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# A Second PDZ-Containing Serine Protease Contributes to Activation of the Sporulation Transcription Factor $\sigma^K$ in *Bacillus subtilis*

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**Gene expression late during the process of sporulation in *Bacillus subtilis* is governed by a multistep, signal transduction pathway involving the transcription factor $\sigma^K$, which is derived by regulated proteolysis from the inactive proprotein pro-$\sigma^K$. Processing of pro-$\sigma^K$ is triggered by a signaling protein known as SpoIVB, a serine protease that contains a region with similarity to the PDZ family of protein-protein interaction domains. Here we report the discovery of a second PDZ-containing serine protease called CtpB that contributes to the activation of the pro-$\sigma^K$ processing pathway. CtpB is a sporulation-specific, carboxyl-terminal processing protease and shares several features with SpoIVB. We propose that CtpB acts to fine-tune the regulation of pro-$\sigma^K$ processing, and we discuss possible models by which CtpB influences the $\sigma^K$ activation pathway.**

Sporulation by the gram-positive bacterium *Bacillus subtilis* is a highly coordinated process, involving multiple pathways of intercellular signaling (for recent reviews, see references 19 and 27). Upon commitment to sporulate, the developing cell (the sporangium) divides asymmetrically to generate compartments of unequal size. The larger compartment is known as the mother cell, and the smaller one is referred to as the forespore (the prospective spore). Initially the mother cell and the forespore lie adjacent to each other, but as sporulation progresses the mother cell engulfs the forespore in a phagocytosis-like process, generating a free protoplast within the mother cell. As a result of engulfment, the forespore is surrounded by two membranes, the inner forespore membrane and the mother-cell membrane that surrounds the forespore, which is known as the outer forespore membrane. Throughout the course of development the forespore and the mother cell communicate with each other to ensure that gene expression in one compartment is coordinated with gene expression in the other. Four sporulation-specific RNA polymerase sigma factors are activated in a spatially and temporally restricted fashion. Shortly after asymmetric division the transcription factor $\sigma^F$ is activated selectively in the forespore. The $\sigma^F$ factor is then responsible for the activation of $\sigma^E$ in the mother cell. At a later stage, $\sigma^E$ is required for the activation of $\sigma^G$ in the engulfed forespore. Finally, $\sigma^G$ sets in motion a chain of events that triggers the activation of $\sigma^K$ in the mother cell. While the overall pathway of these intercompartmental signal transduction pathways has been elucidated (at least in the cases of the pathways governing the activation of $\sigma^E$ and $\sigma^K$), many of the mechanistic details concerning how $\sigma$ factor activation is achieved are unknown.

The late-appearing, mother-cell-specific transcription factor $\sigma^K$ is synthesized as an inactive precursor protein known as pro-$\sigma^K$, with a 20-residue inhibitory extension at its N terminus (5, 18). A signaling protein (SpoIVB) synthesized in the forespore under the control of $\sigma^G$ triggers the proteolytic activation of $\sigma^K$ (see Fig. 1B) (4). The pro-$\sigma^K$ processing enzyme is likely to be the membrane-embedded metalloprotease SpoIVFB (not to be confused with the similarly named signaling protein SpoIVB), which is synthesized in the mother cell and localizes to the outer forespore membrane. SpoIVFB is held inactive in a multimeric membrane complex by two other proteins that are synthesized in the mother cell, SpoIVFA and BofA. SpoIVFA anchors the pro-$\sigma^K$ processing complex in the outer forespore membrane and is thought to serve as a platform for bringing the processing enzyme SpoIVFB into proximity with the inhibitor protein BofA (6, 10, 20, 22, 29–34, 42). Inhibition imposed on SpoIVFB by BofA and SpoIVFA is relieved by the SpoIVB signaling protein, which is believed to be secreted from the forespore into the space between the inner and outer forespore membranes (9, 38).

