gene because of abbreviation of the *dnaE* RBS in the P4-directed transcript (241). Promoter changeover thus appears to alter the expression pattern of the operon.

Aside from the changes in P23, which are of unknown significance, there is the potential for the continued synthesis of σ^A in post-exponential-phase cells but reduced expression of *dnaE* (primase). This has been proposed to be in keeping with the cessation of chromosome replication that occurs at this time (241). Recently, a fifth promoter (P5) was discovered in primer extension and promoter probe studies (242). Unlike the other four promoters, transcription from P5 does not become evident until 3 to 5 h after the onset of sporulation (242). Mutations in the genes for σ factors (σ^E, σ^G, σ^H, and σ^K) that are known to be needed during sporulation did not block transcription from P5, although mutations in the sporulation gene activator-repressor system (Spo0A) severely reduced expression from P5 (242). The existence of P5 is taken as evidence that σ^A has an ongoing role during the later stages of sporulation (242). It has not been determined whether an unknown form of RNAP is needed for transcription from P5 or if a known holoenzyme (e.g., E-σ^A), in concert with DNA-binding proteins, triggers P5 activation. There is a sixth promoter (P6) in the *sigA* operon which is incompletely defined but appears to be used when *B. subtilis* is exposed to elevated temperatures (315). P6 is positioned in the operon so as to allow only the expression of σ^A (Fig. 2). P6 may represent a mechanism for the synthesis of σ^A during thermal stress, as occurs for a σ^{70} in *E. coli* following heat shock (299).

E-σ^A-transcribed genes. σ^A is the most abundant sigma factor found in purified RNAP from vegetatively growing *B. subtilis* (63, 270) and also the most similar to σ^{70}, the primary *E. coli* σ factor (120). σ^A and σ^{70} have a similar consensus promoter sequence (120) and are the host sigma factors that

direct the transcription of bacteriophage genes following infection by their respective viruses (64, 120, 176, 278). These similarities argue that σ^A is the *B. subtilis* counterpart of σ^{70} and, as such, likely to direct the transcription of most of the *B. subtilis* genes expressed during growth in rich medium. In addition to expressing these "housekeeping genes," σ^A is also involved in specialized gene expression. The DNA damage-inducible (*din*) promoter regions of *B. subtilis* contain σ^A-like recognition sequences (44), as do at least some of the genes that are required for the heat shock response (40, 180, 321) and the *B. subtilis* stationary-phase functions of degradative enzyme synthesis (225) and the development of competence for DNA transformation (68). σ^A also plays a role in the expression of early sporulation genes. Several catabolite-resistant sporulation (*crsA*) mutations, which both overcome the repressive effects of glucose on sporulation and partially suppress some mutations in sporulation regulatory genes (*spo0B*, *spo0E*, *spo0F*, *spo0K*, and *spoIIN*/*spoIIG279*), have been found to be base changes within the coding sequence for *sigA* (155, 156, 177, 238). Conversely, intergenic suppressors of a *crsA* mutation were isolated and mapped to the *spo0A*, *spo0B*, and *spo0E* genes (156). Spo0A is a DNA-binding protein which functions as a transcriptional activator-repressor of post-exponential-phase gene expression (28, 127). It is critical for the initiation of sporulation, with several of the other stage 0 sporulation gene products participating in Spo0A activation. Genetic evidence for an interaction between *sigA* and components of the *spo0A* regulatory machine suggested that σ^A has a role in expressing genes that are involved in the initiation of sporulation (238). Explicit evidence for this role came with the discovery that specific base substitutions in the promoter elements of the *spo0A*-dependent sporulation genes *spoIIG* and *spoIIE* could be suppressed by amino acid substitutions in σ^A (158, 161, 258, 334). Transcription of *spoIIG* and *spoIIE* does not begin until after the onset of sporulation; hence, at least some σ^A activity persists into the early stages of this process (101, 109, 159, 334).

Although there is clear evidence that σ^A has a function in early spore gene expression, its role at later times in sporulation is controversial. The loss of σ^A activity and the disappearance of extractable E-σ^A from sporulating *B. subtilis* was an early indication of this organism's RNAP heterogeneity (25, 182); however, there are reports of some investigators being able to isolate E-σ^A from sporulating *B. subtilis* (63). Although E-σ^A is not routinely isolated from sporulating *B. subtilis*, immunological probes can detect σ^A in these cells (73, 301). Anti-σ^A antibody immunoprecipitates similar amounts of σ^A from crude extracts of cells that either were growing vegetatively or had progressed 3 to 4 h into sporulation (301). The segregation of σ^A from RNAP during RNAP purification was found to be a chloramphenicol-sensitive phenomenon. Addition of chloramphenicol to sporulating *B. subtilis* rapidly restores both σ^A activity in the treated cells and the ability to isolate E-σ^A from their extracts (266). From these results, sporulating cells were hypothesized to contain an unstable (half-life, 11 min) inhibitor of σ^A that can become depleted after chloramphenicol treatment to allow the reformation of E-σ^A (266). This hypothetical σ^A inhibitor and its proposed instability could account for the disparity among investigators' abilities to isolate E-σ^A from cultures of sporulating *B. subtilis*. The loss of the putative inhibitor from some preparations may be responsible for the reformation of E-σ^A.

The role, if any, of σ^A in late spore gene expression remains undefined. The isolation of E-σ^A from sporulating *B. subtilis* (63) and the discovery of a promoter (P5) within the *sigA* operon which may direct the synthesis of σ^A late in sporulation (242) offer the possibility that E-σ^A could be needed late in development. Presumably, its role at this time would involve directing the transcription of σ^A-dependent genes whose expression must persist throughout sporulation. However, the difficulty with which E-σ^A is isolated from sporulating cells (25, 182) and the loss of bacteriophage gene expression (25, 185) following the onset of sporulation suggest that once the early sporulation genes are expressed, σ^A may become largely inactive. If this is so, the possible synthesis of σ^A late in sporulation (242) could represent a device to provide the spore with σ^A that is inactivated and stored for use during germination and outgrowth (33).

Sigma Factor σ^B

Isolation and characterization. σ^B, the first alternate σ factor to be found in bacteria, was detected as a subunit of an RNAP holoenzyme that transcribed a cloned sporulation gene (*0.4Kb*/*spoVG*) in vitro (113). *spoVG* could not be transcribed in vitro by purified E-σ^A but was transcribed by partially purified RNAP that had been prepared from sporulating *B. subtilis* (113). The enzyme responsible for the transcription, resolved by sequential chromatography on phosphocellulose and DNA-cellulose, was found to be a minor component of the total RNAP population (114). It had the subunit composition of core RNAP plus an additional protein (σ^{37}/σ^B) with an apparent molecular mass of 37,000 Da. σ^B, unlike σ^A, did not separate from core RNAP during chromatography on phosphocellulose (113). Although this characteristic aided in the initial purification of E-σ^B, it complicated the isolation of σ^B for reconstitution studies. Dissociation of highly purified E-σ^B with 6 M urea followed by fractionation of the subunits by phosphocellulose chromatography was used to isolate the σ^B protein (114). The fractionated σ^B subunit, when added back to purified core RNAP, reconstituted a holoenzyme that would selectively bind and initiate transcription from promoters (*ctc* and *spoVG*) that were not recognized by E-σ^A (114). The probable promoter sequences of these genes argued that E-σ^B was recognizing a nucleotide sequence element (Table 1) that was distinct from that used by E-σ^A (208–211, 297). E-σ^B was initially isolated from early sporulating *B. subtilis*; however, subsequent studies demonstrated that it is primarily a vegetative-cell σ factor. The levels of E-σ^B and E-σ^A purified from vegetatively growing or sporulating *B. subtilis* decrease in parallel as the cells proceed into development, so that by 2 h after the onset of sporulation, the levels of both enzymes are markedly reduced (112). Although E-σ^B's period of abundance parallels that of E-σ^A, the amount of E-σ^B present in vegetatively growing or early sporulating cells is less than 5% of the E-σ^A level (112, 113). Thus, compared with σ^A, σ^B is a minor-abundance sigma factor.

Cloning the σ^B structural gene. The gene that encodes σ^B was independently cloned and sequenced by two laboratories, using either a deduced oligonucleotide sequence (18) or anti-σ^B polyclonal antibodies (75) as probes to identify the σ^B-encoding region. The nucleotide sequence of *sigB* specifies a protein of 30,143 Da whose predicted amino acid sequence shows significant homology with that of known σ proteins (18, 75). Disruption of the *sigB* sequence has no obvious effect on the ability of *B. subtilis* to grow or sporulate (18, 75).

Sequence analysis of the region surrounding *sigB* revealed the presence of three additional open reading frames, which were initially called *orfV*, *orfW*, and *orfX* but renamed *rsb* (regulator of sigma B) once their role in σ^B control was established (Fig. 3) (75, 151). *rsbV*, *rsbW*, and *rsbX* are calculated to encode proteins of 12,000, 18,000, and 22,000 Da, respectively

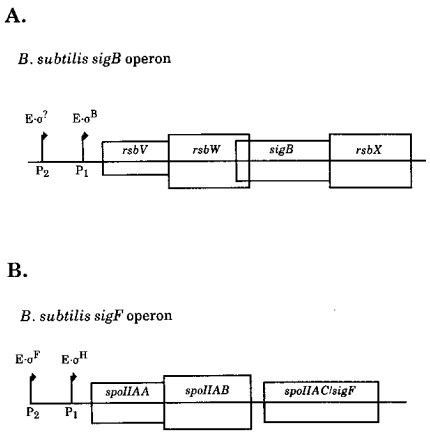

FIG. 3. *B. subtilis sigB* and *sigF* operons. The *sigB* (A) and *sigF* (*spoIIA*) (B) operons are drawn based on the data of Kalman et al. (151) and Fort and Piggot (91), respectively. *rsbV/W* and *spoIIAA/AB* encode homologous proteins, with the initiation codon of the downstream member of the pair (i.e., *rsbW* and *spoIIAB*) overlapping the termination codon of the upstream member (*rsbV* or *spoIIAA*). Thirteen codons of the *sigB* structural gene are contained within *rsbW*, while the *sigF* structural gene is separated by 11 bp from the *spoIIAB* termination codon. The *sigB* operon contains a fourth gene (*rsbX*) not found in the *sigF* operon. *rsbX*'s initiation codon overlaps the termination codon of *sigB*. *sigB* and *sigF* both have at least two promoters, with the principal promoters of each operon being dependent on E-σ^B and E-σ^H, respectively.

(151). The gene order is *rsbV-rsbW-sigB-rsbX*, with the initiation codons for *rsbW* and *rsbX* overlapping the termination codons for *rsbV* and *sigB*, respectively, and the first 13 codons of *sigB* lying within, but out of frame with, the last codons of *rsbW* (151). Primer extension and S1 nuclease mapping demonstrated that the major *sigB* operon RNA initiates 32 nucleotides upstream of *rsbV* and terminates 34 nucleotides downstream of *rsbX* (151). *sigB* is therefore the third gene of a four-gene operon. A comparison of the sequence of the *sigB* operon with sequences in a protein data bank revealed a striking similarity of the genetic organization of the *sigB* operon to that of the *B. subtilis sigF* operon (*spoIIA*) (151). Both sigma factor genes are preceded in their respective operons by two highly homologous proteins (RsbV, SpoIIAA; RsbW, SpoIIAB) (Fig. 3). RsbV has 32% amino acid sequence identity to SpoIIAA, and RsbW has 27% identity with SpoIIAB (151). From these similarities, it was speculated that these protein pairs might regulate their respective sigma factors by a similar molecular mechanism and that the *spoIIA* and *sigB* operons could control divergent branches of post-exponential-phase gene expression (151).

σ^B regulation. The σ^B structural gene is the third gene in a four-gene operon whose principal promoter is itself dependent on E-σ^B (151). The nucleotide sequence of the promoter has the consensus sequence at −10 and −35 found in other σ^B-dependent promoters (151). In addition, the activity of the promoter, measured either by means of a *sigB-lacZ* transcriptional fusion (151) or by Northern (RNA blot) analysis (13), is eliminated in *B. subtilis* strains with null mutations in the σ^B structural gene. At least one additional promoter for the *sigB* operon, which does not depend on σ^B for its activity, is likely to exist. Plasmids which form transcriptional fusions and express the *E. coli lacZ* gene upon integrating into active operons were found to display β-galactosidase activity when they entered the *sigB* operon upstream of the σ^B coding region (151). This integration event should have disrupted σ^B synthesis, and yet transcription into the reporter gene persisted. There is no obvious termination site upstream of the *sigB* operon. This leaves open the possibility that the transcript of an upstream promoter or operon could extend into *sigB*. The RNAP responsible for this transcript and the conditions under which it contributes to *sigB* expression are unknown. Under routine culture conditions, expression of the *sigB* operon is σ^B dependent and likely originates from the promoter immediately upstream of *rsbV*.

The σ^B-dependent promoter of the *sigB* operon is principally regulated by the activity of σ^B itself. Although σ^B's activity does not change dramatically under conventional culture conditions (13, 151), it has the potential to be silenced or activated to very high levels by the products of the other genes within the *sigB* operon (13–15, 75, 133, 151, 312). The most promoter-distal gene of the *sigB* operon was the first to be implicated as a regulator of σ^B activity. Two groups independently discovered that null mutations in *rsbX* caused both an elevation in the expression of σ^B-dependent promoters and an inhibition of the growth of *B. subtilis* strains which carried the mutations (75, 133). The growth inhibition observed in the RsbX$^-$ strains was relieved by secondary mutations within the σ^B coding region (75, 133). RsbX was proposed to be a negative regulator of σ^B synthesis or activity, with the heightened σ^B activity in its absence being detrimental to *B. subtilis* growth (75, 133, 151). Roles for RsbV and RsbW as additional regulators of σ^B activity were first proposed on the basis of their similarity to regulatory genes contained within *spoIIA*, the σ^F operon (151). A direct test of the significance of *rsbV* and *rsbW* in regulating σ^B came from studies in which their coding sequences were inactivated by directed frameshift (13) or in-frame deletion (23) mutations. Mutations in *rsbW*, like those in *rsbX*, resulted in elevated expression from σ^B-dependent promoters. In contrast, σ^B-dependent promoter activity in *B. subtilis* strains with mutations in *rsbV* was found to be virtually nonexistent, resembling the level of activity found in mutant strains which lack σ^B itself. RsbW and RsbX thus acted as negative regulators of σ^B, while RsbV behaved as a positive regulator. *rsbV* mutations were found to be epistatic to *rsbX* mutations but subordinate to mutations in *rsbW*. These results were interpreted as evidence that RsbW is the primary negative regulator of σ^B, with RsbV being needed to counteract its inhibitory effects (13, 23). Because RsbV must be present for RsbX to exert its effect, its site of negative regulation is thought to be upstream of RsbV/RsbW in the regulatory pathway (13, 15, 23).

The above-referenced results did not distinguish between an effect of the *rsbV*, *rsbW*, and *rsbX* proteins at the σ^B-dependent promoter of *sigB* alone or at additional promoters that are recognized by E-σ^B. Although the mutations in *rsbV*, *rsbW*, and *rsbX* have identical effects on the activity of both the σ^B-dependent *sigB* and *ctc* promoters, the effect of the mutations on *ctc* transcription could be indirect, i.e., a consequence of their influence on *sigB* expression and the resulting changes in σ^B levels. Studies in which the σ^B-dependent promoter of *sigB* was deleted and replaced with an inducible σ^A-dependent promoter (P_{SPAC}) argued that the *rsbV/rsbW* regulatory system is not limited to the expression of *sigB* (15, 23). In *B. subtilis* strains in which *sigB* expression is dependent on P_{SPAC}, the σ^B-dependent *ctc* transcription remains sensitive to the *rsbV/rsbW* network: i.e., even with a P_{SPAC}-driven *sigB* operon, *ctc* transcription is barely detectable in an *rsbV* mutant strain and expressed at very high levels in an *rsbW* mutant strain (15, 23). On the basis of this finding, RsbV and RsbW were concluded to be general regulators of σ^B activity. Unlike the RsbW-RsbV pair, RsbX mutations had no apparent effect on σ^B activity when the *sigB* operon was expressed from P_{SPAC} (15, 23). Although this finding suggested that RsbX controls *sigB*

operon expression and not the state of σ^B activity, such a notion was not easily reconciled with the observation that σ^B-dependent promoters are expressed at very high levels in $RsbX^-$ $RsbW^+$ $RsbV^+$ strains. It would be expected that if a loss of RsbX merely elevated the expression of the *sigB* operon, the coordinately enhanced synthesis of RsbW as well as σ^B would continue to inhibit σ^B.

