Supplemental Data

A Branched Pathway Governing the Activation of a Developmental Transcription Factor by Regulated Intramembrane Proteolysis

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Supplemental Experimental Procedures

Expression, Purification, and Reconstitution of IVB Protease Activity In Vitro

Expression of the catalytically active IVB protease in *E. coli* is toxic and results in production of very low levels of IVB protein (Wakeley et al., 2000). For this reason, all previous biochemical investigations of the signaling protease have been performed using IVB protein derived from in vitro transcription-translation reactions. To investigate how IVB regulates pro-σ^K processing, we decided to revisit production of the protein in *E. coli*. Using anti-IVB antibodies raised against a catalytic mutant of IVB (IVB^{S378A}), we monitored expression of wild-type IVB after induction in *E. coli*. We discovered that after 20 minutes of induction, some full-length IVB could be detected and over time, this protein was rapidly proteolyzed to smaller forms (Fig. S1A). The instability of IVB is probably due to autoproteolysis because the catalytic mutant was stable and produced at high levels (data not shown). In order to purify enough protein for biochemical characterization, 4 L of culture was induced for 20 minutes. Upon Ni^{2+}-agarose purification, we obtained 50-60µg of three proteolytic fragments of the IVB protease (Fig. S1B). As a negative control for our biochemical analysis, we performed a "mock purification" from 4 L of *E. coli* containing the empty expression plasmid (Fig. S1B).

It has been shown previously, using in vitro transcribed-translated protein that IVB is capable of cleaving itself (in *cis*) and cleaving a catalytically inactive mutant IVB^{S378A} in *trans* (Wakeley et al., 2000). To test *cis*-cleavage activity, IVB was incubated at room temperature over a 6 hour time course. The cleavage products were resolved by SDS-PAGE and detected by Coomassie staining. Consistent with the idea that IVB has autoproteolytic activity, the three IVB products sequentially collapsed to a single species over time (Fig. S1C). To determine whether IVB could cleave itself in *trans*, we incubated the purified protease with the catalytic mutant IVB^{S378A}. The purified IVB efficiently cleaved the catalytic mutant over the 4 hour time course (Fig. S1D). Moreover, the IVB^{S378A} cleavage product was similar in size to the one observed using in vitro transcription-translation reactions (Wakeley et al., 2000). Importantly, the cleavage product was not detected when IVB^{S378A} was incubated with the mock-purified protein or with buffer alone (Fig. S1D). Taken together, these results indicate that our purified

IVB protein is catalytically active and has similar activities to those that have been described previously.

Plasmid Construction

The plasmids used in this study are listed in Table S3.

pKM35 [*Δss-IVB-his*$_6$] was generated in a two-way ligation with a *Nco*I-*Hind*III PCR product containing the *IVB* gene without its signal sequence (oligonucleotide primers oDR303 and oDR307 and template DNA pVO57 (Oke et al., 1997)) and pET28a(+) (Novagen) cut with *Nco*I and *Hind*III.

pDT73 [*his*$_6$-*A*$_{EC}$] was generated by inserting a *Bam*HI-*Xho*I fragment from pDR171 (Doan et al., 2005) encoding the extracellular domain of A into pET28a(+) (Novagen) between *Bam*HI and *Xho*I.

pQP51-1 [*Δss-IVB*^{S378A}*-his*$_6$] was generated in a two-way ligation with a *Nco*I-*Hind*III PCR product containing the *IVB*^{S378A} gene without its signal sequence (oligonucleotide primers oDR303 and oDR307 and template DNA pQP50-1) and pET28a(+) (Novagen) cut with *Nco*I and *Hind*III. pQP50-1 was generated by introducing the catalytic mutant S378A into the gene encoding *IVB* by site directed mutagenesis in pVO57 (Oke et al., 1997).

pDR211 [*Δss-ctpB-his*$_6$] was generated in a two-way ligation with an *Nde*I-*Sal*I PCR product containing the *ctpB* gene without its putative signal sequence (oligonucleotide primers oDR400 and oDR401 and genomic DNA as template) and pET24b(+) (Novagen) cut with *Nde*I and *Xho*I.

pDR212 [*Δss-ctpB*^{S309A}*-his*$_6$] was generated in a two-way ligation with an *Nde*I-*Sal*I PCR product containing the *ctpB*^{S309A} gene without its putative signal sequence (oligonucleotide primers oDR400 and oDR401 and pQP169-S309A (Pan et al., 2003) as template) and pET24b(+) (Novagen) cut with *Nde*I and *Xho*I.