The SpoIVB signaling protein has three distinct domains (Fig. 1A). Near the extreme N terminus is a hydrophobic sequence that probably facilitates secretion of SpoIVB across the inner forespore membrane (38). The middle region of SpoIVB contains a PDZ domain, a modular protein-protein interaction domain implicated in protein targeting and complex assembly (14, 15). Near its C terminus SpoIVB contains a serine protease domain of the PA(S) clan (13, 28). It has recently been shown that SpoIVB has serine peptidase activity and that the protease activity is necessary for its signaling function in vivo (13). SpoIVB undergoes self-cleavage (and may also be cleaved by additional unidentified proteases) at sites close to its N terminus to produce multiple proteolytic products. Autoproteolysis of SpoIVB may facilitate its release from the inner forespore membrane into the intermembrane space, and it has also been proposed that the signaling-active form is among the cleavage products (38).

Interestingly, the overall domain organization of the SpoIVB signaling molecule is very similar to that of the carboxyl-terminal processing proteases (or tail-specific proteases), which are conserved in many gram-negative and gram-positive bacteria and in chloroplasts and mitochondria from eukaryotes (25, 35, 36). Members of the Ctp protease family contain an N-terminal secretion signal, followed by a PDZ domain and a

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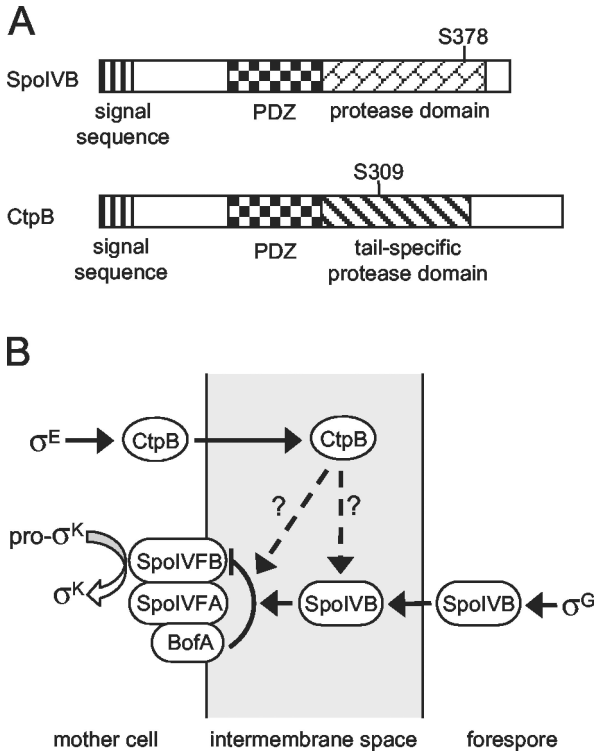


FIG. 1. (A) Anatomy of SpoIVB and CtpB. Conserved domains are represented by shaded boxes, and the putative catalytic serine residue in each protein is indicated. (B) Model for the regulation of pro-$\sigma^K$ processing. The forespore signaling molecule SpoIVB is secreted into the intermembrane space, where it relieves inhibition imposed on the putative pro-$\sigma^K$ processing enzyme SpoIVFB by BofA and SpoIVFA. We hypothesize that the mother-cell-synthesized CtpB protein is also secreted into the intermembrane space, where it regulates pro-$\sigma^K$ processing. CtpB could act directly on the pro-$\sigma^K$ processing complex to enhance the relief of inhibition, or it could affect pro-$\sigma^K$ processing indirectly by positively regulating SpoIVB activity. The two possible models are indicated by the dashed arrows. We have previously suggested that integral membrane proteins in the mother cell are not inserted into the engulfing septal membrane. Rather, according to our model, they are inserted into the cytoplasmic membrane and then reach the engulfing membrane by diffusion (34). We do not know whether this would be true for secreted proteins, but for purposes of simplicity we have depicted CtpB as being secreted directly across the engulfing septal membrane.

tail-specific serine protease domain (Fig. 1A) (21). It has been demonstrated that one of the Ctp family members, *Escherichia coli* Tsp, utilizes its PDZ domain to selectively bind to the nonpolar C termini of its substrates (3).

In this study, we describe a sporulation-specific carboxyl-terminal processing protease in *B. subtilis* named CtpB. We show that CtpB is synthesized in the mother-cell compartment under the control of $\sigma^E$ and probably functions to fine-tune the signal transduction pathway that controls pro-$\sigma^K$ processing.