This apparent paradox was resolved by the discovery of an additional *sigB* regulatory gene, *rsbU*, whose product is essential for the RsbX-regulated pathway (312). This gene, lying immediately upstream of the *sigB* operon, was disrupted during the construction of the strains used in some of the earlier *rsbX* studies (15). If *rsbU* is intact, inactivation of RsbX results in a dramatic increase in σ^B-dependent transcription regardless of whether the *sigB* operon is expressed from its normal promoter or P_{SPAC} (312). The increase in σ^B activity following a loss of RsbX still requires a functional RsbV. The data are consistent with RsbX's being an indirect regulator of σ^B activity, inhibiting an RsbU-dependent process that stimulates RsbV's ability to counteract RsbW. Although important details of the interactions of RsbU, X, V, and W are largely missing, the basic mechanism by which RsbW inhibits σ^B-dependent transcription is becoming clear.

Antibodies specific for either RsbW or σ^B have been found to coprecipitate both proteins from crude cell extracts (14). In addition, gel filtration studies, coupled with Western (immunoblot) analyses, demonstrated that the binding of σ^B to RNAP and its association with RsbW are mutually exclusive (14). In reconstitution experiments, in which partially purified σ^B and RsbW were added to core RNAP in vitro, RsbW efficiently blocked σ^B-dependent transcription, but only if it was incubated with σ^B prior to the addition of core RNAP (14). Taken together, these results support a model (14) in which RsbW functions as an anti-σ factor, binding to σ^B and preventing σ^B's association with core RNAP.

RsbV's role as a positive regulator appears to involve keeping σ^B free from RsbW-σ^B complexes. Gel filtration chromatography and nondenaturing gel electrophoresis studies visualized complexes of RsbW-RsbV that were distinct from the RsbW-σ^B complexes (72). The data support a model in which RsbV binds directly to RsbW and blocks its ability to form the RsbW-σ^B complex. Thus, σ^B activity would appear to be controlled by the differential association of RsbW with either σ^B or RsbV. The factors determining whether RsbW will bind to either RsbV or σ^B are largely unknown; however, two conditions have been proposed to influence RsbW's binding decision. The first circumstance was suggested from in vitro studies of the RsbW homolog SpoIIAB, the anti-σ^F factor (5). These studies showed that SpoIIAB preferentially binds to σ^F or SpoIIAA (an RsbV counterpart) in response to the ATP/ADP ratio present in the reaction mixture. A high ATP/ADP ratio favors SpoIIAB binding to σ^F, while a low ratio results in preferential association of SpoIIAB with SpoIIAA (5). RsbW is hypothesized to react similarly to ATP/ADP ratios, with σ^B induction triggered by a drop in ATP, which shifts RsbW to RsbV and the release of σ^B. A second factor that could potentially influence RsbW's binding decision is the phosphorylation state of RsbV. Both RsbV and SpoIIAA can be kinased in vitro by their respective anti-σ partners (RsbW and SpoIIAB, respectively) (72, 202). In the case of RsbV, variants of RsbV with isoelectric focusing (IEF) properties identical to those of phosphorylated and unphosphorylated forms of RsbV have also been visualized when crude *B. subtilis* extracts were subjected to IEF and analyzed by Western blot (72). An IEF analysis of the proteins present in gel filtration fractions containing RsbV-RsbW complexes or RsbV alone revealed that only the unmodified form of RsbV was associated with RsbW (72). The presumed phosphorylated variant of RsbV was present in those fractions which did not contain RsbW. Apparently, the phosphorylation of RsbV by RsbW diminishes RsbV's ability to associate with RsbW. It is not known whether the RsbW-dependent phosphorylation of RsbV represents a device to prevent binding of the modified RsbV to RsbW or a consequence of a reaction that RsbW must catalyze in order to change its specificity for RsbV or σ^B.

σ^B-dependent transcription is activated when *B. subtilis* enters the stationary phase of growth or is subjected to any of several environmental insults, including heat shock, O_2 limitation, or exposure to ethanol or high salt (13, 16, 21, 23, 313). Although all of these conditions involve a release of σ^B from RsbW inhibition, different pathways may be involved in effecting this release. Heat shock-induced σ^B activation, in contrast to the σ^B activation that occurs upon entry into stationary phase or following treatments with ethanol or high salt, can at least partially occur without a functional RsbV gene (16, 21). Either the RsbW-σ^B complex itself is thermolabile, or RsbW is dissociated from σ^B by an alternative release factor that has not yet been found. If this second possibility is correct, RsbW could be a common target for distinct factors that respond to different environmental cues. Further complexity comes from the observation that even the RsbV-dependent σ^B activation response can require additional components. The induction of σ^B by ethanol and salt stress but not by entry into the stationary phase requires RsbU as well as RsbV (312). This suggests that entry into stationary phase and ethanol or salt stress are either generating different signals which influence RsbW's binding decision or ultimately generate the same signal, with entry into stationary phase initiating this signal without the need for RsbU. It is unknown which regulatory protein receives and responds to the σ^B activation signal; however, RsbW's central role makes it a probable target.

Although the induction of σ^B-dependent genes by several different environmental stresses argues that σ^B is a component of the *B. subtilis* general stress response, it does not appear to be essential to *B. subtilis*'s survival under any of these conditions. The dispensability of σ^B is most clearly illustrated during heat shock. The *B. subtilis* homologs of the essential *E. coli* heat shock genes have been identified (6, 166, 180, 262). As would be expected from the finding that *B. subtilis* strains with null mutations in *sigB* are no more temperature sensitive than their wild-type counterparts, it has been observed that $SigB^+$ and $SigB^-$ strains produce the principal heat-inducible proteins in similar amounts (16, 313). There are, however, additional proteins which are present after a temperature shift in the wild-type strain but are absent in a *sigB* null mutant (16, 313). Thus, although σ^B is not required for the expression of essential heat shock genes, it is activated by heat shock to elevate its own synthesis and the synthesis of several other heat-inducible proteins. Although the role of most of the σ^B-dependent gene products is unknown, they presumably provide a beneficial but nonessential function that aids *B. subtilis* in adopting to heat shock and other stresses.

The observation that both *rsbV* and *rsbW* and their homologs *spoIIAA* and *spoIIAB* have overlapping termination and initiation codons with identical sequences at the junction of each suggested the possibility that each gene pair may be translationally coupled (151). Presumably, this would be a device to ensure equimolar synthesis of proteins that act together in vivo. Experimental support for translational coupling in the *spoIIA* system came when a mutation in the RBS of *spoIIAA* was found to confer the phenotype of a strain deficient in both *spoIIAA* and *spoIIAB* and significantly reduce the expression of

a downstream *spoIIAB*::*lacZ* translational fusion (227). A corresponding loss in *rsbW* activity was not seen, however, when a frameshift mutation was introduced into *rsbV* (13). The *rsbV* mutation had a distinct phenotype that was opposite that elicited by *rsbV rsbW* double mutations (13). In experiments in which antibody probes specific for RsbV, RsbW, and σ^B were used to monitor possible polar effects of the mutations on the accumulation of downstream gene products, the *rsbV* frameshift mutation was shown to have no effect on RsbW levels, but an *rsbW* frameshift mutation dramatically reduced the abundance of σ^B (15). The RBS and coding region for the first 13 amino acid residues of σ^B are within the *rsbW* open reading frame (151). This overlap suggests that the reduction in σ^B levels could be due to translational coupling between σ^B and the gene product, RsbW, that is critical to its regulation. It should be noted, however, that RsbW and σ^B appear to form a complex in vivo. Thus, it is possible that σ^B is more susceptible to degradation in the absence of RsbW, and its low levels in the RsbW strain reflects its enhanced turnover.

E-σ^B-transcribed genes. σ^B was initially purified based on its ability to transcribe the early sporulation gene *spoVG* (*0.4Kb*) in vitro (113). This transcription was likely fortuitous. σ^B is dispensable for sporulation (18), and an in vivo role for σ^B in transcribing this gene is in doubt (70). *spoVG* is one of three genes (*spoVG*, *aprE* [subtilisin], and P43 [function unknown]) that are transcribed by E-σ^B in vitro but have not been shown to be expressed by this form of RNAP in vivo (106, 209, 225, 325). All three are transcribed by several forms of RNAP, and in the cases of *spoVG* and *aprE*, there is evidence that σ^B does not contribute significantly to their expression under the growth conditions that are normally employed (70, 225).

ctc (catabolite controlled) represents another gene of unknown function that is transcribed by E-σ^B. However, unlike the examples given above, there is good evidence that *ctc*'s σ^B-dependent promoter is active in vivo (18, 133, 134, 247, 298). *ctc* was fortuitously discovered as a σ^B-dependent transcription unit on a DNA fragment which contained the cloned *spoVG* gene (222). It was initially thought that *ctc* was *spoVC*, a sporulation gene that is closely linked to it (212); however genetic and sequencing studies revealed that they are distinct (132, 308). The 5′ terminus of *spoVC* begins 60 bp downstream of the 3′ end of the *ctc* coding sequence (132). Although *ctc* and *spoVC* are separate genes, they may have synergistic functions. If a strain contains a mutation in *spoVC* as well as a disrupted *ctc*, a growth lag is observed when the culture is shifted from 37 to 48°C (308). Disruption of *ctc* alone does not affect growth but inhibits sporulation at elevated temperatures (48°C). These data suggested that *ctc* could have a role to play, albeit a minor one, during thermal stress. The *ctc* promoter was also found to be activated during heat shock, salt stress, glucose or oxygen limitation, oxidative stress, and cessation of growth under conditions that inhibit the activity of the tricarboxylic acid cycle (TCA) (16, 21, 134, 313). *ctc* induction following growth arrest has been shown to require only the minimum *ctc* promoter element (134). Induction of *ctc* under this and the other activation conditions is likely due to the activation of σ^B by its partial release from RsbW-dependent inhibition (15, 23). Although the σ^B-dependent promoter of *ctc* is the major contributor to *ctc* expression, there appears to be a second *ctc* transcript that initiates upstream of the σ^B-dependent promoter (17). Immediately upstream of the −35 region of the *ctc* promoter is the structural gene for the purine and pyrimidine nucleotide biosynthetic enzyme phosphoribosyldiphosphate synthetase (218). It is likely that this operon's transcript contributes to *ctc* expression.

Another σ^B-controlled gene is *gsiB* (313). This gene was identified as one of several glucose starvation-inducible (*gsi*) transcription units that were isolated on the basis of their activation following glucose deprivation (213). Although originally proposed to be transcribed by E-σ^A (213), *gsiB* has a promoter element that is highly homologous to σ^B-dependent promoters and fails to be induced in vivo in a *B. subtilis* strain lacking σ^B (313). *gsiB* appears to be a monocistronic operon encoding a 13,789-Da hydrophilic protein, organized in five tandem units of 20 amino acids (213). The product shows no strong homology to known proteins, and its loss does not confer an obvious phenotype on *B. subtilis*. *gsiB*, like *ctc*, is induced in response to a number of environmental stresses (313).

A gene (*csbA* [controlled by sigma B]) with induction properties similar to those of *ctc*, i.e., it is maximally expressed during early stationary phase in medium that nutritionally suppresses the TCA cycle, was identified during the screening of a random Tn*917 lacZ* library for *lacZ* fusions whose maximum expression required an intact *sigB* gene (24). The *csbA* reading frame encodes a 76-residue product of unknown function that is both hydrophobic and basic (24). A σ^B-dependent promoter was identified 83 bp upstream of the *csbA* coding sequence, with a weaker σ^A-like promoter located between the σ^B promoter and the *csbA* open reading frame (24). Screening a similar Tn*917 lacZ* library for β-galactosidase expression in a *B. subtilis* strain with an *rsbX* mutation (i.e., a strain with heightened σ^B activity) led to the isolation of 11 additional operons that appeared to be wholly or partly controlled by σ^B (22). The *lacZ* fusions mapped to six different loci which displayed diverse patterns of expression during logarithmic and stationary growth phases, with the σ^B-dependent aspect of their transcription largely confined to stationary-phase cells (22). The high frequency with which independent loci were detected by this means suggested to the investigators that σ^B controls a large stationary-phase regulon (22).

One of the fusions (*gta*) isolated in the Tn*917 lacZ* screening was found to encode UDP-glucose pyrophosphorylase, the enzyme that catalyzes the synthesis of UDP-glucose (310). *gta* is the first gene with a known function that has been shown to be transcribed by E-σ^B in vivo. It has been proposed that UDP-glucose could be important for *B. subtilis* to respond to stationary-phase stress and that the transcription of *gta* by E-σ^B reflects a role for σ^B in this process (310).

At present, 17 *B. subtilis* genes have been identified as having σ^B-like promoter elements upstream of their coding sequences. In addition, one of two cloned *Bacillus licheniformis* alkaline phosphatase genes has been reported to have a σ^B-like promoter that can be used by E-σ^B in vitro (130). Most, if not all, of the *B. subtilis* genes that are transcribed by E-σ^B are transcribed at a heightened level when *B. subtilis* is exposed to environmental stress, have additional promoters that are recognized by other RNAP holoenzymes, and, for those that have been tested, are nonessential for growth or sporulation under normal laboratory conditions. If the *B. subtilis* genes that are known to rely on σ^B for their expression in vivo are representative of the σ^B regulon as a whole, their expression patterns suggest that σ^B participates in general stress response (heat shock, osmotic shock, entry into stationary phase, etc.). However, the apparent nonessential role of σ^B in permitting *B. subtilis* to deal with these stresses leaves its function in this process obscure. The multiple promoters of E-σ^B-transcribed genes may contribute to the cryptic nature of σ^B's role. If the bulk of the σ^B regulon can be transcribed by alternative means, σ^B may enhance some stress responses without being critical to any of them.

Sigma Factor σ^C

Isolation and characterization. E-σ^C was detected as an in vitro transcriptional activity that initiates RNA synthesis from a site (P2) on the *spoVG/0.4Kb* gene which is distinct from the site (P1) where E-σ^B initiates transcription (145, 209). RNAP purified by phosphocellulose and DNA-cellulose chromatography gives only the σ^B-dependent transcript from the *spoVG* template; however, in the absence of phosphocellulose chromatography, a less purified enzyme yields dual transcripts from *spoVG* (145). The E-σ^C activity was separated from E-σ^B by gradient elution from DNA-cellulose and purified further by DEAE-Sephacel chromatography, followed by gradient elution from a second DNA-cellulose column (145, 209). SDS-PAGE analysis of the proteins present in the fractions eluting from the second DNA-cellulose column revealed a 32,000-Da protein whose abundance paralleled the transcriptional activity at P2. This protein was present in very small amounts and detectable only by high-sensitivity silver staining (145). The 32-kDa protein (σ^{32}/σ^C) band was cut from the gel, renatured, and shown to confer on core RNAP the ability to initiate transcription at the P2 promoter of *spoVG* (145). As implied by the need to use silver stain to detect it, σ^C is a very low abundance protein. From the apparent ratio of σ^C to RNAP core subunits and on the assumption that there are approximately 5,000 molecules of RNAP per cell, it has been estimated that there are only 30 molecules of σ^C per *B. subtilis* cell, 10-fold lower than the estimate for σ^B (145). σ^C protein cannot be directly detected without extensive purification; however, its activity can be measured in partially purified extracts. *B. subtilis* was surveyed for σ^C activity in different growth states by using P2 promoter activity as an assay. From this measurement, σ^C, like σ^B, was found to be present in growing cells, early sporulating cells, and stationary-phase cells that were blocked by mutation (*spo0A*) from entering sporulation (145). The low abundance of σ^C has discouraged its study. No report has been published on this σ factor since its discovery. Its function, structural gene, and regulation remain undefined.