pNC1 [*amyE::P*$_{spoIVF}$*-A (spec)*] was generated in a two-way ligation with an *Eco*RI-*Bam*HI PCR product containing the *A* gene (oligonucleotide primers oDR69 and oNC3 and template DNA pSC224 (Cutting et al., 1991)), and pDG1730 (Guerout-Fleury et al., 1996) cut with *Eco*RI and *Bam*HI.

pNC8 [*lacA*::*P*$_{spoIVF}$*-B (erm)*] was created in a three-way ligation with an *Eco*RI-*Hind*III PCR product containing the *spoIVF* promoter (oligonucleotide primers oDR67 and oDR68 and template DNA pSC224 (Cutting et al., 1991)), a *Hind*III-*Bam*HI PCR product containing the *B* gene with the *spoIVFA* RBS and an ATG start codon (oligonucleotide primers oNC2 and oNC5 and template DNA pSC224), and pDR183 (Doan et al., 2005) cut with *Bam*HI and *Eco*RI.

pNC52 [*lacA::P*$_{spoIVF}$*-B-gfp (erm)*] was created in a two-way ligation with a *Bam*HI-*Sph*I fragment from pDR91 (Rudner and Losick, 2002) encoding the C-terminal domain of B fused to GFP and pNC8 cut with *Bam*HI and *Sph*I.

pNC12 [*bofA::tet*] was created in a two-way ligation with an *Xba*I-*Bam*HI PCR product containing the downstream chromosomal region of the *bofA* gene (oligonucleotide primers oNC13 and oNC14 and genomic DNA from PY79 as a template), and pNC11 cut with *Xba*I and *Bam*HI. pNC11 was created by ligating an *Xho*I-*Eco*RI PCR product containing the downstream chromosomal region of the *bofA* gene (oligonucleotide primers oNC11 and oNC12 and genomic DNA from strain PY79 as a template), into pDG1515 (Guerout-Fleury et al., 1995) between *Xho*I and *Eco*RI.

pNC59 [*IVB::phleo*] was created in a two-way ligation with a *Pst*I-*Sal*I PCR product containing the upstream chromosomal region of the *IVB* gene (oligonucleotide primers oNC39 and oNC40 and genomic DNA from strain PY79 as a template), and pNC54 cut with *Pst*I and *Sal*I. pNC54 was created in a two-way ligation with an *Eco*RI-*Xba*I PCR product containing the downstream chromosomal region of the *IVB* gene (oligonucleotide primers oNC41 and oNC44 and genomic DNA from strain PY79 as a template), to pKM80 (K. Marquis and D.Z.R., unpublished) cut with *Eco*RI and *Xba*I. pKM80 is a plasmid designed to make insertion-deletions with the phleomycin resistance gene.

Purification of IVB-His$_6$

The *IVB* gene lacking its signal sequence was fused to a C-terminal His$_6$-tag (pKM35) and expressed in *E. coli* BL21 DE3 pLysS. Cells were grown in 4 L LB at 37˚C to an OD_{600} of 0.9 and induced by the addition of IPTG to 1 mM, and harvested after 20 minutes. All subsequent manipulations were carried out at 4˚C. Cells were harvested by centrifugation and resuspended in 1/100th volume buffer I (50 mM Tris-HCl pH 8, 1 M NaCl, 5 mM 2-Mercaptoethanol, 10 mM Imidazole). A crude extract was prepared by freeze-thawing the cells followed by the addition of Lysozyme (250 μg/ml) and sonication. A soluble fraction was made by 100,000X g spin and was loaded on a 1ml Ni^{2+}-NTA agarose (Qiagen) column equilibrated with buffer I. Bound protein was first washed with buffer II (50 mM Tris-HCl pH 8, 1 M NaCl, 5 mM 2-Mercaptoethanol, 10% Glycerol, 20 mM Imidazole) and then buffer III (20mM Tris-HCl pH 8, 25 mM NaCl, 5 mM 2-Mercaptoethanol, Glycerol 10%, 20 mM Imidazole), followed by elution in buffer III containing 150 mM Imidazole. Peak fractions were aliquoted and flash frozen in N_2(l). The activity of individual preparations were normalized using purified IVB^{S378A} as a substrate. The purified protease retains full catalytic activity after freeze-thawing.

Purification of CtpB-His$_6$

The *ctpB* gene lacking its putative signal sequence was fused to a C-terminal His$_6$-tag (pDR211) and expressed in *E. coli* BL21 DE3 pLysS. Cells were grown in LB at 37˚C to an

OD_{600} of 0.6 and induced by the addition of IPTG to 1 mM, and harvested after 40 minutes. All subsequent manipulations were essentially the same as those for the purification of IVB-His_6. A similar protocol was used to purify $CtpB^{S309A}$-His_6

Purification of His_6-A_{EC}

The last 173 amino acids of the *A* gene (corresponding to the extracellular domain of A) were fused to an N-terminal His_6-tag (pDT73) and expressed in *E. coli* BL21 DE3 pLysS. Cells were grown in LB at 37˚C to an OD_{600} of 0.4, induced by the addition of IPTG to 1 mM, and harvested after 2 hours. All subsequent manipulations were essentially the same as those for the purification of IVB-His_6.