## MATERIALS AND METHODS

**General methods.** All *B. subtilis* strains (Table 1) are derived from the prototrophic strain PY79 (41). The *E. coli* strain used was DH5α. *B. subtilis* cells were induced to sporulate by resuspension in Sterlini-Mandelstam medium for β-galactosidase activity assays and immunoblot analysis as described previously (33). Sporulation efficiency was determined by heat resistance (80°C for 20 min) from 27-h cultures sporulated by exhaustion in Difco sporulation medium (12).

TABLE 1. *B. subtilis* strains

| Strain | Relevant genotype |
|---|---|
| PY79 | Prototrophic |
| RL813 | *amyE*::*spoIID-lacZ cat* |
| RL832 | *spoIIIGΔ1 spoIIIG-lacZ cat* |
| QPB159 | Δ*ctpA*::*tet* |
| QPB160 | Δ*ctpA*::*tet spoIIIGΔ1 spoIIIG-lacZ cat* |
| QPB161 | Δ*ctpB*::*tet* |
| QPB162 | Δ*ctpB*::*tet spoIIIGΔ1 spoIIIG-lacZ cat* |
| QPB170 | Δ*ctpA*::*cat* Δ*ctpB*::*tet* |
| QPB179 | Δ*ctpA*::*tet* Δ*ctpB*::*erm spoIIIGΔ1 spoIIIG-lacZ cat* |
| QPB203 | Δ*ctpA*::*cat* Δ*ctpB*::*tet thrC*::*cotD-lacZ erm* |
| QPB209 | Δ*ctpA*::*tet thrC*::*cotD-lacZ erm* |
| QPB210 | Δ*ctpB*::*tet thrC*::*cotD-lacZ erm* |
| QPB211 | *thrC*::*cotD-lacZ erm* |
| QPB212 | Δ*ctpA*::*tet amyE*::*sspB-lacZ cat* |
| QPB213 | Δ*ctpB*::*tet amyE*::*sspB-lacZ cat* |
| QPB214 | *amyE*::*sspB-lacZ cat* |
| QPB228 | Δ*ctpA*::*tet* Δ*ctpB*::*erm amyE*::*sspB-lacZ cat* |
| QPB613 | Δ*bofA*::*neo thrC*::*cotD-lacZ erm* |
| QPB615 | Δ*bofA*::*neo* Δ*ctpA*::*cat* Δ*ctpB*::*tet thrC*::*cotD-lacZ erm* |
| QPB675 | *amyE*::$P_{ctpA}$-*lacZ cat* |
| QPB679 | *amyE*::$P_{ctpB}$-*lacZ cat* |
| QPB680 | *spoIIIGΔ1 amyE*::$P_{ctpB}$-*lacZ cat* |
| QPB682 | *spoIIAC*::*kan amyE*::$P_{ctpB}$-*lacZ cat* |
| QPB683 | *spoIIGB*::*erm amyE*::$P_{ctpB}$-*lacZ cat* |
| QPB749 | Δ*ctpB*::*tet amyE*::*ctpB spec thrC*::*cotD-lacZ erm* |
| QPB750 | Δ*ctpB*::*tet amyE*::*ctpB-S309A spec thrC*::*cotD-lacZ erm* |
| QPB776 | Δ*ctpA*::*tet amyE*::*spoIID-lacZ cat* |
| QPB777 | Δ*ctpB*::*tet amyE*::*spoIID-lacZ cat* |
| QPB778 | Δ*ctpA*::*tet* Δ*ctpB*::*erm amyE*::*spoIID-lacZ cat* |