E-σ^C-transcribed genes. There are two genes at which E-σ^C is known to initiate transcription in vitro, *spoVG/0.4Kb* and *ctc* (145). In neither case has it been shown that E-σ^C functions at these promoters in vivo. The transcriptional start site of *spoVG* that is used by E-σ^C in vitro can be detected as an in vivo initiation site by S1 nuclease analysis of cellular RNAs (145); however, it is not known whether E-σ^C is actually responsible for this in vivo transcript. There are no known null mutations in the σ^C structured gene, and hence, the contribution of E-σ^C to this transcript is, at present, untestable. E-σ^C also initiates transcription at the *ctc* promoter; however, unlike the situation at *spoVG*, where E-σ^B and E-σ^C have distinct start sites, both enzymes initiate *ctc* transcription at the same point on the *ctc* template (145) and mutations in the promoter region which diminish its use by E-σ^B also reduce its use by E-σ^C (296). The contribution of E-σ^C to *ctc* expression in vitro does not appear to be significant under the culture conditions that are normally employed. Null mutations in *sigB* virtually eliminate *ctc* transcription (13, 23, 134). If E-σ^C transcribes *ctc* in vivo, either its contribution is minor or the proper environmental stimulus for its activation has not yet been determined. A comparison of the *ctc* promoter with the P2 promoter of *spoVG* revealed conserved sequences that were different from those shared by *ctc* and other σ^B-recognized promoters (Table 1) (145).

Sigma Factor σ^D

Isolation and characterization. Preparations of RNAP from exponentially growing *B. subtilis* were found to recognize an in vitro promoter on *E. coli* bacteriophage T7 DNA that is not used by E-σ^A (324). Fractionation of the preparation by chromatography on heparin-agarose separated the RNAP into two peaks of activity. The high-salt-eluting fraction was composed of E-σ^A, while the fraction which eluted at low ionic strength had a relatively low level of RNAP but a novel transcriptional activity on T7 DNA (324). SDS-PAGE analysis of the fractions containing the novel activity demonstrated the absence of σ^A but revealed the presence of a polypeptide of 28,000 Da (σ^D), with a direct coincidence between content of this protein and the novel T7 transcription. RNAP with this unique specificity was used to transcribe pooled plasmid DNAs from a library of cloned *B. subtilis* chromosomal DNA (103). Plasmids that functioned as highly active templates for this enzyme were selected and shown to contain promoters used by the novel RNAP but not by E-σ^A. By employing one of these "strong" promoters as a sensitive assay, a reconstitution experiment was performed in which the 28,000-Da protein was cut from an SDS-polyacrylamide gel, renatured, and shown to confer the distinct promoter specificity on core RNAP (122). The 28,000-Da protein thus appeared to be a novel σ factor (σ^D). E-σ^D represents only 1.2% of the RNA polymerase isolated during an enzyme purification (122). Quantitative immunoblot analysis was used to estimate the cellular abundance of σ^D at 220 $\pm$ 50 molecules per cell for late-logarithmic-phase *B. subtilis* (122). This level is roughly comparable to that of σ^B.

Cloning the σ^D structural gene. Tryptic peptide fragments of purified σ^D were sequenced, and a degenerate oligonucleotide, specific for a portion of σ^{28}, was synthesized and used to identify the σ^{28} sequence in a *B. subtilis* subgenomic library (121). Sequence analysis of the cloned DNA revealed an open reading frame for a 29,500-Da protein that contained the sequence identified in the tryptic fragments. The σ^D open reading frame was preceded by a sequence found in σ^A-dependent promoters; however, normal σ^D transcription appears to depend on promoters upstream of this region (121). *sigD* is located immediately downstream of a chemotaxis locus. Transposon insertions or the introduction of transcription terminators at sites up to 24 kb upstream of *sigD* reduce its expression (195). *sigD* is believed to depend upon promoter sequences >24 kb upstream of its structural gene and to be part of a large operon (>26 kb) which encodes structural proteins that form the flagellar hook-basal body complex and chemotaxis regulatory proteins (195).

E-σ^D-transcribed genes. Disruption of the resident copy of *sigD* with an integrating plasmid results in a viable mutant strain that is sporulation proficient but grows as filamentous chains of cells that are nonmotile (121). Western blot analyses demonstrated a lack of flagellin protein in these cells, leading to the proposal that σ^D controls flagellin production (121). Direct support for σ^D as a positive regulator of flagellar synthesis came with the cloning of the *B. subtilis* flagellin gene (*hag*) and the findings that it is transcribed by E-σ^D in vitro and that its transcription in vivo is eliminated by mutations that block σ^D synthesis (203). Several known σ^D-dependent genes are clustered near *hag* in operons with organizations similar to those of the *E. coli* operons which encode flagellar and chemotactic genes (42, 96, 121). The *sigD* gene itself was found to be allelic to the *B. subtilis flaB* gene (194). *sigD* resides within the *fla-che* region of the *B. subtilis* chromosome as part of a large operon containing at least 30 genes whose products are involved in flagellar or chemotaxis functions (223).

A role for σ^D in the expression of the methyl-accepting chemotaxis proteins was suggested by the findings that some *sigD* mutant cells lack methyl-accepting chemotaxis proteins and that other mutant *sigD* cells, which synthesize reduced

levels of σ^D, are deficient in chemotaxis, although they accumulate nearly normal amounts of flagellin (194).

Electron microscopic studies of the filamentous cells formed by *sigD* mutants show normal division septa, indicating that the absence of σ^D results in a deficiency in autolysin activity rather than a defect in cell division (121). An autolysin defect was supported by the observation that the principal *B. subtilis* autolysin activities in extracts of a *sigD* mutant are reduced relative to those found in extracts of a wild-type strain (194) and that autolysin genes are transcribed, in part, from σ^D-dependent promoters (170, 175, 193).

Additional σ^D-dependent promoters, isolated by screening a collection of *B. subtilis* genomic fragments for in vitro promoter activity, were analyzed for unique versus duplicate clones (103). The distribution implied that there were a relatively small number (25 to 30) of σ^D-dependent promoters in *B. subtilis* (103). A comparison of the sequence upstream of a number of σ^D-dependent promoters revealed a consensus sequence (Table 1) which is distinct from that found to be conserved in promoters used by the other forms of *B. subtilis* RNAP (103, 104, 119). The specificity of E-σ^D overlaps that of the *E. coli* RNAP species (E-σ^{32}) that recognizes heat shock promoters. E-σ^D and E-σ^{32} can initiate transcription on each other's templates in vitro (27). There is, however, no evidence that E-σ^D-recognized promoters in *B. subtilis* are temperature inducible or that σ^D participates in the *B. subtilis* heat shock response (194). Unlike the case of the overlapping specificities of the *B. subtilis* σ^A and *E. coli* σ^{70} proteins, for which the similarities appear to reflect similar functions, the similarities between σ^D- and σ^{32}-recognized promoters are thought to be fortuitous (194).

The unique sequence of σ^D-recognized promoters permitted a nucleic acid database search for genes with upstream σ^D promoters. This led to the preliminary identification of σ^D-like promoters upstream of the gene (*degR*) for a regulator of protease and exoenzyme secretion and the gene (*epr*) for an extracellular protease (119). Most *Bacillus* protease and exoenzyme genes do not have identifiable σ^D promoter sequences, and so the significance of the σ^D-recognized sequence at *degR* and *epr* is unclear (119).

An analysis of the transcription pattern of several σ^D-dependent promoters, including *hag*, demonstrated transient peak expression at the end of exponential growth (T_0) (102, 103, 119). This parallels the abundance of σ^D protein, which appears to be maximum at T_0 and decreases thereafter (J. Helmann, unpublished; cited in reference 119). Although σ^D levels and the expression of σ^D-dependent promoters are maximum at the end of exponential growth, this stationary-phase increase is independent of a number of genes (*spo0*, *abrB*, and *sin*) whose products normally control stationary-phase gene expression (196). The factors that are responsible for the stationary-phase elevation in σ^D levels are unknown. σ^D-dependent transcription declines after the onset of sporulation, and inactivation of *sigD* itself causes no overt defect in sporulation (121). These results argue that σ^D's contribution to sporulation, if any, is modest.

Present evidence demonstrates that σ^D is primarily involved in the expression of flagellar, motility, and autolysin genes and their regulators.

Sigma Factor σ^H

Isolation and characterization. The sporulation gene *spoVG* was the template used to detect the activities of both σ^B and σ^C (113, 145). The RNAs synthesized in vivo from *spoVG* initiate at the same sites as the transcripts generated in vitro by E-σ^B and E-σ^C; however, the in vitro transcriptions fail to entirely mimic the in vivo condition (37). In vitro, the *spoVG* promoter is relatively inefficient when transcribed by E-σ^B or E-σ^C, yet its activity level during early sporulation is very high. This disparity implied that another RNAP form or a positive regulator was contributing to *spoVG* transcription in vivo. The σ^B-dependent promoter of *ctc* is a more efficient target for E-σ^B than is the *spoVG* promoter. The preference of E-σ^B for *ctc* is so great that under conditions of template excess, *ctc* will sequester the available E-σ^B and prevent transcription of *spoVG* (222). By employing this template system as an assay, a search was initiated for a form of RNAP that could use the *spoVG* promoter in the presence of the *ctc* promoter (37). RNAP prepared from early-stationary-phase cells and fractionated by gradient salt elution from a DNA-cellulose column was resolved into two activities in the mixed-template competition reaction on the *ctc* and *spoVG* promoters. The low-salt-eluting activity primarily recognized the *ctc* promoter, while fractions eluting at higher salt concentrations predominantly transcribed *spoVG* (37). SDS-PAGE analysis of the proteins present in each of the fractions demonstrated the presence of σ^B in the early fractions and the coincidence of a 30,000-Da protein with the *spoVG* transcription activity that was present in the high-salt fractions (37). This 30,000-Da protein band was cut from the gel, renatured, and demonstrated to direct core RNAP to the *spoVG* promoter. From this result, the 30,000-Da protein was designated a new σ factor (σ^{30}) (37). As a test of the notion that E-σ^{30} might be the major species of RNAP responsible for *spoVG* expression in vivo, mutant *B. subtilis* that failed to express *spoVG* was examined for the presence of E-σ^{30}. In general, the effects of the mutations on E-σ^{30} levels were found to parallel the effect of the mutations on *spoVG*. An exception was *spo0H*. A *B. subtilis* strain with a missense allele of *spo0H* (*spo0H81*) contained E-σ^{30} but failed to transcribe *spoVG*, and a strain with a deletion of *spo0H* lacked detectable σ^{30}. These findings argued that σ^{30} could be Spo0H (37).

The structural gene for Spo0H was cloned from *B. subtilis* based on its ability to complement the Spo$^-$ phenotype of a *spo0H* mutant (320). The *spo0H* sequence encoded an open reading frame for a 26,097-Da polypeptide with significant homology to known prokaryotic sigma factors (69). Rabbit polyclonal antibodies prepared against the *B. licheniformis spo0H* gene reacted with Spo0H from *B. subtilis* and with the σ^{30} subunit of purified E-σ^{30} (70). The immunological evidence for σ^{30} as the *spo0H* gene product prompted a change in designation from σ^{30} to σ^H (70). E-σ^H as the *spoVG*-transcribing enzyme was confirmed by the observation that a block on *spoVG* expression caused by a mutant *spo0H* allele could be suppressed in an allele-specific fashion by a single base substitution in the *spoVG* promoter (340).

Regulation of σ^H. E-σ^H was not detected in *B. subtilis* strains with mutations in the stage 0 genes *spo0A* and *spo0F* but was readily found if the *spo0A* strain contains a second mutation (*abrB*) which restores part of the *spo0A* cell's early sporulation phenotype (37). This result implies that σ^H synthesis is controlled by post-exponential-phase gene regulators. Spo0A is both a negative and positive regulator of post-exponential-phase genes which is activated through a transphosphorylation cascade that includes Spo0F, Spo0B, and SpoIIJ (*kinA*) (28, 127). *abrB* encodes a negative regulator of certain sporulation genes and is itself downregulated by activated Spo0A (288, 289). A translational fusion of the *E. coli lacZ* gene to *spo0H* was used to study the regulation of σ^H synthesis in more detail (320). With this reporter system, *spo0H* was found to be expressed during vegetative growth with increasing synthesis as

B. subtilis enters the mid-logarithmic stage of growth. The increased expression of *spo0H* requires the *spo0A*, *0B*, *0E*, and *0F* gene products unless the strain also contains a mutation in *abrB*. The *abrB* mutation results in constitutive high expression of *spo0H* during vegetative growth and bypasses the requirement for the *spo0A* and *spo0B* gene products (69, 320). In addition, a mutation in *spo0A* (*sof-1*) which circumvents requirements for *spo0B*, *spo0E*, and *spo0F* gene products (128) permits *spo0H* expression in an *spo0F* background. Taken together, these results argue that *spo0H* is negatively regulated by AbrB and released from this inhibition by the effects of Spo0A on *abrB* expression (273).

Sequence analysis identified a σ^A consensus promoter that is used in vitro by E-σ^A immediately upstream of the σ^H structural gene (70). The relevance of this potential promoter was verified by primer extension experiments, which detected an RNA initiating at this site in vivo (319). Thus, *spo0H*/*sigH* is likely to be contained in a σ^A-dependent operon that is negatively controlled by AbrB (320). Northern blot analyses indicate that the *spo0H*/*sigH* transcript is 1,300 bases (319). This is approximately 600 bases larger than the σ^H coding region. The function of the bases that do not directly encode σ^H is unknown.

Regulation of σ^H abundance is not limited to *sigH* transcription. *spo0H* mRNA levels observed during growth do not directly correspond to σ^H levels, suggesting that *spo0H* has a posttranslational regulatory component (118, 320). This idea was tested in experiments in which *spo0H* transcription was artificially controlled by using an inducible promoter (P_{SPAC}) (118). σ^H levels increased significantly and σ^H-dependent genes were activated only when the *sigH* operon was induced under conditions that also induced sporulation (118). Pulse-labeling and turnover studies revealed that sporulation conditions stimulated the rate of σ^H synthesis relative to total protein synthesis approximately fivefold and increased its half-life to 90 to 130 min from the 20- to 30-min half-life found in growing cells (118). It was concluded that the increase in σ^H which occurs at the end of exponential growth is principally due to a posttranscriptionally regulated elevation in σ^H synthesis coupled with decreased σ^H turnover (118). The mechanisms by which this regulation is accomplished are unknown.

E-σ^H-transcribed genes. The role of σ^H in *B. subtilis* transcription has not been fully defined. Although σ^H is essential for sporulation, it also is present during growth, when it may direct the transcription of a subset of vegetative genes. A substantial number of genes with σ^H-dependent promoters have been detected, and many have been characterized. The largest number of σ^H-controlled genes were identified in a study in which a reporter gene was randomly inserted into the *B. subtilis* chromosome and used to monitor gene expression at the integration site in the presence and absence of σ^H (142). Eighteen *csh* (controlled by sigma H) genes whose expression was influenced by σ^H levels were found (142). Most of strains carrying the σ^H-dependent reporter gene fusions, which are likely to have inactivated the gene at the insertion site, displayed no overt phenotype; however, two of the insertion mutations caused a sporulation defect. A third insertion mutation caused a growth defect, but this phenotype was only seen when the insertion mutation was combined with a mutation in *spo0H* (142). One of the sporulation mutants also had an impaired ability to become competent for genetic transformation. On the basis of the reporter gene activity, the expression patterns of the 18 *csh* genes are similar in that all are induced within 30 min after the onset of sporulation (142). The level of expression, amount of induction, and degree of dependence on σ^H for expression differ among the *csh* genes. It was suggested that these differences could be due to additional regulatory factors acting at these promoters as well as the presence of multiple promoters at some of the genes (142).