Preparation of Crude Membrane Vesicles

50 ml cultures were harvested at 3.5 hours after the initiation of sporulation (by resuspension) and washed two times with 1X SMM (0.5 M Sucrose, 20 mM $MgCl_2$, 20 mM Maleic acid pH 6.5) (Harwood and Cutting, 1990) at room temperature. Cells were resuspended in 1/10 volume 1X SMM and protoplasted with Lysozyme (0.5 mg/ml). Protoplasts were collected by centrifugation and flash frozen in N_2(l). Thawed protoplasts were disrupted by osmotic lysis with 3 ml hypotonic buffer (buffer H) (20 mM Hepes pH 8, 25 mM NaCl, 1 mM DTT, with protease inhibitors: 1 mM PMSF, 0.5 µg/ml leupeptin, 0.7 µg/ml pepstatin). $MgCl_2$ and $CaCl_2$ were added to 1 mM and lysates were treated with DNAseI (10 µg/ml) (Worthington) and RNAseA (20 µg/ml) (USB) for 1 hour on ice. Lysates were loaded on a 1.8 ml sucrose cushion (20 mM Hepes pH 8, 25 mM NaCl, 0.8 M Sucrose) and spun at 100,000X g for 1 hour at 4˚C in an SW55Ti rotor (Beckman). The supernatant was carefully removed and the pellet was dispersed in 200 µl buffer G (buffer H with 10% Glycerol), distributed in 25 µl aliquots and flash frozen in N_2(l).

Detergent Solubilization of Membrane Proteins

25 µl crude membranes were diluted 20-fold with buffer S [buffer H + 1.5 mg/ml *E. coli* phospholipids (Avanti), 20% Glycerol] (Driessen and Wickner, 1990) and membrane proteins were solubilized by the addition of the nonionic detergent Digitonin to a final concentration of 0.5%. Detergent-solubilized membrane fractions were rotated at 4˚C for 1 hour. Soluble and insoluble fractions were separated by centrifugation at 100,000X g for 1 hour at 4˚C.

Cleavage Assay with His-A_{EC} and IVB^{S378A}

Approximately 1.5 µg of the purified proteins were mixed with ~100 ng of purified IVB (or 50ng of CtpB) (per time point) and incubated at room temperature. The reaction was stopped by the addition of 2X SDS-sample buffer and analyzed by SDS-PAGE followed by Coomassie staining.

Cleavage Assay with Crude Membrane Vesicles

25 µl crude membranes derived from osmotic lysis were diluted 8-fold with buffer H. 4 µl of the diluted membrane vesicles (equivalent to 125 µl of cells) were mixed with ~50 ng of purified IVB (per time point) and incubated at room temperature. The reaction was stopped by the addition of 2X SDS-sample buffer, resolved by SDS-PAGE and analyzed by immunoblot using anti-GFP antibodies. Virtually all the GFP-A protein could be cleaved within 4 hours in the membrane vesicle assay if three times more IVB protease was used. This result suggests that the membrane vesicles derived from the outer forespore membrane generated by osmotic lysis are almost entirely right-side-in.

Cleavage Assay with Detergent-Solubilized Membrane Proteins

25 µl crude membranes were diluted 4-fold with buffer S and membrane proteins were solubilized by the addition of the nonionic detergent Digitonin to a final concentration of 0.5%. Detergent-solubilized membrane proteins were rotated at 4˚C for 1 hour. Soluble and insoluble fractions were separated by centrifugation at 100,000X g for 1 hour at 4˚C. 4 µl solubilized membrane proteins (equivalent to 250µl of cells) were mixed with ~50 ng of purified IVB (per time point) and incubated at room temperature. The reaction was stopped by the addition of 2X SDS-sample buffer and analyzed by SDS-PAGE followed by immunoblot using anti-GFP or anti-A antibodies.

Coimmunoprecipitation from Detergent-Solubilized Membrane Fractions

The soluble fraction from the Digitonin-treated membrane preparation was mixed with 10 µl affinity-purified anti-GFP antibody resin and rotated for 4 hours at 4˚C. The resin was pelleted at 5Krpm and washed four times with 1 ml buffer S + 0.5% Digitonin. Immunoprecipitated proteins were eluted by the addition of 50µl of 2X SDS-sample buffer and heated for 15 minutes at 50˚C. GFP-A was either directly immunoprecipitated after Digitonin-solubilization or after a 4 hour incubation with the recombinant IVB protease.