**Strain and plasmid construction.** To construct *ctpA* and *ctpB* transcriptional *lacZ* fusions, QPO275 (ggcAAGCTTcagcttcgataccaatcagca; uppercase letters indicate the restriction; endonuclease site) and QPO276 (aatcGGATCCtcttcttaca ttattatcatggacg) were used to amplify the promoter region of *ctpA* by PCR, and QPO278 (atgGAATTCgtttaaagtggtcggcgtttc) and QPO279 (aatcGGATCCgtatg ccagactgtctttacc) were used for the amplification of the promoter region of *ctpB*. $P_{ctpA}$ and $P_{ctpB}$ were digested with *Hin*dIII/*Bam*HI and *Eco*RI/*Bam*HI, respectively, and cloned into pDG1661 (11) to generate pQP116 and pQP117. The resulting plasmids were linearized and transformed into PY79 to create QPB675 and QPB679. To construct the *ctpB* complementation plasmid, *ctpB* and its promoter region were amplified with QPO278 and QPO376 (atcGGATCCgcac aaagaaacaggagatgaa). The PCR product was digested with *Eco*RI/*Bam*HI and cloned into pDG1730 (11) to produce pQP169. To construct the S309A missense mutation in *ctpB*, QPO377 (ggataaaggaagtgccgctgcatcagaaattcttg), QPO378 (ca agaatttctgatgcagcggcacttccttttatcc), and pQP169 were used for site-directed mutagenesis (26) to generate pQP169-S309A. pQP169 and pQP169-S309A were linearized and transformed into QPB210 to produce QPB749 and QPB750, respectively. The *ctpA* and *ctpB* deletion mutants were created by the long flanking homology PCR method as described previously (26). The sequences of the oligonucleotide primers used to generate the deletions are available upon request.

## RESULTS

***ctpB* encodes a protein homologous to carboxyl-terminal processing proteases.** In the course of our search for the protease responsible for the degradation of the anti-$\sigma^F$ factor SpoIIAB (26), we discovered that a deletion mutation in the *yvjB* gene caused a mild but reproducible reduction in sporulation efficiency (46% ± 6% compared to wild type; also see Table 2). Sequence alignment shows that the *yvjB* gene encodes a protein of 480 amino acids that shares extensive sequence similarity with members of the carboxyl-terminal processing protease family, including *E. coli* Tsp (29% identity, 46% similarity) and *Synechocystis* CtpA (33% identity, 57%

TABLE 2. Sporulation efficiency[a]

| Strain | CFU ($10^8$) | | Spores[d] ($10^8$) | Sporulation efficiency[e] (%) |
|---|---|---|---|---|
| | $T_0$[b] | $T_{24}$[c] | | |
| wt[f] | 7.6 | 6 | 5.6 | |
| Δ*ctpA* | 7.4 | 6.6 | 4.4 | 79 ± 15 |
| Δ*ctpB* | 7.4 | 3.9 | 2.6 | 46 ± 6 |
| Δ*ctpA* Δ*ctpB* | 8 | 3.1 | 2 | 36 ± 6 |

[a] The averages of three independent sporulation assays are shown. Strains used were PY79, QPB159, QPB161, and QPB170. See Table 1 for a description of the strains used in this study.
[b] The number of CFU at 0 h of sporulation ($T_0$).
[c] The number of CFU at 24 h of sporulation ($T_{24}$). The reduction in CFU of the Δ*ctpB* and Δ*ctpA* Δ*ctpB* mutants is likely due to the lysis of cells that failed to form spores.
[d] Heat-resistant (80°C for 20 min) CFU.
[e] The number of spores compared to the number of wild-type spores.
[f] wt, wild type.

similarity) (35, 36). *yvjB* is one of two genes in *B. subtilis* predicted to encode a carboxyl-terminal processing protease. One has been described previously and is called *ctpA* (23). Therefore, we have renamed *yvjB* as *ctpB*. *B. subtilis* CtpA and CtpB share 42% identity and 64% similarity in their amino acid sequences. Interestingly, a *ctpA* deletion mutant did not affect sporulation significantly (Table 2), suggesting that CtpA and CtpB perform separate functions in *B. subtilis*.

**Expression of *ctpB* depends on the mother-cell-specific $\sigma^E$ factor.** To determine whether *ctpB* is under sporulation control, we monitored the expression of *ctpB* by using a *ctpB-lacZ* transcriptional fusion. *ctpB* was not expressed during vegetative growth (data not shown) but was induced 2 h after the initiation of sporulation, with maximal expression at 3 h (Fig. 2, upper panel). For comparison, we also examined the expression of *ctpA* with a *ctpA-lacZ* transcriptional fusion. *ctpA* was expressed in vegetatively growing cells and appeared to be shut off upon entry into sporulation (Fig. 2, upper panel, and data not shown).