The idea that multiple factors regulate σ^H-dependent promoters is supported by the varied expression patterns of the known E-σ^H-read genes: *spoIIA* (327, 328), *spoVG* (37), *citG* (87, 240, 294), *sigA* (38), *spo0F* (237), *kinA* (237), and *spo0A* (237, 272). These genes encode products that participate in diverse processes. *spoIIA* and *sigA* encode sporulation-specific (78, 283) and vegetative-cell (239) sigma factors, respectively. *spo0A*, *spo0F*, and *kinA* (*spoIIJ*) specify functions needed for, or are components of, the sporulation signal transduction pathway (28, 127). *citG* is the structural gene for the TCA cycle enzyme fumarase (240), and *spoVG* is an early sporulation gene which appears to play a role during the forespore septation process (192). The expression patterns of these genes, which are partially or exclusively dependent of E-σ^H for their transcription, reflect the diversity of their functions. The σ^H-dependent *spoIIA*, *spoVG*, *sigA*, *spo0A*, and *spo0F* promoters become active after the end of exponential growth (38, 222, 237, 328), while the σ^H-dependent *citG* and *kinA* promoters are active in the middle and late stages of exponential growth (237, 294). Activating σ^H synthesis from an inducible promoter (P_{SPAC}) fails to induce the expression of either the sporulation-expressed *spoVG* (118) or the vegetatively expressed *citG* (240) genes, arguing that additional factors are involved. In the case of *spoVG* and *spoIIA*, the additional regulatory factors have been identified (AbrB and Spo0A, respectively) (28, 251, 327, 342). The control of most σ^H-dependent promoters whose expression increases at the end of exponential growth may ultimately be accounted for by a combination of release from the effects of repressors (e.g., AbrB and Sin) and the positive effects of the Spo0A induction pathway (273). The regulators of the vegetatively expressed σ^H-dependent promoters are more obscure. The σ^H-dependent promoter of *citG* was found to vary during growth and sporulation, but unlike the developmentally regulated σ^H promoters, its post-exponential-phase induction does not depend on the *spo0A* pathway (240). *citG* is also regulated by carbon source during vegetative growth, with a 50-fold increase in expression in lactate medium relative to the level seen in glucose-glutamine medium (240). The basis of this regulation is unknown. Aside from the cloned genes whose transcription has been shown to require σ^H, there is genetic evidence that some σ^H-dependent process is involved in the induction of competence (4, 68, 142, 272) and the DNA damage-inducible response (SOB) (331).

The present collection of σ^H-dependent promoters argues that σ^H has an important role in post-exponential-phase gene expression but that its activity is not limited to this stage of culture growth. σ^H is needed for the vegetative functions of TCA cycle enzyme synthesis and DNA repair as well as the mutually exclusive post-exponential-phase processes of competence development and sporulation. When the expression of σ^H-dependent promoters has been studied in detail, σ^H has been found to play an essential but insufficient role in their activation.

Sigma Factor σ^L

σ^L is the most recently discovered *B. subtilis* sigma factor. It has not yet been isolated for biochemical studies but is known by its in vivo activity and the similarities of its cloned structural gene to known sigma factors (60). *sigL* was identified during a study of the levanase operon of *B. subtilis*. Levanase is an exofructosidase that hydrolyzes both levan and inulin for the production of free fructose. The levanase structural gene

(*sacC*) is the fifth gene in an operon whose promoter-proximal gene products form a fructose phosphotransferase system (197, 198). A positive regulatory gene (*levR*) homologous to the NifA/NtrC regulatory proteins of *Klebsiella pneumoniae* lies upstream of the levanase operon's promoter (59). NifA and NtrC are positive activators of promoters that are recognized by a minor species of RNAP, E-σ^{54} (171). σ^{54}-dependent promoters require these positive activator proteins for expression and have conserved sequences at bp −12 and −24 rather than at the more typical −10 and −35 positions (171). It was determined that the levanase promoter is only weakly similar to σ^{A}-dependent promoters but is very similar to the σ^{54}-recognized promoters of enteric bacteria (59). The levanase promoter was found to be identical to the σ^{54}-recognized promoter at 11 bases of the 12-base consensus sequence (Table 1) and to have a potential activator site, at which LevR might bind (59).

The dependence of levanase transcription on LevR and a σ^{54}-like RNAP was tested by reconstituting its transcription in *E. coli*. Expression of a *levD*::*lacZ* fusion was found to be dependent on both *levR* and the *E. coli ntrA* gene product (σ^{54}) (59). The discovery that *E. coli* E-σ^{54} could transcribe the levanase gene prompted a search for a similar enzyme in *B. subtilis*. A merodiploid strain that contained two copies of a *levR* allele (*sac*L8) which confers constitutive expression on the levanase operon, as well as two copies of the levanase operon: one containing a *levD-lacZ* fusion, and the second expressing levanase (*sacC*) was used for the search (60). In this background, levanase expression could be monitored by both β-galactosidase and levanase activity, and the isolation of *levR* mutants would be minimized. Mutants which failed to express the levanase operon were isolated (60). The gene affected in these mutants was cloned by complementation, sequenced, and found to encode a 49,644-Da protein that was homologous to σ^{54} of *E. coli*. Its coding sequence was designated *sigL* (60).

A null mutation was constructed in *sigL* and introduced into *B. subtilis*, where it abolished expression of *sacC* (60). Other bacterial species that lack σ^{54} are viable but have very diverse pleiotropic phenotypes, including defects in nitrogen metabolism (171). The *sigL* mutant was tested for growth in minimal medium containing different nitrogen sources and found to be indistinguishable from the parent strain except when the amino acids arginine, ornithine, valine, and isoleucine were provided as nitrogen sources. These amino acids supported the growth of *sigL*$^{+}$ but not *sigL* strains (66). The current evidence limits the role of σ^{L} to the expression of genes for a subset of degradative enzymes (e.g., levanase and amino acid degradation). The loss of *sigL* does not affect sporulation, competence, or mobility (60). Whether σ^{L}, like its counterpart in other bacteria, has more wide-ranging activities remains to be demonstrated.

SPORULATION-SPECIFIC SIGMA FACTORS

Nutrient deprivation triggers *B. subtilis* to differentiate into an alternate cell type, the dormant spore. During the process of sporulation, groups of genes are sequentially activated and silenced to lead the cell through a series of intermediate developmental stages (reviewed in references 76, 187, and 232). The process of sporulation begins at the end of exponential growth and requires approximately 8 to 10 h to complete under standard laboratory conditions. Cells that have ceased exponential growth in a sporulation medium (sporulation onset, T_0) but have not undergone any obvious morphological change are classified as being at the earliest stage of sporulation, stage 0. By 1.5 to 2 h into sporulation ($T_{1.5}$ to T_2), the cell has partitioned itself into two compartments by invagination of the plasma membrane to form a septum near one pole of the cell. Completion of the septum defines stage II of sporulation. The peripheral septal membrane then migrates toward the forespore pole of the cell, engulfing the smaller compartment as a double-membraned entity (the forespore protoplast) within the mother cell. This event signals stage III, which is normally completed by $T_{3.5}$. During stage IV, a thick peptidoglycan layer (cortex) is placed between the two membranes surrounding the forespore protoplast. This is followed by the deposition of layers of coat proteins on the outer membrane that envelopes the cortex (stage V). Development of spore resistances (heat, organic solvents, radiation, etc.) occurs fully during stage VI (T_6 to T_7). At stage VII, the mature spore is released by lysis of the mother cell.

Genes whose products are sporulation specific and essential to the spore-forming process are designated by the stage at which the absence of their products blocks sporulation. For example, a strain with a sporulation-specific mutation that prevents a cell from forming the asymmetric septum and proceeding to stage II would be termed a stage 0 mutant, with the gene containing the mutation designated *spo0X*. Likewise, a cell that can form the septum but not a forespore protoplast would contain a stage II mutation (*spoIIX*).

The phenotypes of null mutations in the four known sporulation-specific sigma factors of *B. subtilis* (σ^{E}, σ^{F}, σ^{G}, and σ^{K}) bear out the prediction that cascades of sigma factors might be responsible for the sequential pattern of gene expression that occurs in sporulation (184, 287). The loss of any of these proteins blocks spore development at a particular stage in the process. The inability to form active σ^{E} or σ^{F} confers a stage II terminal phenotype on *B. subtilis*, while σ^{G}- and σ^{K}-deficient strains arrest sporulation at stages III and IV, respectively. As will be described below, the sporulation sigma factors not only are important regulators of temporal gene expression but also appear to play pivotal roles in activating compartment-specific gene expression in the mother cell and forespore.

Sigma Factor σ^{E}

Isolation and characterization. As *B. subtilis* proceeds into sporulation, dramatic changes occur in the subunit composition of its extractable RNAP. Aside from the disappearance of the vegetative-cell sigma subunits, novel polypeptides become associated with RNAP. One of the more obvious new polypeptides is a protein with an apparent molecular mass of 27,000 to 29,000 Da (P^{29}) (112, 181, 216). Purified RNAP carrying the 27,000- to 29,000-Da protein can be readily separated from other forms of RNAP by its unusually high affinity for DNA cellulose (112, 181, 216). When the in vitro transcription properties of $E \cdot P^{29}$ are compared with those of E-σ^{A} or core RNAP, it is found to display a distinct response to Mg^{2+} and KCl and a characteristic activity on poly(dA-dT) or bacteriophage DNA templates (181, 216). $E \cdot P^{29}$'s novel transcriptional properties and sporulation-specific appearance prompted speculation that P^{29} might be a sporulation-specific transcription factor which could participate in the control of spore gene expression (181, 216). Evidence that P^{29} was a sigma-like protein came from experiments in which RNAP carrying P^{29} was shown to have a unique profile of in vitro transcriptional specificity on cloned *B. subtilis* DNA compared with that displayed by other *B. subtilis* holoenzymes (E-σ^{A} and σ^{B}). A reconstitution experiment in which P^{29} was separated from the other $E \cdot P^{29}$ subunits by SDS-PAGE, renatured, and added to core RNAP demonstrated that the unique transcriptional specificity

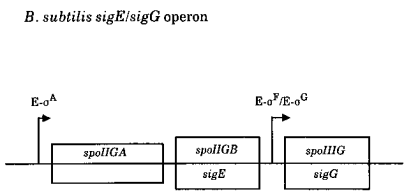

FIG. 4. *B. subtilis sigE* and *sigG* operons. The bicistronic *sigE* and monocistronic *sigG* operons are drawn with the forms of RNAP responsible for each operon's transcription illustrated (152, 159, 160, 201, 290). The *sigE* operon's mRNA can extend through *sigG*; however, the *sigG* portion of the transcript does not appear to be translated (201, 290) (see text).

of $E \cdot P^{29}$ was due to P^{29} (112). P^{29} therefore became σ^{29} and was later renamed σ^E.

Cloning of σ^E structural gene. The structural gene for σ^E was cloned by investigators who were seeking to isolate early sporulation genes (20, 163). The sequence of a cloned portion of the *B. subtilis* chromosome that complemented a stage II sporulation mutation (*spoIIG*) was determined to encode a 27,652-Da polypeptide with a 65-amino-acid region that is highly homologous to an internal part of the *E. coli* σ^{70} protein (285). From this analysis, it was proposed that *spoIIG* could encode σ^E or a not yet characterized sporulation sigma factor (285). Support for the idea that σ^E is the *spoIIG* product came from an immunological study in which an anti-σ^E antibody detected a σ^E-like protein in an *E. coli* strain that expressed the cloned *spoIIG* sequence and σ^E variants in a *B. subtilis* strain with a mutant *spoIIG* allele (*spoIIG41*) (304). Direct sequencing of the amino terminus of σ^E and its alignment with the *spoIIG* open reading frame verified *spoIIG* (*sigE*) as the σ^E coding sequence (172).

Regulation of σ^E. E-σ^E is isolated only from *B. subtilis* cells that have progressed approximately 2 h into sporulation (112) and appears to be maximally present between T_2 and T_4 (307). As determined by Western blot and reporter gene expression studies, σ^E synthesis is controlled at two levels. The first level is transcriptional control, with the promoter of the *spoIIG* operon becoming active only after the onset of sporulation. The second level of regulation is posttranslational, with the primary product of *spoIIG* being an inactive precursor form (pro-σ^E) of σ^E. Conversion of pro-σ^E to the active sigma factor occurs at approximately T_2 of sporulation. Thus, formation of σ^E depends not only on the expression of its structural gene but also on the subsequent activation of its product.

(i) Pro-σ^E synthesis. Synthesis of pro-σ^E parallels the transcription of the *spoIIG* operon, which only occurs during sporulation in cells that contain the wild-type alleles of at least several stage 0 (*spo0A*, *0B*, *0E*, *0F*, and *0H*) and stage II (*spoIIJ*, *IIL*, and *IIN*) sporulation genes (146, 159). Experiments in which reporter gene plasmids were integrated into the *B. subtilis* chromosome at the *spoIIG* locus demonstrated both that *spoIIG* transcription is induced at the onset of sporulation and that the *spoIIG* operon includes a promoter-proximal gene (*spoIIGA*) which, like *sigE* (*spoIIGB*), is essential for sporulation (Fig. 4) (149, 159). The start site of the *spoIIG* transcript was precisely mapped by RNase protection and primer extension techniques (158). Once the initiation site was localized, transcriptional factors which acted at this site were sought. Given that *spo0H*, which encodes σ^H, is required for *sigE* expression in vivo (159), it seemed possible that E-σ^H could be the *sigE*-transcribing enzyme. However, this notion was not supported by an in vitro experiment in which E-σ^A but not E-σ^H initiated *spoIIG* transcription (158). Compelling evidence for E-σ^A as the transcribing enzyme for *sigE* came with the discovery that the deleterious effects of a base substitution at the −10 region of the *spoIIG* promoter on *spoIIG* transcription could be suppressed in an allele-specific fashion by a change of an amino acid residue in the −10 recognition segment of σ^A (161). E-σ^H's role in *sigE* expression is likely to be indirect.

The *spoIIG* promoter sequence resembles a σ^A "consensus promoter" except for the spacing between the putative −35 and −10 regions of *spoIIG*, which is 22 bp instead of the usual 17 or 18 bp (158). This unusual spacing was investigated by a mutagenesis experiment in which substitutions at sites in and around positions −35 and −87 were found to reduce promoter activity (259). These regions resemble the target site for the Spo0A activator protein. Gel mobility shift and DNase protection experiments showed that Spo0A could bind to these sites in vitro (9, 259) and that Spo0A stimulates in vitro transcription from the *spoIIG* promoter (19, 258). It is now thought that the sequence originally hypothesized to be a "displaced" −35 consensus sequence is in fact an Spo0A binding site. Many if not all of the other loci required for *sigE* transcription participate in the induction or activation of Spo0A at the onset of sporulation (28, 127, 273). A simple model for the regulation of *spoIIG* transcription envisions the activation of Spo0A and its binding to the *sigE* promoter as the sporulation-specific regulatory event that induces the vegetative-cell holoenzyme E-σ^A to initiate the synthesis of pro-σ^E at the onset of sporulation (258).

(ii) Pro-σ^E processing. An unusual aspect of σ^E synthesis is its formation from a precursor protein (pro-σ^E). Pro-σ^E was detected in Western blot analyses of sporulating *B. subtilis*, in which an anti-σ^E monoclonal antibody reacted not only with σ^E (apparent mass, 29 kDa) but also with a larger protein (P^{31}; apparent mass, 31 kDa) which had a similar peptide substructure (307). Two comparable proteins were also observed in extracts prepared from other sporulating *Bacillus* species (305). Synthesis of P^{31} was found to occur earlier in sporulation than that of σ^E, with no *B. subtilis* strain synthesizing σ^E without first accumulating P^{31}, although several Spo$^-$ strains could synthesize P^{31} but not σ^E (307). A pulse-chase experiment revealed that [^{35}S]methionine is "chased" into σ^E concomitantly with the disappearance of previously labeled P^{31} (172) and that the induced expression of the *sigE* gene in *E. coli* gives rise to the synthesis of a single protein with the mobility of P^{31} on SDS-polyacrylamide gels (304). These findings indicated that a precursor-product relationship existed between P^{31} and σ^E (172). When anti-σ^E antibody was used to monitor P^{31}'s fate during purification of *B. subtilis* RNAP, the bulk of P^{31}, unlike σ^E, was found to separate from RNAP during the purification process. A small portion (5%) of the P^{31} did remain associated with the RNAP and conferred on the resulting E-P^{31} the chromatographic properties normally associated with E-σ^E (306). Although E-P^{31} had physical properties similar to those of E-σ^E, its transcriptional activity was distinct: E-P^{31} could synthesize RNA in vitro from the nonspecific template poly(dA-dT), but it was inactive on σ^E-dependent promoters (306). In this regard, E-P^{31} resembled core RNAP rather than a holoenzyme.