To investigate which sporulation-specific sigma factor was required for *ctpB* transcription, we examined *ctpB-lacZ* expression in mutants of different sigma factors. *ctpB-lacZ* expression required both the early-acting, forespore-specific transcription factor $\sigma^F$ and the early-acting, mother-cell-specific factor $\sigma^E$ (Fig. 2, lower panel). A mutation in the late-appearing forespore-specific factor $\sigma^G$ did not affect *ctpB* expression. Because $\sigma^F$ is required for the activation of $\sigma^E$, these results suggest that *ctpB* is under $\sigma^E$ control in the mother cell and that the effect of the $\sigma^F$ mutant is indirect. To investigate whether *ctpB* is indeed expressed in the mother cell, we visualized *ctpB* expression by fluorescence microscopy by using a transcriptional fusion of the gene (*gfp*) for the green fluorescent protein to the promoter of *ctpB*. The results showed that fluorescence was confined to the mother cell (data not shown). Taken together, these results indicate that *ctpB* expression is under the control of $\sigma^E$. Consistent with our observations, *ctpB* was found to be in the $\sigma^E$ regulon by DNA microarray analysis (the ratio of *ctpB* mRNA levels in the presence and absence of $\sigma^E$ was ~2.7) (8). Moreover, an examination of the *ctpB* promoter region identified a sequence [acatgaa$(n)_{14}$catatact] which is similar to the consensus promoter sequence recognized by $\sigma^E$ [*nn*at*nnn*$(n)_{14}$cata*nnn*t] (8).

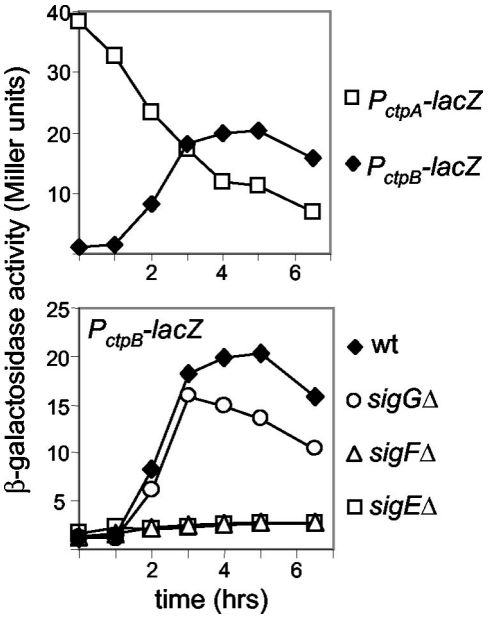


FIG. 2. Analysis of *ctpB* expression during sporulation. Cells containing transcriptional *lacZ* fusions to the promoters of *ctpA* or *ctpB* were induced to sporulate, and samples were analyzed for β-galactosidase activity at the indicated times after the initiation of sporulation. Strains used were QPB675 and QPB679 (top panel) and QPB679, QPB680, QPB682, and QPB683 (bottom panel). wt, wild type.

**CtpB is required for efficient activation of the mother-cell transcription factor $\sigma^K$.** To determine at what stage CtpB activity was influencing sporulation, we examined the activities of the four compartment-specific sigma factors in a Δ*ctpB* mutant. The expression levels of a $\sigma^F$-dependent (*spoIIIG*), a $\sigma^E$-dependent (*spoIID*), and a $\sigma^G$-dependent (*sspB*) promoter fused to *lacZ* were all unaffected by the Δ*ctpB* mutation (or by Δ*ctpA* or Δ*ctpA* Δ*ctpB*) (Fig. 3A to C). However, the expression of a $\sigma^K$-dependent (*cotD*) promoter fused to *lacZ* was delayed by about 1 h in the Δ*ctpB* mutant (Fig. 3D and 4B). Although the onset of *cotD-lacZ* expression was delayed in the Δ*ctpB* mutant, at later times the transcriptional fusion reached similar expression levels as those observed in the wild type (Fig. 4B). Thus, these results indicate that CtpB is required for efficient activation of the mother-cell transcription factor $\sigma^K$.