σ^E has been independently sequenced by several groups, who identified either residue 28 (204, 207a) or residue 30 (172) of the *sigE* open reading frame as the σ^E amino terminus. σ^E is therefore likely to be derived from pro-σ^E by the removal of 27 to 29 amino acids from the pro-σ^E amino terminus. It is not known whether the ambiguity in the σ^E amino terminus is due to differences among *B. subtilis* strains, a lack of precision in the processing reaction, or a secondary loss of two amino acids from one of the σ^E samples subsequent to processing. In order

to test whether a proteolytic event alone could change pro-σ^E into a protein with σ^E-like properties, a fusion protein (P^{31}*) containing most of pro-σ^E joined at its amino terminus to 12 amino acids of the *E. coli* lipoprotein was overproduced in *E. coli*, partially purified, and converted in vitro into a protein with electrophoretic mobility similar to that of σ^E by treatment with *Staphylococcus aureus* V8 protease (172). A preferred cleavage site for *S. aureus* V8 protease exists two to four residues upstream of the putative pro-σ^E processing sites (172, 304). Protease-treated P^{31}*, but not untreated P^{31}*, was capable of directing *B. subtilis* core RNAP to specifically initiate RNA synthesis at a σ^E-recognized promoter in vitro (172). Thus, although it is possible that further modifications occur to σ^E in vivo, proteolytic modification is sufficient for the activation of σ^E in vitro.

Mutant *sigE* alleles which fail to encode the first 15 or 16 amino acids of pro-σ^E synthesize a σ^E-like protein that is active without processing (148, 283a). A deletion analysis of the pro-σ^E amino terminus revealed that the first 15 residues of the "pro" sequence contains elements that are essential for silencing not only its σ^E activity but also its recognition by the processing apparatus. Deletions into this region both increase the expression of a σ^E-dependent reporter gene in the absence of processing and reduce the ability of the mutant product to be processed (229). Deletions which extend into the next 15 amino acids lessen the accumulation of a *sigE* product in *B. subtilis* and, as a consequence, reduce σ^E-dependent reporter gene activity (148, 229). Mutant *sigE* genes whose translated sequence begins at either of the two putative pro-σ^E processing sites (i.e., residue 27 or 29) specify products that are virtually undetectable in *B. subtilis* (148, 229). It is unlikely that the absence of the pro sequence hinders σ^E accumulation by impairing the translation of σ^E mRNA. Missense or insertion mutations 20 to 50 nucleotides downstream of the translational start site also substantially reduce the accumulation of the *sigE* product (148, 254). The data are most consistent with the pro sequence either being necessary for a posttranslational step in σ^E synthesis (e.g., facilitating proper protein folding) or stabilizing a labile σ^E in a proprotein form until it is processed and joined to RNAP. If the second possibility is correct, the hypothetical lability of free σ^E may represent a device to ensure its disappearance after T_4 of sporulation, when new σ factors replace σ^E to establish the pattern of late gene expression.

The failure of σ^E to accumulate when synthesized without the pro sequence is partly due to its amino-terminal sequence. A deletion of the *sigE* coding sequence that extends 10 amino acids beyond the mature σ^E amino terminus enhances the accumulation of product approximately 10-fold over the level observed for the product of a gene whose amino terminus is similar to that of the "processed" σ^E (H. Carlson, unpublished; cited in reference 229). The ability of the σ^E pro sequence to facilitate σ^E accumulation appears to be specific. σ^K, like σ^E, is made as a proprotein (see below). If the σ^K pro sequence is exchanged for the σ^E pro sequence, the resulting chimeric *sigK/sigE* allele fails to specify a product that can accumulate in *B. subtilis* (36). There is apparently a particular quality to the σ^E pro sequence that is important for σ^E accumulation.

The pro sequence of σ^E contains most, if not all, of the target elements required for its removal. Placement of the σ^E pro sequence on the sporulation sigma factor σ^K results in a chimeric proprotein that is processed in *B. subtilis* at the time in development when pro-σ^E processing normally occurs (36). Mutational studies were undertaken to identify regions of the pro sequence that were essential for recognition by the processing apparatus (229). These experiments suggested that the presence of particular amino acids at the cut site itself is not essential for processing and that pro-σ^E processing requires, at a minimum, an element within the first 15 amino acids of the amino terminus of the pro sequence and at least one specific residue (Glu) close to the processing site (position 25) (229).

The activity which processes pro-σ^E, like the synthesis of pro-σ^E itself, is developmentally regulated and appears in sporulating *B. subtilis* approximately 1 h after the onset of pro-σ^E synthesis (307). The importance of the processing reaction as the device which determines when σ^E is activated can be seen when a *B. subtilis* strain with a *sigE* allele that directly encodes an active product (i.e., missing the first 15 codons of the pro sequence) is allowed to sporulate. Such a strain is Spo^- and blocked in the earliest stages of sporulation (stage 0) (231). Apparently, transcriptional control alone results in the synthesis of σ^E at a time when its activity disrupts the normal pattern of early spore gene expression. The time in sporulation at which pro-σ^E is converted into σ^E coincides with the time at which the developing cell divides into mother cell and forespore compartments. There is considerable speculation that these two events are related and that some aspect of the septation process functions either directly or indirectly as a signal for the activation of σ^E (79, 125, 186, 284, 304). One of the strongest arguments for a dependence of pro-σ^E processing on septation is the observation that mutant *B. subtilis* cells that are depleted of the products of essential septation genes (e.g., *ftsZ* and *divIC*) fail both to form spore septa and to convert pro-σ^E into σ^E (11, 179). It is assumed that the lack of septation-essential gene products inhibits pro-σ^E processing indirectly, via the block on septum formation (11, 179). Although septation and pro-σ^E processing appear to be normally coupled, intact septa per se may not be essential for processing of pro-σ^E to occur. It has been reported that penicillin, at concentrations that block normal stage II septation, fails to block pro-σ^E processing (147). It is not known how penicillin uncouples these two processes.

Aside from *ftsZ* and *divIC*, the products of at least four additional genes (*spoIIGA*, *spoIIAA*, *spoIIAC*, and *spoIIE*) are needed for pro-σ^E processing (146, 149, 284, 307). Of these four genes, *spoIIGA*, the upstream gene of the operon that encodes pro-σ^E, is most likely to encode a protein that participates directly in the processing reaction (284). Forced expression of both *spoIIGA* and *spoIIGB/sigE*, but not *spoIIGB/sigE* alone, in vegetative *B. subtilis* cells leads to a low but measurable level of σ^E-dependent promoter activity (284). This has been interpreted as evidence that SpoIIGA is the pro-σ^E processing enzyme (284). The idea that SpoIIGA plays a direct role in pro-σ^E processing is supported by the isolation of a missense mutation in *spoIIGA* that suppresses the impaired processing of a mutant *sigE* (*sigE25EK*) allele's product (231). The predicted amino acid sequence of SpoIIGA suggests that it could be both a membrane-associated protein and a protease (200, 284). Consistent with SpoIIGA's potential membrane association is the finding that a chimera of SpoIIGA and the *E. coli lacZ* gene product cosediments during ultracentrifugation with the membrane components of a crude *B. subtilis* extract (230). The predicted and observed properties of SpoIIGA, when taken with the apparent coupling of pro-σ^E processing to the forespore septation event, have led to the suggestion that SpoIIGA could be the pro-σ^E processing enzyme and be activated to cleave pro-σ^E upon becoming embedded in the newly formed forespore membrane. Such a process would tie σ^E activation to the spore's morphological development (284). Although the simplicity of this model is attractive, it is probably an oversimplification. Both *spoIIE* and *spoIIA* mutants form septa but fail to process σ^E (137).

The role of *spoIIA* products in pro-σ^E processing is likely to

be indirect. *spoIIA* encodes the sporulation-specific sigma factor σ^F and its regulators (91, 283). σ^F, like σ^E, is activated after septation (191, 227). It is not essential for the transcription of any of the operons known to be required for the conversion of pro-σ^E into σ^E (11, 109, 158, 329). Thus, if σ^F's contribution to σ^E synthesis depends on its function as a transcription factor, the processing reaction will require the product of at least one unidentified σ^F-dependent operon. Although σ^F's known function as a transcription factor is the simplest explanation for its role in pro-σ^E processing, other possibilities exist. A subclass of *spoIIAC* mutants [*spoIIAC*(P)] appear to encode an inactive σ^F that still permits pro-σ^E processing (137). From the phenotype of this class of mutants, it has been proposed that σ^F could have an indirect role in pro-σ^E processing that does not depend on its function as a transcriptional activator (137, 227). What this alternative role might be remains undefined.

spoIIE is also known to be essential for σ^E synthesis. The sequence of SpoIIE does not predict its function but reveals hydrophobic domains found in membrane-associated proteins (334a). *B. subtilis* cells which lack SpoIIE form septa containing abnormally large amounts of cell wall material (137, 232). If a "normal" septum proves to be a prerequisite for pro-σ^E processing, SpoIIE may be needed for this reaction only insofar as it is important to the formation of the normal forespore septum (284). It is worth noting that SpoIIE is not only required for the processing of pro-σ^E but also plays an essential role in the activation of σ^F (191, 227). Recalling that active σ^F (SpoIIAC) is itself needed for pro-σ^E processing, it is unclear whether SpoIIE's role in pro-σ^E processing is limited to the activation of σ^F or if it also plays a direct part in the pro-σ^E processing reaction.

The expression patterns of genes known to depend on E-σ^E or E-σ^F for their transcription (34, 66, 191) (see below) argue that these holoenzymes are likely to be active in different compartments of the sporulating cell, with E-σ^E participating in mother cell-specific transcription (66) and E-σ^F devoted to forespore-specific gene expression (191). To account for this compartmentalized activity, sophisticated models of σ factor activation have been developed in which the formation of the septum is proposed to trigger not only the temporal activation of σ^E and σ^F but also their compartment-specific activation (79, 125, 186, 191). One model depicts septation as an event that leads to the activation of σ^F only in the forespore compartment. This results in the forespore-specific expression of σ^F-dependent genes, which then communicate with SpoIIGA on the mother cell side of the forespore membrane, signaling it to process pro-σ^E only in the mother cell compartment. Proposed mechanics for the individual steps involved in this process have been covered in several recent reviews (76, 79, 125, 186).

An alternative model, based on a fluorescence microscopy study of compartment-specific gene expression, proposes that σ^F and σ^E are initially active on both sides of the forespore membrane but later become selectively inactivated in one or the other cell compartment to ultimately restrict σ^E-dependent transcription to the mother cell and σ^F transcription to the forespore (34). Western blot analyses designed to detect processed σ^E in extracts enriched for mother cell or forespore material and test the competing models have yielded conflicting results (35, 162). Regardless of which model for σ factor localization is correct, there is general agreement that σ^E and σ^F ultimately direct mother cell- and forespore-specific gene expression, respectively.

E-σ^E-transcribed genes. The biochemical qualities of σ^E as a novel sigma factor were defined in vitro by using a fortuitous promoter-like sequence on the *E. coli* plasmid pMB-9 and several regions of cloned *B. subtilis* DNA (112). These templates are transcribed weakly by E-σ^E in vitro and are unlikely to depend on E-σ^E in vivo (112). A *B. subtilis* promoter (G4) that is efficiently used by E-σ^E in vitro and dependent on this form of RNAP in vivo was identified in a *B. subtilis* clone bank by using RNA transcribed by E-σ^E in vitro from total *B. subtilis* DNA as a hybridization probe (249). G4 was used in a mutational analysis which defined the sequences that are important for σ^E-dependent promoter recognition (Table 1) (245, 248). A second σ^E-dependent promoter (*bvx*) was discovered on a DNA fragment that carried the cloned *B. subtilis rrnB* operon (117). The functions of the operons controlled by the *G4* and *bvx* promoters are unknown.

Seventeen known sporulation genes (*spoIID*, *spoIIM*, *spoIIID*, *spoIIIA*, *spoIVA*, *spoIVCA*, *spoIVCB*, *spoIVF*, *bofA*, *spoVB*, *spoVD*, *spoVE*, *spoVJ*, *spoVM*, *spoVR*, *spoVID*, and *cotE*) have been cloned, sequenced, and shown to depend on E-σ^E for at least a part of their expression. Most of these genes had been previously known by mutation and cloned either on the basis of the wild-type allele's ability to complement a mutant phenotype or by selection for an antibiotic resistance marker present on the transposon that generated the mutation. Two of the genes (*spoVID* and *spoVR*) were specifically identified in a search for σ^E-dependent promoters (10, 12), while *cotE* was identified by using an oligonucleotide probe that was developed from the sequence of the *cotE* (337) protein. These genes were assigned to the σ^E regulon based on their meeting several, if not all, of the following criteria: (i) failure to be expressed in *B. subtilis* strains lacking σ^E, (ii) transcription by E-σ^E in vitro, (iii) the presence of a σ^E consensus sequence in their promoters, and (iv) their expression in vegetative *B. subtilis* following the artificially induced synthesis of an active form of σ^E.

spoIID was the first known gene to be identified as part of the σ^E regulon (253). The explicit function of the *spoIID* product is not certain; however, *B. subtilis* strains which lack SpoIID fail to completely remove the cell wall material that lies in the periphery of the spore septum and, as a consequence, fail to progress to forespore engulfment (137). The possibility that the *spoIID* product may directly participate in the removal of septal wall material is suggested by sequence similarity between it and a modifier of amidase activity (D. Karamata, unpublished; cited in reference 125). Although SpoIID's putative role in modification of the septum that separates the mother cell from the forespore leaves open the possibility that it could be needed in both compartments, there is genetic evidence that its expression in the mother cell compartment is sufficient for sporulation (139) and immunoelectron microscopic evidence that its expression is restricted to that compartment (66). The electron microscopic study provided compelling visual documentation of *spoIID* expression only in the mother cell compartment and focused attention on the idea that σ^E could be a mother cell-specific σ factor (66).

spoIIM encodes a 29-kDa protein, with basic and hydrophilic elements, that is required for the expression of forespore genes transcribed by E-σ^G (274). Cell fractionation studies documented the presence of β-galactosidase from an *spoIIM-lacZ* fusion in both the mother cell and forespore compartments, with the mother cell extract containing approximately twice as much β-galactosidase per milligram of protein as the forespore extract (275). Genetic studies argued that expression of *spoIIM* in the forespore is essential to sporulation but that its transcription is independent of the forespore-specific σ factors σ^F and σ^G (275). The dependence of *spoIIM* transcription on σ^E, when taken with the need for *spoIIM* expression in the forespore compartment, implies that *spoIIM* is transcribed by E-σ^E

in the forespore. This conflicts with the notion that σ^E is a mother cell-specific σ factor. However, if, as one study suggests (34), σ^E is initially activated in both compartments and only restricted to the mother cell later in sporulation, *spoIIM* may be transcribed by σ^E in the forespore immediately after σ^E activation, with this early expression being adequate to satisfy the forespore's requirement for its product. Although this is a simple explanation of the data, it is still possible that E-σ^E is solely a mother cell-specific enzyme. If this is so, an alternate, undiscovered way of expressing *spoIIM* in the forespore that does not depend on σ^E would be needed.

spoIIID is another sporulation-essential gene transcribed by E-σ^E. The *spoIIID* gene was independently cloned by two laboratories (167, 281) and found to encode a previously identified sporulation transcription factor that modulates the expression of both E-σ^E- and E-σ^K-dependent genes (165). Aside from dependence on E-σ^E, maximum expression of *spoIIID* also requires an intact copy of *spoIIID*, suggesting autoregulation of *spoIIID* transcription (167, 281). The *spoIIID* promoter has been used in studies of σ factor-promoter interactions in which amino acid substitutions in σ^E were found to suppress the negative effects of specific base pair changes in the *spoIIID* promoter (295). There is genetic evidence that *spoIIID* needs to be expressed only in the mother cell compartment for it to perform its sporulation-essential function (61), and cell fractionation data have localized the β-galactosidase product of an *spoIIID-lacZ* fusion gene to the mother cell compartment (167).

spoIIIA defines an operon of at least three genes (138) which is required for a sporulating cell to advance beyond the engulfment stage (232). The explicit functions of the *spoIIIA* products are unknown, although sporulation-essential expression of *spoIIIA* is required only in the mother cell (139). Mutations in *spoIIIA* lead not only to a significant reduction is the transcription of several of the mother cell-expressed coat protein genes (255, 339) but also to variable reductions in forespore-specific gene expression (49, 136, 152, 199). It has been proposed that *spoIIIA* provides a mother cell-specific function that indirectly affects forespore development (136, 138).

Several σ^E-dependent genes (*spoIVCA*, *spoIVCB*, *spoIVFA*, *spoIVFB*, and *bofA*/*ski-4*) are involved in the synthesis of σ^K, the late-acting mother cell σ factor (see below). σ^K is encoded by a composite gene (*sigK*) formed when two truncated genes are joined by the excision of an intervening sequence (286). *spoIVCB*, the amino-terminal portion of this composite gene, and *spoIVCA*, which encodes the recombinase that catalyzes the excision, are both initially transcribed by E-σ^E, although later expression of *sigK* occurs by E-σ^K (164, 169, 256).

spoIVF encodes two genes (A and B) that participate in the processing of σ^K from its inactive precursor form (pro-σ^K) (53, 188). Mutations (*bofB* [bypass of forespore]) which uncouple the processing of pro-σ^K from events occurring in the forespore (51) have been mapped to *spoIVFA* (53). From the phenotypes of missense and null mutations in the two *spoIVF* cistrons and the predicted properties of their products, SpoIVFA and SpoIVFB are hypothesized to lie in the outer forespore membrane, where they could function in intercompartmental coupling between forespore and mother cell, i.e., SpoIVFB is thought to be a protein that promotes pro-σ^K processing but is prevented from acting by SpoIVFA unless a signal from the forespore triggers its release (53). A second gene (*bofA*/*ski-4*), in which mutations are found that uncouple pro-σ^K processing from forespore events, also appears to be transcribed by E-σ^E (140, 250). This gene was identified by two laboratories studying different regulatory phenomenon: the coupling of pro-σ^K processing to forespore events (*bofA*) (250) and the suppression of sporulation by the overexpression of a histidine protein kinase, KinA (*ski-4*) (140). BofA is hypothesized to cooperate with SpoIVFA to inhibit SpoIVFB-dependent pro-σ^K processing (250).

The *spoVB*, *spoVD*, *spoVE*, *spoVM*, and *spoVR* genes are all transcribed from one or more σ^E-dependent promoters, with some of these genes also requiring the mother cell-specific transcriptional regulatory protein SpoIIID for their expression (12, 55, 178, 204, 235, 300). Mutations in these genes lead to the production of spores containing a defective cortex, the modified peptidoglycan stratum laid down between the membranes that separate the forespore from the mother cell. In keeping with their role in synthesizing this specialized cell wall material, two of these genes (*spoVD* and *spoVE*) have been found to be homologous to penicillin-binding proteins (i.e., peptidoglycan synthesizing) or morphogenic proteins of other bacteria (55, 135, 150).

spoVK (*spoVJ*) is also part of the σ^E-dependent regulon, with an additional requirement for the SpoIIID protein for its expression (84). RNA analysis revealed two overlapping *spoVK* transcripts (93). The putative promoter for one of the transcripts resembles a consensus promoter for E-σ^E, while the second resembles that of a σ^K-dependent promoter (93). Cell fractionation experiments have localized SpoVK expression principally to the mother cell compartment (93). The function of *spoVK* is unknown, but mutations at this locus result in immature spores that are resistant to lysozyme (an early spore resistance marker) but sensitive to organic solvents (a later marker) (126). Dipicolinic acid (DPA), a compound associated with the spore's resistance to heat (76), appears to be synthesized in *spoVK* mutants but is not incorporated into the prespore, leading to the suggestion that SpoVK is involved in DPA transport into the developing spore (126).

spoIVA (232, 252, 280), *spoVID* (10), and *cotE* (337, 339) encode morphogenic proteins that are needed for the proper assembly of the spore coat, a proteinaceous structure that surrounds the spore and contributes to its resistance properties. All three are transcribed from σ^E-dependent promoters, with *cotE* having dual σ^E promoters, one of which requires SpoIIID for its activation (10, 252, 280, 337, 339). Electron microscopic studies revealed that SpoIVA and CotE surround the forespore, likely sitting close to the outer forespore membrane, from where they direct the assembly of the spore coat (73).

With the possible exception of *spoIIM*, all of the σ^E-dependent *spo* genes that have been analyzed in detail are believed to be expressed exclusively in the mother cell compartment. Additional genes encoding extracellular enzymes (e.g., alkaline phosphatase and DNase) which are likely to be expressed in the mother cell for excretion also contain potential σ^E promoter sequences (reviewed in reference 76). The restricted expression of σ^E-dependent genes to the mother cell compartment is the principal argument for σ^E's being a mother cell-specific σ factor.

Sigma Factor σ^F

Cloning and characterization. The existence of the novel sigma factor that would become σ^F was deduced from the sequence of the sporulation gene *spoIIAC* (78, 283). The *spoIIA* locus was cloned on the basis of the wild-type sequence's ability to complement Spo^- mutations which mapped to this site (183, 260). The original cloned DNA was found to contain only a portion of *spoIIA* (183) and was used as a hybridization probe to clone the entire transcription unit (233). Plasmid integration (233) and DNA sequencing (91) studies

revealed that *spoIIA* was a polycistronic operon that included three open reading frames. Although there was a sequencing error in the third open reading frame (*spoIIAC*), which resulted in an underestimate of its product's size (78, 283), the similarity between this open reading frame and the sequence of known sigma factors was still appreciated (78). The similarities became even more apparent once the sequencing error was discovered (283). From the homologies between *spoIIAC* and other bacterial sigma factors, as well as the phenotype of *B. subtilis* mutants that lacked it, SpoIIAC was assumed to be a sporulation-essential sigma factor which had not yet been detected biochemically (283). The idea that *spoIIAC* encoded a sigma-like protein was supported by genetic studies which found that missense mutations that compromised SpoIIAC's sporulation-essential activity were localized to regions of its predicted DNA-binding or core RNAP-binding domain (335, 336). Discovery of the *spoIIAC* product occurred unexpectedly during a search for a form of RNAP that could transcribe the forespore-specific *sspE* gene (292). Genetic and biochemical evidence suggested that neither SpoIIAC nor σ^E (the only other sporulation-specific sigma factor known at that time) was directly involved in *sspE* transcription, and so a novel sporulation-specific sigma factor that could direct the transcription of *sspE* in vitro was sought (292). RNAP from cells expressing *sspE* was purified by heparin-agarose chromatography, glycerol gradient centrifugation, and DNA-cellulose chromatography, with the *sspE*-transcribing portion of the preparation subsequently fractionated by preparative SDS-PAGE (292). A reconstitution experiment revealed that the renatured products of two different protein bands could independently stimulate *sspE* transcription in vitro (292). Sequence analysis demonstrated that the stimulating proteins were likely the products of the *spoIIAC* and *spoIIIG* genes and were designated σ^F and σ^G, respectively (292). Although E-σ^F is able to transcribe *sspE* in vitro, it is not likely to be responsible for its expression in vivo. A *B. subtilis* strain which lacks σ^G but contains σ^F displays no *sspE-lacZ* activity in vivo (292). σ^F and σ^G have similar promoter recognition properties, which is assumed to account for their overlapping in vitro activities (Table 1) (291, 292).

σ^F regulation. (i) Transcriptional regulation. The σ^F structural gene is the promoter-distal cistron of an operon whose principal transcriptional start site is located 27 nucleotides upstream of the first open reading frame (Fig. 3) (327). *spoIIA-lacZ* expression studies revealed that *spoIIA*-dependent β-galactosidase expression is absent from vegetatively growing *B. subtilis* but begins to appear 30 to 60 min after the induction of sporulation (82, 327). This expression is dependent upon the products of all known *spo0* loci but none of the later *spo* loci that were tested (82, 327). Similar results were obtained when *spoIIA*-specific mRNA was analyzed; however, these experiments also revealed two RNA transcripts originating from the *spoIIA* sequence (261). The smaller transcript (1.6 kb) appears early (T_1) and persists, while a larger second transcript (2.6 kb) does not appear until T_3 (261). The larger RNA is thought to originate from a promoter located approximately 1 kbp upstream of the proximal promoter (261, 264, 329) and includes an open reading frame (*dacF*) whose predicted product has extensive sequence homology with DD-carboxypeptidases (329). Sequences upstream of the transcriptional start site for the larger transcript are similar to those of σ^F-dependent promoters (264) and resemble sequences found upstream of a gene (0.3 kb) which is expressed late in sporulation in the forespore (224, 329). *dacF* expression is eliminated in $SigF^-$ and reduced in $SigG^-$ strains of *B. subtilis* and induced in vegetatively growing *B. subtilis* by the artificial synthesis of σ^F or σ^G (264). This argues that the *dacF* promoter is principally transcribed by E-σ^F and that the synthesis of σ^F from this promoter is thus autoregulated (264). Integrating plasmids, used to delimit the size of the essential *spoIIA* control region, showed that at least 52 bp upstream of the start site are essential for *spoIIA* expression but that the region upstream of bp −168 is unnecessary for maximum spore formation (329). It therefore appears that the upstream σ^F-dependent promoter is dispensable for adequate *spoIIA* expression under laboratory conditions. The region approximately −35 and −10 bp upstream of the principal *spoIIA* transcriptional start site shows homology to *B. subtilis* E-σ^H-recognized promoters (329). *spoIIA* can be efficiently transcribed by E-σ^H in vitro (328). In vivo, *spoIIA* transcription depends on the presence of σ^H (329) and the activated form of Spo0A (28). These results are consistent with *spoIIA*'s being part of the σ^H regulon, with Spo0A as an additional regulator. The requirement for Spo0A in *spoIIA* transcription resembles a similar requirement in *spoIIG* transcription. Thus, although *spoIIA* (*sigF*) and *spoIIG* (*sigE*) differ in the form of RNAP (E-σ^H and E-σ^A, respectively) that recognizes their promoters, they both likely receive their sporulation-specific activation signal via the *spo0A* phosphorelay system. Given that *spoIIA* and *spoIIG* are both transcribed at the same time in sporulation (101, 334), under the control of the same activation system (Spo0A), their dependence on separate holoenzymes is puzzling.

(ii) Posttranslational regulation. Even though transcriptional regulation determines when σ^F appears in the developing cell, its activation requires additional factors. A genetic study revealed that the two upstream genes of the *spoIIA* operon, *spoIIAA* and *spoIIAB*, encode potent regulators of σ^F activity (263). Overexpression of *spoIIAB* was found to inhibit σ^F-directed gene expression, while mutations in *spoIIAB* stimulate σ^F-dependent genes. Furthermore, it was observed that a mutation in *spoIIAA* that blocks σ^F-directed transcription could be suppressed by a mutation in *spoIIAB*. These results were interpreted as evidence for SpoIIAB's being an antagonist of σ^F, with SpoIIAA counteracting its negative effects (263). It was also noted that mutant σ^Fs, which become capable of recognizing promoters that are normally transcribed by other RNAP holoenzymes (i.e., E-σ^B and E-σ^G), remain sensitive to control by SpoIIAA and SpoIIAB in initiating transcription from these previously unrecognized promoters (191, 263). This implied that SpoIIAA and SpoIIAB functioned at the level of E-σ^F activity rather than at the level of a particular promoter sequence (263). Evidence that SpoIIAB affects σ^F-dependent transcription by a direct protein-protein interaction came from an in vitro cross-linking study in which purified SpoIIAB was shown to physically associate with σ^F (74). Incubation of σ^F with SpoIIAB also inhibits σ^F-dependent transcription in vitro (74, 202). From these findings, SpoIIAB was viewed as an anti-sigma factor, with SpoIIAA suggested to function as an anti-anti-σ factor (74).

The expected importance of SpoIIAB in properly regulating σ^F activity during sporulation was confirmed in a study in which a *spoIIAB* deletion mutation was found to cause hyperexpression of genes that are normally expressed in the forespore and arrest sporulation at its earliest stage (stage 0) (48). Thus, the appearance of active σ^F at the time when the operon encoding it becomes transcriptionally active results in a disruption of the sporulation program. This is the same phenomenon that occurs if an active form of σ^E is synthesized directly from *spoIIG* (231). Apparently, the synthesis of σ^F and σ^E in inactive forms is essential for establishing the correct program of early spore gene expression. The posttranscriptional regulation of σ^F and σ^E thus represents a device that permits the operons that encode them to be controlled by the principal early spore

gene regulator (Spo0A) while preventing them from prematurely reprogramming RNAP as they accumulate.

It has been proposed that the SpoIIAA/SpoIIAB regulatory system, like the pro-σ^E processing system, plays a critical role in controlling not only the timing of σ factor activation, but also the cell compartment in which this activation takes place. Just as the pro-σ^E processing reaction is believed to restrict σ^E transcription to the mother cell, the SpoIIAA/SpoIIAB system has been proposed to restrict σ^F-directed gene expression to the forespore (5, 191, 227, 263). The mechanism by which SpoIIAA causes the release of σ^F from SpoIIAB-mediated inhibition is not known; however, in vitro, SpoIIAB has been shown to preferentially bind to either SpoIIAA or σ^F, depending on the ratio of ADP to ATP that is present in the reaction mixture. A high ADP/ATP ratio favors the formation of the SpoIIAB-SpoIIAA complex, while a low ratio facilitates SpoIIAB binding to σ^F (5). A model has been proposed in which ATP levels could selectively drop in the forespore compartment while remaining relatively high in the mother cell, thereby activating σ^F only on the forespore side of the septal membrane (5). It is unknown whether the hypothetical changes in ATP levels occur in vivo or what processes could cause such an ATP change in the forespore. Other experiments demonstrated that SpoIIAB has a kinase activity that can phosphorylate SpoIIAA in vitro (202). This modification appears to inhibit SpoIIAA's ability to bind to SpoIIAB (5). The role of SpoIIAA phosphorylation in the in vivo control of σ^F and the signals that instigate it are unclear.

Although details of σ^F regulation still need to be resolved, there is general agreement that the transcription of σ^F-dependent genes eventually becomes restricted to the forespore compartment. As is the case with the restriction of σ^E activity to the mother cell, there are competing models in which σ^F is either selectively activated only in the forespore compartment because of a unique environment in that compartment (5, 191) or initially active in both compartments and restricted to the forespore as a consequence of its eventual inactivation in the mother cell (34). Regardless of which model is correct, activation of σ^F appears to require the septation event and the product of at least one sporulation-essential gene (*spoIIE*) (191, 227). The product of a second sporulation gene (*spoIIIE*) is also normally needed for the expression of σ^F-dependent genes (92); however, the SpoIIIE requirement can be bypassed if the E-σ^F-transcribed gene (e.g., *spoIIIG* or *gpr*) is moved to a different site on the *B. subtilis* chromosome (291). The fact that σ^F-dependent transcription can occur in the absence of SpoIIIE argues that SpoIIIE is not required for σ^F's release from SpoIIAB (191). A recent electron microscopic study demonstrated convincingly that SpoIIIE plays a role in the proper partitioning of the *B. subtilis* chromosome to the forespore (330). This result implies that the requirement for SpoIIIE in σ^F-dependent transcription is likely to be indirect and a result of the need for SpoIIIE in transferring an intact chromosome to the compartment where σ^F is maximally active.