**The active-site serine is required for the proper function of CtpB.** Carboxyl-terminal processing proteases are members of the SM clan of serine peptidases (28) that exhibit a serine-lysine catalytic dyad (17, 21). Serine 309 of *B. subtilis* CtpB corresponds to the active-site serine 430 of *E. coli* Tsp. To test whether serine protease activity is required for CtpB function during sporulation, we generated a *ctpB* mutant in which the putative catalytic-site serine residue was replaced with an alanine. We introduced either a wild-type *ctpB* gene or the *ctpB-S309A* gene into *B. subtilis* at a nonessential locus in the Δ*ctpB* mutant. The wild-type *ctpB* gene, but not *ctpB-S309A*, was able to restore efficient $P_{cotD}$-*lacZ* expression and sporulation to the Δ*ctpB* mutant (Fig. 4A and data not shown). These results indicate that the proteolytic activity of CtpB is required for efficient activation of $\sigma^K$ during sporulation.

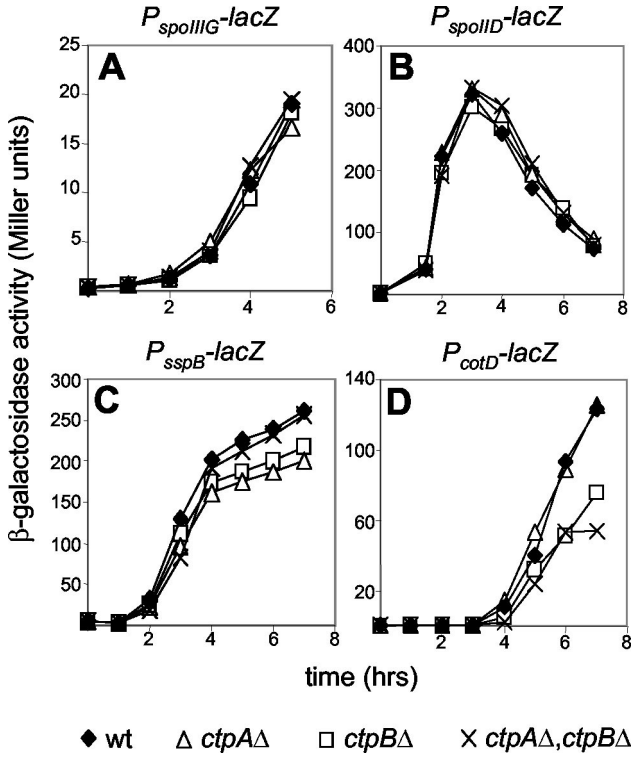


FIG. 3. Compartment-specific sigma factor activity in the absence of CtpB. Cells containing transcriptional *lacZ* fusions to the indicated promoters were induced to sporulate, and samples were analyzed for β-galactosidase activity at the indicated times after the initiation of sporulation. (A) Strains (RL832, QPB160, QPB162, and QPB179) containing the $\sigma^F$ activity reporter $P_{spoIIIG}$-*lacZ*. (B) Strains (RL813, QPB776, QPB777, and QPB778) containing the $\sigma^E$ activity reporter $P_{spoIID}$-*lacZ*. (C) Strains (QPB214, QPB212, QPB213, and QPB228) containing the $\sigma^G$ activity reporter $P_{sspB}$-*lacZ*. (D) Strains (QPB211, QPB209, QPB210, and QPB203) containing the $\sigma^K$ activity reporter $P_{cotD}$-*lacZ*. wt, wild type.

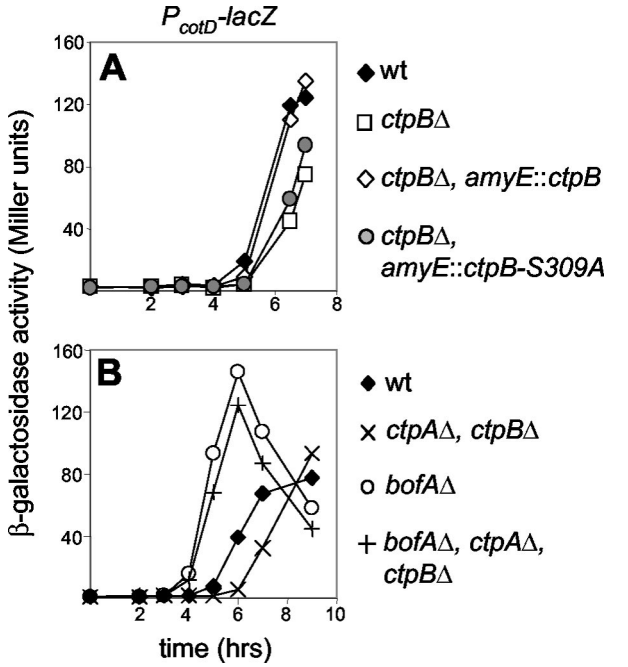


FIG. 4. Requirement for CtpB in $\sigma^K$ activation. Cells containing the $\sigma^K$ activity reporter $P_{cotD}$-*lacZ* were induced to sporulate, and samples were analyzed for β-galactosidase activity at the indicated times after the initiation of sporulation. (A) The putative catalytic serine residue in CtpB is required for efficient activation of $\sigma^K$. Strains used were QPB211, QPB210, QPB749, and QPB750. (B) The delay in $\sigma^K$ activation caused by Δ*ctpB* can be suppressed by a Δ*bofA* mutant. Strains used were QPB211, QPB203, QPB613, and QPB615. wt, wild type.

**CtpB is required for efficient signaling in the pro-$\sigma^K$ processing pathway.** $\sigma^K$ activity is controlled at the level of proteolytic cleavage of an inactive precursor (pro-$\sigma^K$). The putative pro-$\sigma^K$ processing enzyme SpoIVFB is held inactive by BofA and SpoIVFA until a signal is received from the forespore (33). The forespore signaling protein SpoIVB is predicted to be secreted into the intermembrane space between the mother cell and forespore where it somehow relieves inhibition imposed on SpoIVFB by BofA (38). Since the CtpB mutant delayed the timing of $\sigma^K$ activity (Fig. 4B) and the overall organization of the CtpB protein is very similar to that of SpoIVB (Fig. 1A), we hypothesized that CtpB might play a role in this signal transduction pathway. To test this, we analyzed whether a Δ*bofA* mutant could bypass the delay in $\sigma^K$ activity observed in the Δ*ctpB* mutant. Consistent with the idea that CtpB acts in this signaling pathway to promote efficient pro-$\sigma^K$ processing, premature $\sigma^K$ activity was observed in the Δ*ctpB* mutant when *bofA* was mutated (Fig. 4B).

Next we examined pro-$\sigma^K$ processing directly in the Δ*ctpB* mutant. Wild-type and Δ*ctpB* cells were induced to sporulate and pro-$\sigma^K$ processing was assessed by immunoblot analysis. In wild-type cells, mature $\sigma^K$ appeared at 220 min after the start of sporulation, and >80% of pro-$\sigma^K$ had been processed by 280 min (Fig. 5). In the Δ*ctpB* mutant, the appearance of mature $\sigma^K$ was delayed by about 1 h. These data indicate that the CtpB is required for timely and efficient processing of pro-$\sigma^K$.

## DISCUSSION

Activation of the mother-cell-specific transcription factor $\sigma^K$ is controlled by an intricate signal transduction pathway that operates at the level of proteolytic processing of an inactive precursor protein. The pro-$\sigma^K$ processing enzyme is held inactive in a multimeric complex in the outer forespore membrane until a signal is received from the forespore compartment. This signal triggers relief of inhibition imposed on the

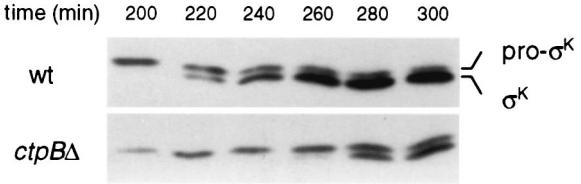


FIG. 5. Pro-$\sigma^K$ processing is delayed in a Δ*ctpB* mutant. The figure shows results of immunoblot analysis of unprocessed and mature $\sigma^K$ in wild-type (wt; PY79) and Δ*ctpB* (QPB161) sporulating cells at indicated times after the initiation of sporulation.

processing enzyme, resulting in proteolytic activation of $\sigma^K$. In this study, we present evidence that the carboxyl-terminal processing protease CtpB acts in this signaling pathway to fine-tune the timing of $\sigma^K$ activation.