E-σ^F-transcribed genes. Besides the *dacF* promoter, which appears to be part of an extended *sigF* operon (264), *spoIIIG* and *gpr* are the only additional examples of genes that are known to rely on σ^F for at least a portion of their expression (227, 293). As with *dacF*, both of these genes can also be transcribed by the E-σ^G form of RNAP (227, 293). *spoIIIG*, the σ^G structural gene, was identified as a sigma-like open reading frame immediately downstream of the operon (*spoIIG*) that encodes σ^E (152, 201). As its designation implies, disruption of the *spoIIIG* reading frame blocks sporulation at stage III (152, 201). A *spoIIIG-lacZ* reporter system, used to estimate the time in development when *spoIIIG* transcription becomes active, synthesized β-galactosidase 2.5 h after the onset of sporulation and continued to generate β-galactosidase during the next 2 h (152). Fractionation of these sporulating cells into mother cell- and forespore-enriched extracts segregated the β-galactosidase primarily into the forespore extract (152). Genetic experiments implied that *spoIIIG* expression in the forespore compartment was necessary if a cell is to sporulate successfully (139). The data were consistent with *spoIIIG*'s encoding a forespore-specific sigma factor (152). The notion that *spoIIIG* expression is dependent on σ^F is based both on in vitro experiments in which E-σ^F was found to transcribe *spoIIIG* (292), in vivo experiments in which induction of σ^F synthesis in vegetatively growing *B. subtilis* activated *spoIIIG* transcription (227), and genetic studies in which mutations which inactivate σ^F were shown to block *spoIIIG* expression (92, 152, 227).

The last gene known to be transcribed by E-σ^F in vivo is *gpr*. *gpr* is the coding sequence for an endopeptidase which degrades the spore's protein reserve (small, acid-soluble spore proteins [SASPs]) upon germination (293). *B. subtilis gpr*, as judged from the compartment-specific partitioning of the product of a *gpr-lacZ* fusion, is expressed primarily if not exclusively in the forespore (292). *gpr* synthesis is abolished in a *spoIIAC* (*sigF*) mutant and reduced 50% in a *spoIIIG* (*sigG*) mutant (293). Thus, *gpr* is likely to be transcribed by both E-σ^F and E-σ^G. Aside from *spoIIIG* and *gpr*, several forespore-specific genes (e.g., *gerA* and *sspE*) that appear to be primarily transcribed by E-σ^G in vivo can also be transcribed by E-σ^F, for unknown reasons, if their chromosomal location is altered (291). At least one of them, *sspE*, can also be transcribed by E-σ^F in vitro (292). The consensus sequences for σ^F- and σ^G-dependent promoters (Table 1) are very similar (291, 293), and it is possible that one of σ^F's functions is to initially turn on some of the operons which ultimately will be transcribed by E-σ^G.

There is evidence that at least one σ^F-dependent gene remains to be discovered. The processing of pro-σ^E into its active form requires the *spoIIAC*/*sigF* gene product (146, 284); however, no operon known to be needed for the processing reaction has been found to require σ^F for its expression. Thus, either the σ^F protein itself somehow directly participates in the processing reaction (137, 227) or there is an undiscovered σ^F-dependent operon whose products are required for pro-σ^E processing. From a small number of identified target genes, σ^F-dependent transcription appears to be limited to the early expression of forespore-specific genes.

Sigma Factor σ^G

Identification and cloning. The σ^G structural gene was discovered immediately downstream of the σ^E-encoding (*spoIIG*) operon as an open reading frame (*spoIIIG*) that contained sequence elements in common with known sigma factors (152, 201). The σ^G protein itself was uncovered, along with σ^F, during a search for factors that could activate the transcription of the forespore-specific *sspE* gene (292). RNAP prepared from sporulating cells and purified by column chromatography was observed to transcribe *sspE* in vitro. When the proteins contained in this RNAP fraction were separated by SDS-PAGE, renatured, and added back to core RNAP, two different proteins were found that were capable of directing RNAP to the *sspE* promoter (292). A comparison of their amino-terminal sequences with the sequences of known sporulation genes identified one as SpoIIAC (σ^F) and the second as SpoIIIG (σ^G) (292).

σ^G regulation. From the accumulation of β-galactosidase

from an *spoIIIG-lacZ* translational fusion, expression of *spoIIIG* begins between 2 and 2.5 h after the onset of sporulation and requires the products of all of the stage 0 sporulation genes as well as wild-type *spoIIA*, *spoIIE*, and *spoIIIE* operons (92, 290). In contrast to the delay in β-galactosidase accumulation obtained with the *lacZ* translational fusion, transcriptional fusions of *lacZ* to the *spoIIIG* region result in β-galactosidase synthesis within the first hour of sporulation (290). The transcriptional fusion result was consistent with S1 nuclease protection assays of *spoIIIG*-specific RNA, which detected *spoIIIG* transcripts 1 h after the onset of sporulation (201). Measurements of the amount of β-galactosidase expressed from different *spoIIIG-lacZ* transcriptional fusions, constructed by joining various lengths of upstream regions of *spoIIIG* to *lacZ*, revealed that the early synthesis of *spoIIIG* mRNA is due to readthrough from the upstream *spoIIG* operon (290). The same conclusion could be drawn from the size of the *spoIIIG* mRNA that is synthesized early (201). It is thought that the *spoIIIG* portion of this early RNA is not translated because of a region, approximately 50 bases upstream of the *spoIIIG* translational initiation site, which can base pair with the initiation site and block its availability to ribosomes (201, 290). A second *spoIIIG* mRNA, beginning 27 nucleotides upstream of the *spoIIIG* initiation codon, becomes evident between T_2 and T_4 (201, 290). This transcript lacks the upstream sequences needed to form the translational block on σ^G expression and appears at the time when the product of *spoIIIG-lacZ* translational fusions becomes assayable (290). The "late-appearing" *spoIIIG* transcript is the only one essential for providing the cell with σ^G. Introduction of a wild-type *spoIIIG* gene, expressible from its late-activated promoter but lacking the ability to synthesize the early transcript, suppresses the Spo phenotype of a *spoIIIG* null mutation (290). This principal *spoIIIG* promoter can be activated in vivo during vegetative growth by the induced expression of either σ^G or σ^F (227, 263, 291). In vitro, both E-σ^G and E-σ^F transcribe *spoIIIG*; however, E-σ^F's preference for *spoIIIG* is 20-fold higher than that of E-σ^G (290). Furthermore, mutations which inactivate σ^F but not those which affect only σ^G block *spoIIIG* expression (227, 290). Thus, the activity of the principal *spoIIIG* promoter appears to depend on σ^F. As such, *spoIIIG* expression would be expected to be controlled by the σ^F regulators *spoIIAA* and *spoIIAB* and limited to the forespore compartment (191, 263).

The question of why *spoIIIG* is cotranscribed with *spoIIG* early in sporulation if this mRNA is dispensable for σ^G synthesis and fails to be translated has been discussed by Sun et al. (290). They suggest the possibility of two independent mechanisms for σ^G synthesis: one dependent on E-σ^F, and a second involving a hypothetical, developmentally regulated translation of the polycistronic *spoIIG-spoIIIG* mRNA to yield σ^G, which then directs further *spoIIIG* expression (290). The notion that *spoIIIG* could be expressed by E-σ^G, with its initial synthesis driven by readthrough transcription from *spoIIG*, was in fact proposed when *spoIIIG* was first identified as a potential σ factor (152). It has been hypothesized (290) that although E-σ^F is the principal *spoIIIG*-transcribing enzyme under normal laboratory conditions, there could be other growth conditions in which the E-σ^G pathway is favored. There is evidence that *spoIIAB* not only may be a negative regulator of σ^F but also may directly affect the activity of σ^G (94, 162, 244). Thus, if a E-σ^G-dependent *spoIIIG* expression pathway exists, the environmental factors that modulate σ^F activity through SpoI IAA and SpoIIAB might still be in control, directing the activity of σ^G (290).

Aside from SpoIIAA/SpoIIAB control, σ^F-dependent expression of *spoIIIG* also depends on the *spoIIIE* and possibly the *spoIIIA* products (92, 152, 291). Although required for sporulation, *spoIIIE* is constitutively expressed at a low rate during growth. It has consensus sequences for mononucleotide binding as well as several potential membrane-spanning domains (92). *spoIIIE* mutations block *spoIIIG* transcription and subsequent forespore gene expression but do not seem to directly affect mother cell gene regulation (92). It has been shown that σ^F-dependent *spoIIIG* expression is freed from a need for SpoIIIE but not from a need for release from SpoI IAB if *spoIIIG* is moved to an alternate site on the *B. subtilis* chromosome (291). Recent microscopic analyses have revealed that SpoIIIE is needed for the proper segregation of the *B. subtilis* chromosome into the forespore compartment (330). An intriguing model has been proposed in which the defective DNA segregation that occurs in *spoIIIE* mutants is hypothesized to result in only a specific segment of the *B. subtilis* chromosome's entering the forespore. This partitioning of a limited portion of the chromosome into the forespore would explain why a forespore-specific gene's chromosomal location determines whether or not it is expressed in a *spoIIIE* mutant. If it resided in the region of the chromosome that entered the forespore, it would be expressed. Otherwise it would remain in the mother cell and not become active (330). An additional factor that could influence forespore gene expression comes from the observation that the forespore chromosome condenses early in development (268). This has promoted the suggestion that chromosome condensation, with its potential for limiting RNAP access, may establish accessibility domains which are altered in the *spoIIIE* mutant and that only genes that are physically located in an accessible region of the chromosome can be expressed (291).

The σ^E-dependent, and presumably mother cell-specific, *spoIIIA* gene has also been implicated as a regulator of *spoIIIG* expression (152) as well as that of other forespore genes (81, 199, 224, 246); however, contradictory evidence has been published (136) leaving SpoIIIA's role in forespore gene expression in question. A third stage III *spo* gene, *spoIIIJ*, may encode a regulator of σ^G activity. It is not required for the expression of the σ^G structural gene but is needed for the transcription of genes that are dependent on E-σ^G (77). *spoIIIJ* consists of a bicistronic operon that is expressed primarily in vegetative cells, with the promoter-proximal cistron needed for sporulation (77). How SpoIIIJ controls E-σ^G-dependent gene expression is unknown.

E-σ^G-transcribed genes. Several genes are known to depend on σ^G for their expression. The transcription of these genes, like the synthesis of σ^G itself, appears to be restricted to the forespore compartment. Among the E-σ^G-transcribed genes is σ^G's own coding sequence (*spoIIIG*) (290). σ^G is not required for the expression of *spoIIIG*. E-σ^G transcribes *spoIIIG* in vitro but with a lower efficiency than does E-σ^F, the RNAP form thought to be the principal *spoIIIG*-transcribing enzyme (290). σ^G has been speculated to enhance the expression of *spoIIIG* or to be the principal *spoIIIG*-transcribing enzyme under undefined sporulation conditions in which σ^F activity may not be high (152, 290).

The first gene demonstrated to be transcribed by E-σ^G was *sspE* (292). *sspE* is one of five genes (*sspA* through *sspE*) which encode a group of SASPs that constitute 10 to 20% of the protein found in dormant spores (89). These proteins are rapidly degraded as a source of amino acids upon spore germination. In addition, the SASPs play a role in the resistance of dormant spores to UV light (269). All five of these genes have been cloned, sequenced, and mapped on the *B. subtilis* chromosome (45–47, 110). Translational fusions of four of the *ssp*

genes to *lacZ* revealed that all are similarly regulated and produced β-galactosidase, which accumulated almost exclusively in the forespore compartment (199). These genes are not expressed in mutant *B. subtilis* lacking σ^G but are expressed in vegetatively growing *B. subtilis* if a source of σ^G is provided (292). They have common conserved sequences at bp −35 and −10 which are believed to be the target sequences for E-σ^G (Table 1) (217). A late sporulation gene (0.3 kb) that is turned on later than the other *ssp* genes has been recently redesignated as a sixth *ssp* gene (*sspF*) (224). The *sspF* promoter is less well conserved than the other *ssp* promoters; however, *sspF* expression can be induced by the synthesis of σ^G in vegetative cells, and so its transcription is thought to rely on σ^G (224).

In addition to the *ssp* genes, the gene (*gpr*) encoding the principal endoprotease responsible for the degradation of the SASPs is also recognized by E-σ^G. Both E-σ^F and E-σ^G effectively initiate *gpr* transcription at the same site in vitro (293). In vivo, *gpr-lacZ* transcription is eliminated in the absence of σ^F and reduced by half, with early *gpr* expression unaffected, in mutant *B. subtilis* cells that lack σ^G (293). Thus, *gpr* appears to be transcribed first by E-σ^F, with its late expression dependent on E-σ^G (293).

The glucose dehydrogenase structural gene (*gdh*) is likely to be part of the σ^G regulon. Glucose dehydrogenase is a forespore-specific enzyme that becomes evident 2 to 3 h into sporulation (97, 199), with its appearance paralleling the accumulation of the *gdh* mRNA (174). The promoter of the cloned *gdh* gene resembles known σ^G-recognized promoters (215, 246, 311). E-σ^G transcribes *gdh* in vitro (215), and glucose dehydrogenase accumulation in sporulating cells is blocked in a *spoIIIG*/*sigG* null mutant (292).

spoIVB is a gene whose product is required for activation of the mother cell-specific sigma factor σ^K (49). Although it is essential for a mother cell-specific event, its expression appears to be limited to the forespore compartment, where it is induced at the engulfment stage of development (49). *spoIVB* contains a σ^G consensus promoter, is not expressed in *spoIIIG*/*sigG* mutant strains, and is induced in vegetatively growing *B. subtilis* if σ^G is present (49, 309).

spoVA is an operon with an expression pattern similar to that of genes dependent on σ^G (81). *spoVA* mutations result in the production of immature spores that are partially resistant to toluene and lysozyme but sensitive to heat and chloroform and fail to accumulate DPA in the prespore (80). The *spoVA* locus has been cloned (260, 261) and, from its sequence, is believed to encode a polycistronic mRNA including at least five genes (90). Genetic analyses have shown that *spoVA* must be expressed in the forespore if sporulation is to proceed (61, 139). Cell fractionation studies demonstrated that the product of a *spoVAA-lacZ* fusion accumulates predominantly, if not exclusively, in the forespore compartment (81). The promoter region of *spoVA* contains a consensus σ^G promoter sequence (207, 217), which is recognized by E-σ^G in vitro (217).

Germination of *B. subtilis* spores to recover the vegetative state can be induced by a variety of amino acids and sugars (reviewed in reference 206). Mutations at more than a dozen loci (*ger*) which alter the germination properties of the cells which carry them have been isolated. At least two of these *ger* loci (*gerA* and *gerD*) appear to be part of the σ^G regulon (85, 157). The *gerA* and *gerD* genes have been cloned, sequenced, and predicted to encode proteins with features found in membrane-associated proteins (86, 333). The putative promoter regions of both operons resemble σ^G-consensus promoters (85, 157). *gerA* and *gerD* are not expressed in *spoIIIG* mutant cells, are turned on in vegetatively growing *B. subtilis* by the induction of σ^G synthesis, and are transcribed by E-σ^G in vitro (85, 157).

From its known members, the σ^G regulon appears to encode products that are synthesized within the forespore compartment during the later stages of sporulation to enhance spore survival and facilitate germination.