The forespore signaling protein SpoIVB probably acts directly on the multimeric membrane complex. However, the mechanism by which it triggers pro-$\sigma^K$ processing remains unknown. An attractive model is that, upon secretion into the intermembrane space, SpoIVB binds to BofA or SpoIVFA through its PDZ domain and then utilizes its serine protease domain to cleave one or both of these proteins to relieve the inhibition imposed on the processing enzyme SpoIVFB (13, 14).

The newly discovered CtpB protein shares several intriguing similarities with the SpoIVB signaling protein. They have similar domain structures, are both predicted to be secreted into the intermembrane space, and both regulate pro-$\sigma^K$ processing (Fig. 1). Unlike the SpoIVB protein, which is secreted from the forespore chamber and is essential for intercompartmental signaling, CtpB, if it is secreted, comes from the mother-cell compartment and modulates the signaling back to the mother cell. CtpB could act directly on the pro-$\sigma^K$ processing complex by enhancing relief of inhibition. For example, CtpB could cleave SpoIVFA or BofA after the SpoIVB signal has been received. Alternatively, CtpB could regulate the pro-$\sigma^K$ processing indirectly by enhancing the activity of SpoIVB. SpoIVB exists as multiple proteolytic products derived from autoproteolysis and perhaps cleavage by other proteases. The signaling-active form of SpoIVB has been proposed to be among these cleavage products (38). Consistent with the idea that CtpB might act through SpoIVB by generating one of the signaling-active forms, the appearance of some of the SpoIVB proteolytic products was delayed in a CtpB mutant (data not shown). There is precedent for fine-tuning the pro-$\sigma^K$ processing pathway through the regulation of SpoIVB. The forespore protein BofC has been shown to delay $\sigma^K$ activation, probably by binding to SpoIVB and inhibiting its activity (37). Our data suggest that CtpB acts in the opposite direction, enhancing $\sigma^K$ activation perhaps by facilitating the generation of one or more of the signaling-active forms of SpoIVB.

The pathway controlling pro-$\sigma^K$ processing has features in common with the pathway governing the activation of $\sigma^E$ of *E. coli* (not to be confused with the unrelated *B. subtilis* sigma factor of the same name), a member of the ECF family of sigma factors (24, 40). *E. coli* $\sigma^E$ is a stress-response transcription factor that directs the expression of genes under its control in response to the accumulation of unfolded or misfolded proteins in the periplasm (7). In unstressed cells, $\sigma^E$ is held inactive by an integral membrane protein, the anti-sigma factor RseA, which presumably tethers $\sigma^E$ to the cytoplasmic membrane and prevents it from associating with core RNA polymerase. The $\sigma^E$ factor is released from the membrane through inactivation of RseA by means of two sequential proteolytic cleavage events (1, 2, 16). The first cleavage occurs in the region of RseA that projects into the periplasm and is mediated by a serine protease, DegS, which senses unfolded periplasmic proteins through its PDZ domain (39). The DegS-mediated cleavage renders RseA susceptible to a second intramembrane cleavage that is mediated by a putative membrane-embedded metalloprotease that is significantly similar to the SpoIVFB protease of the pro-$\sigma^K$ processing pathway. Thus, activation of both $\sigma^E$ in *E. coli* and $\sigma^K$ in *B. subtilis* involves PDZ-containing serine proteases (two in the case of $\sigma^K$ as we have seen) and related membrane-embedded proteases. Nonetheless, and interestingly, the logic of the two regulatory systems is different. In the case of $\sigma^E$, proteolysis leads to the destruction of an anti-sigma factor, whereas in the case of $\sigma^K$ the serine proteases act (presumably) on components of the signal transduction pathway and the membrane-embedded protease activates $\sigma^K$ directly by separating it from an inhibitory extension at its N terminus. It will be interesting to compare and contrast both systems as further mechanistic insights emerge into the activation of the stress-response regulatory protein $\sigma^E$ and the developmental transcription factor $\sigma^K$.

## ACKNOWLEDGMENTS

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