Sigma Factor σ^K

Isolation and cloning. σ^K was detected and purified in an in vitro study of compartment-specific transcription using cloned copies of mother cell-expressed genes (*spoIVC* and *cotD*) as templates (165). RNAP partially purified from sporulating cells was shown to initiate transcription at the in vivo start sites of both *spoIVC* and *cotD* (165). DNA-cellulose chromatography separated the RNAP activities into fractions having a preference for one or the other of the two promoters. The proteins found in these samples were separated by SDS-PAGE, eluted from the gel, and tested for their ability to direct core RNAP to each of the two promoters. A single 27-kDa protein present in both peak fractions was responsible for the recognition of both the *spoIVC* and *cotD* promoters; however, it was significantly more effective in directing RNAP to *cotD* than to *spoIVC* (165). This difference in activity occurred even if the 27-kDa protein was purified from an RNAP fraction that had originally demonstrated a preference for *spoIVC*. The basis of this discrepancy was revealed when a 14-kDa protein found in the fraction that originally preferred the *spoIVC* template was shown to stimulate transcription of *spoIVC* by RNAP plus the 27-kDa protein (σ^K) (165). The 14-kDa protein proved to be a DNA-binding protein and the product of the *spoIIID* gene (167, 281). Amino-terminal sequencing of σ^K revealed that its predicted coding sequence matched that of one of the genes (*spoIVCB*) which had been used as a template for its biochemical isolation (165). *spoIVCB* is one of two separate operons, A and B, that make up the *spoIVC* locus (169). The σ^K sequence matched the predicted *spoIVCB* product sequence beginning at residue 21. This suggested that σ^K, like σ^E, is processed from a precursor (165). An additional curiosity associated with *spoIVCB* as the σ^K structural gene was the fact that its open reading frame was inadequate to encode a protein of the size of σ^K (165). This discrepancy was resolved when it was noted (286) that another sporulation locus (*spoIIIC*), whose predicted product is similar to the carboxy-terminal domain of sigma factors but lacks a recognizable sigma factor amino terminus (83), was near *spoIVC*. It was proposed that the σ^K structural gene (*sigK*) could be a composite gene formed from *spoIVCB* and *spoIIIC* (286). This notion was substantiated by Southern blot analyses in which the *spoIVCB-spoIIIC* region of the *B. subtilis* chromosome was seen to rearrange during sporulation, forming a composite *sigK* gene that could be cloned from the chromosome of sporulating cells (286).

σ^K regulation. The synthesis of σ^K is more complexly controlled than that of the other known *B. subtilis* sigma factors. It depends on a sporulation-specific rearrangement of the *B. subtilis* chromosome to form the σ^K structural gene, a highly regulated transcriptional activation to synthesize its mRNA, and a posttranslational processing reaction to convert an inactive pro-σ^K into the active sigma factor (164). This multilevel control represents a device to prevent inappropriate expression of σ^K under nonsporulation conditions, a situation which occurs if σ^K synthesis is controlled by transcription alone (221).

(i) Formation of *sigK*. *sigK* is formed by a site-specific recombination event which joins the previously separated *spoIVCB* and *spoIIIC* genes into a single cistron (286). As

determined by Southern blot analysis of fractionated mother cell and forespore chromosomes, the rearrangement occurs only in the mother cell compartment (286). It is a reciprocal recombination event in which the intervening DNA (approximately 42 kb) is deleted from the chromosome as a circle that can be detected as an extrachromosomal element on gels (168). The recombination occurs at a 5-bp sequence found in both *spoIVCB* and *spoIIIC* (286). This sequence in *spoIVCB* is followed closely by a 21-bp sequence that is present in an inverted form upstream of the same 5-bp sequence in *spoIIIC* (286). The recombinase responsible for the recombination reaction is encoded by a gene, *spoIVCA*, which lies within the excised DNA and whose transcription precedes *sigK* rearrangement (168, 257). The predicted product of *spoIVCA* has an amino terminus that is homologous to site-specific DNA recombinases (257) and binds in vitro to the recombination sites interrupting *sigK* (236). Rearrangement of *spoIVCB-spoIIIC* fails to occur in *spoIVCA* mutants, and the requirement for SpoIVCA in sporulation can be bypassed if the cell is provided with a rearranged copy of *sigK* (168). In keeping with the observation that the recombination event is dependent on the products of the *spoIIG*/*sigE* and *spoIIID* genes (286), in vitro transcription of *spoIVCA* occurs by E-σ^E and is stimulated by purified SpoIIID protein (164, 256). Although SpoIIID is likely to affect *sigK* formation by facilitating the transcription of the recombinase, a direct role for SpoIIID in the *sigK* rearrangement has not been ruled out. The dependence of the *sigK* rearrangement on E-σ^E and SpoIIID ensures that this reaction will be highly controlled. Together, these two transcription factors limit *sigK* rearrangement to the mother cell and postpone its occurrence until σ^E-dependent gene expression is well under way.

(ii) *sigK* transcription. *sigK* expression, studied by using an *spoIVCB-lacZ* fusion, is turned on between the third and fourth hour of sporulation and requires the *spoIIG* (*sigE*) and *spoIIID* gene products (169). As would be expected of a gene dependent on σ^E and SpoIIID, the β-galactosidase produced by the *spoIVCB-lacZ* fusion was enriched in extract fractions containing mother cell components (169). Besides *sigE* and *spoIIID*, *sigK* expression is also at least partially dependent on other *spo* genes, including *sigK* itself (169). A requirement for σ^K in attaining maximal *sigK* transcription was anticipated, given that the *spoIVC* (*sigK*) promoter was one of the assay templates used for the isolation of σ^K (165). *sigK* can be transcribed, in vitro, by either E-σ^E or E-σ^K (165). In vivo, *sigK* transcription is absolutely dependent on *sigE* and partially dependent on *sigK* (169). Although a requirement for E-σ^E in SpoIIID synthesis complicates the interpretation of this in vivo observation, the data suggest that in the presence of the SpoIIID, *sigK* is initially transcribed by E-σ^E and then by E-σ^K (164, 287).

(iii) Pro-σ^K processing. The σ^K amino terminus aligns with the *spoIVCB* gene at the 21st codon of *spoIVCB*'s open reading frame (165). This observation implies that σ^K, like σ^E, is synthesized as a proprotein (pro-σ^K). By using antibodies specific for pro-σ^K/σ^K, the putative pro-σ^K was detected in crude *B. subtilis* extracts, where its appearance preceded that of σ^K by approximately 1 h (i.e., pro-σ^K was first seen at 3 h into the sporulation process, while σ^K was not detected before the fourth hour) (188). The precursor form of σ^K appears to be inactive. Expression of *cotD*, which depends on σ^K for its transcription, only begins at about the time when the processed form of σ^K appears (188). In addition, the artificially induced synthesis of pro-σ^K in vegetatively growing *B. subtilis* fails to lead to the expression of a *cotD-lacZ* reporter gene until the cells proceed into sporulation and the accumulated pro-σ^K is converted into σ^K (188). In vitro, σ^K but not pro-σ^K was found to stimulate core RNAP to transcribe σ^K-dependent genes (188). The observation that pro-σ^K accumulates during sporulation and is converted into σ^K only later in development suggests that pro-σ^K processing is a developmentally regulated event distinct from pro-σ^K synthesis (188).

By using an anti-pro-σ^K/σ^K antibody as a probe, extracts of mutant *B. subtilis* strains were examined to determine the importance of various *spo* genes in the processing reaction. Mutations in eight loci (*spoIIB*, *spoIID*, *spoIIIA*, *spoIIIE*, *spoIIIG*, *spoIVA*, *spoIVB*, and *spoIVF*) permit the accumulation of pro-σ^K but block or reduce its conversion into σ^K (188). The two stage II loci that are needed for processing (*spoIIB* and *spoIID*) encode products that function relatively early in sporulation. They are hypothesized to play a role in modifying the forespore septum as a prerequisite for forespore engulfment (137, 192). Ongoing morphological development has been proposed to be an important regulatory cue for spore gene activation (51, 79, 125, 186). If this is so, then the need for these gene products in pro-σ^K processing likely reflects the failure of the cells which lack them to reach the stage of morphological development which activates the processing machinery and not an indication that their products are directly involved in the processing reaction. Three stage III genes (*spoIIIA*, *IIIE*, and *IIIG*) are needed for pro-σ^K processing. The explicit function of *spoIIIA* is unknown. From its dependence on σ^E for expression, *spoIIIA* is likely to be a mother cell-specific operon (138) and therefore could at least theoretically participate directly in pro-σ^K processing. *spoIIIE* has been better characterized. It is expressed predominantly in vegetatively growing cells (92). *spoIIIE* may play a direct, albeit undefined, role in pro-σ^K processing; however, as described above, it is required for chromosome partitioning to the forespore and the expression of *spoIIIG* (330). Thus, SpoIIIE's role in pro-σ^K processing may be indirect and limited to *spoIIIG* expression (92). *spoIIIG* encodes the forespore-specific σ factor σ^G (152, 201).

The dependence of pro-σ^K processing on σ^G argues that one or more σ^G-dependent (i.e., forespore-expressed) genes are needed for pro-σ^K processing, a circumstance that could coordinate late gene expression between the two compartments (51). The identity of one of these σ^G-dependent genes was revealed when the processing-essential *spoIVB* gene was cloned and analyzed (49, 309). From the time of appearance and disposition of an *spoIVB-lacZ* product, *spoIVB* is induced at the engulfment stage of development in the forespore compartment (49). The principal promoter of *spoIVB* has a σ^G consensus sequence (49, 309). It is not expressed in *spoIIIG*/*sigG* mutant strains, and it is induced in vegetatively growing *B. subtilis* if σ^G is provided (49). Given that *spoIVB* is transcribed in the forespore by E-σ^G, it has been proposed that the *spoIVB* product, or an event under its control, represents the factor which couples the processing of pro-σ^K to σ^G-directed gene expression in the forespore (49). Gene products through which this hypothetical coupling could occur were identified by mutations that released the expression of the σ^K-dependent *cotA* gene from σ^G-dependent gene expression (51). One of these mutations, *bofB* (bypass of forespore), permitted the processing of pro-σ^K in *spoIIIE*, *spoIIIA*, *spoIIIG*, and *spoIVB* mutant strains and was mapped to the *spoIVF* locus (51). *spoIVF* consists of a two-cistron operon (*spoIVFA* and *spoIVFB*) under the control of σ^E (52). The *bof* mutations were found in the 3′ end of the *spoIVFA* cistron. It was shown that both *spoIVF* cistrons are required for spore formation at 37°C (53). The promoter-proximal *spoIVFA* product is dispensable for sporulation at 30°C but its loss confers a Bof phenotype at this temperature (53). These findings suggested that

SpoIVFB is a thermolabile protein that instigates pro-σ^K processing but is both inhibited in its activities and stabilized to heat by SpoIVFA (53). A second σ^E-dependent gene, *bofA*/*ski-4* (140, 250), also discovered in the "bypass of forespore" mutant search (51), appears to cooperate with SpoIVFA in inhibiting the action of SpoIVFB (250). The gene products known to influence pro-σ^K processing have been assembled into a model in which SpoIVFB is thought to promote pro-σ^K processing in response to an SpoIVB-dependent signal from the forespore that releases its inhibition by SpoIVFA/BofA (49, 51). Overproduction of pro-σ^K uncouples σ^K-dependent gene expression from dependence on intercompartmental communication (189). The uncoupled pro-σ^K processing appears to be due to a low-level *spoIVF*-independent processing reaction that depends on one or more σ^E-transcribed genes. It is unknown whether this processing activity represents an additional protease or residual activity of the "normal" pro-σ^K processing enzyme.

E-σ^K-transcribed genes. The genes that rely on E-σ^K for part or all of their expression are expressed in the mother cell compartment at late times in sporulation. They include the regulatory gene *gerE* (52, 338), genes involved in DPA synthesis and accumulation (56, 76, 93), and genes for the synthesis and assembly of the spore coat (52, 54, 252, 337, 339).

gerE mutations result in spores that are germination defective and have aberrant protein coat composition and structure (88, 205). The *gerE* gene was cloned (144) and found to encode a small (74-amino-acid) protein whose synthesis is switched on after T_3 of sporulation (50). *gerE* is transcribed in vitro by E-σ^K (338). The product of a *gerE-lacZ* fusion accumulates in the mother cell compartment of sporulating cells that are able to form an active σ^K (52). *gerE* is a DNA-binding protein that stimulates the transcription of the spore coat genes *cotB*, *cotD*, and *cotC* and inhibit the transcription of *cotA* and *sigK* in vitro (338). In vivo, *gerE* is needed for the expression of *cotB* and *cotC* and full expression of *cotD*, while its absence results in overexpression of *cotA* and the operon (*dpa*) encoding DPA synthetase (52, 56, 255, 339). GerE is proposed to be a regulatory protein that establishes the pattern of late, mother cell-specific transcription (338, 339).

Six genes encoding spore coat proteins (*cotA*, *-B*, *-C*, *-D* [65], *-F* [54], and *-T* [7]) have been identified and cloned. These gene products form the protein coat which is laid down around the developing forespore by the mother cell to provide the mature spore with a protective barrier (232). Their expression has been described as a cascade, with an ordered sequential appearance of both structural and regulatory proteins (339). At least several of these *cot* genes depend on E-σ^K for their transcription, in some instances with an additional level of regulation provided by GerE (7, 255, 338, 339).

Two loci (*spoVK* and *spoVE*) involved in DPA accumulation are also transcribed by E-σ^K (56, 93). *spoVK* (*spoVJ*) has a σ^E-dependent promoter that is responsible for its initial expression but also contains a σ^K-dependent promoter which ensures its expression after T_4 (93). The *spoVE* locus, encoding two genes (*dpaA* and *dpaB*) that together specify DPA synthetase, has a σ^K consensus promoter element, is activated at T_4, when σ^K activity appears, and is silent in *B. subtilis* mutants which are incapable of synthesizing σ^K (56).

In addition to activating a number of late sporulation genes, the appearance of E-σ^K also coincides with a reduction in the expression of at least some earlier *spo* genes. E-σ^K has been hypothesized to trigger a feedback loop that decreases the level of SpoIIID, a mother cell regulatory protein of the preceding sporulation stage, and thereby reduce the transcription dependent on it (111).

CONCLUDING REMARKS

The sigma factors of *B. subtilis* illustrate the complexity to be found in prokaryotic gene regulation. Not only are the structural genes for these proteins expressed under sophisticated systems of transcriptional control; additional layers of post-translational regulation are also present. Particularly noteworthy is the use of proprotein sequences and anti-σ factor proteins to modulate σ factor activity. These devices presumably tie sigma factor activation to cues that are less readily joined to conventional systems of transcriptional or translational regulation. Posttranslational controls on sigma factor activity are most evident during *B. subtilis* sporulation, the process in which multiple σ factors were first discovered. At several stages of the spore-forming process, the activation of preexisting σ factors appears to couple *spo* gene induction to the cell's ongoing morphological development, not only regulating temporal and compartment-specific gene expression but also coordinating gene expression between the mother cell and forespore compartments.

The specific use of either a proprotein sequence or an anti-σ factor as the σ factor's negative regulator has distinct consequences once the sigma factor is activated. Sigma factor activation by the removal of the proprotein sequence is irreversible, while activation by the release of a binding protein could, at least theoretically, be reversed by the reassociation of the σ factor with its regulator. It is probably not a coincidence that the two σ factors which are "permanently activated" by the removal of a pro sequence are either sporulation-specific sigma factors that are short-lived (σ^E) or the terminal σ factor (σ^K) of a cell compartment that is destined to lyse. Silencing of genes dependent on these sigma factors necessitates either degradation of the σ factor or destruction of the cell in which it is active. This lack of flexibility may be useful in committing a terminally differentiating cell to provide spore-essential products in the face of changing environmental signals but is likely unsuitable for processes that must remain responsive to change. In contrast, anti-σ factors, with their capacity to reversibly modulate σ factor activity, would be expected to have the flexibility needed to regulate dynamic processes. The *B. subtilis* σ factors (σ^B, σ^F, and σ^G) that are inhibited by anti-σ factors are found in vegetatively growing cells or the spore compartment, which regenerates to form a new vegetative cell.

Anti-σ factors may prove to be common regulators of σ factor activity. In addition to σ^B, σ^F, and σ^G, it is possible that the sporulation-induced inhibitor of the principal *B. subtilis* σ factor (σ^A) (266) is also a protein of this class. If σ^A does have a corresponding anti-σ factor, it would be a strong argument for wide-ranging control by this species of regulator in *Bacillus* gene expression. Given that an anti-σ factor has also been observed in *Salmonella typhimurium*, where it controls a motility sigma factor (220), it would not be surprising to find that anti-σ factors, like multiple σ factors themselves, are ubiquitous among procaryotes. Anti-σ factors and their corresponding anti-anti-σ factors may well become the next two-component system (282) of bacterial gene regulation.

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