Affinity Purification of Antibodies

Approximately 1 mg of purified antigen was dialyzed into coupling buffer (20 mM Hepes pH 8, 200 mM NaCl, 10% Glycerol) and coupled to 1 ml Affigel-10 resin (BioRad) as described by the manufacturer. After coupling, the antigen resin was washed with 100 mM Glycine pH 2.5 (and then neutralized with 1XPBS) to remove all uncoupled protein. 10-20 ml of antisera were batch-absorbed to the antigen-resin overnight at 4˚C. The resin was loaded into a 5 ml column and washed with 20 ml 1XPBS, 50 ml 1XPBS 500 mM NaCl and 10 ml 0.2X PBS. The affinity-purified antibodies were then eluted with 100 mM Glycine pH 2.5. The peak fractions were pooled and dialyzed with three changes into 1XPBS 50% Glycerol and stored at -20˚C.

Anti-GFP Antibody Resin

Affinity-purified anti-GFP antibody (4 mg) (Rudner and Losick, 2002) were batched absorbed to 1 ml proteinA-sepharose (Amersham) for 1 hour at 4˚C. The antibody resin was washed four times with 1XPBS and the antibody was covalently crosslinked to the proteinA-sepharose by the addition of Disuccinimidyl Suberate (Pierce) to a final concentration of 5 mM. After 30 minutes the reaction was quenched by the addition of Tris pH 7.5 to a final concentration of 100 mM. The antibody resin was washed with 100 mM Glycine pH 2.5 to remove uncrosslinked antibody. The resin was neutralized with 1X PBS and stored at 4˚C.

Supplemental References

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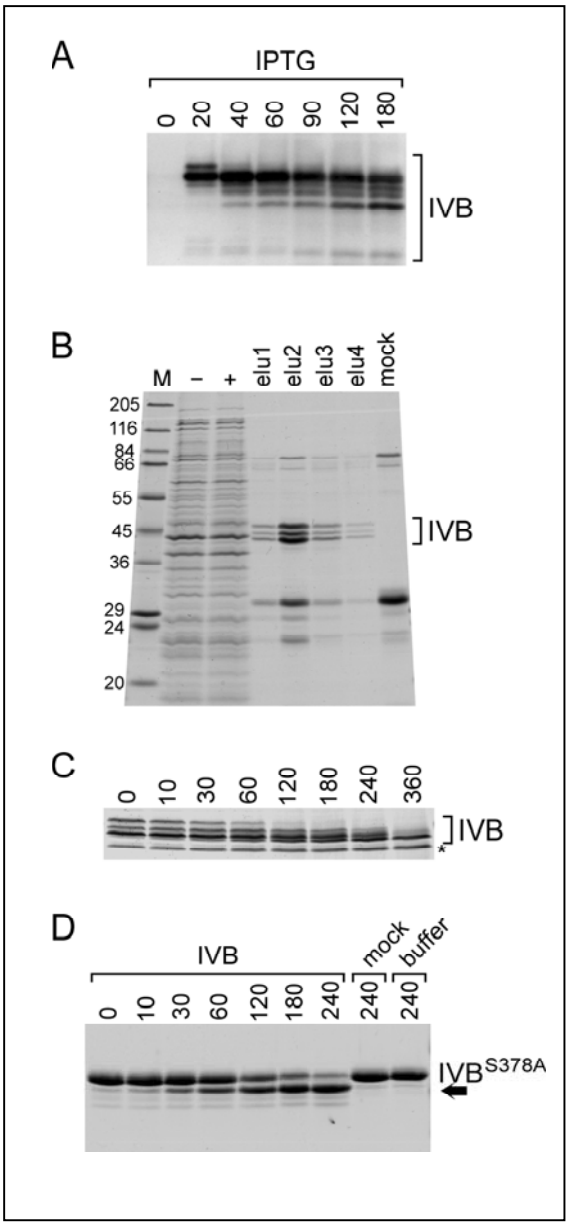

Figure S1. Expression, Purification, and Reconstitution of IVB Protease Activity In Vitro

(A) IVB-His_6 is proteolyzed in *E. coli*. *E. coli* strain BL21 DE3 pLysS containing the IVB-His_6 expression plasmid pKM35 was induced with 0.5mM IPTG. At indicated times (in minutes) after induction, samples were collected and lysates prepared. The lysates were analyzed by immunoblot using anti-IVB antibodies. (B) Purification of IVB-His_6. Cell extracts from *E. coli* cultures before (-) and after (+) induction of IVB-His_6 with IPTG were analyzed by SDS-PAGE and stained with Coomassie blue. Fractions 1-4 after purification on Ni^{2+}-agarose are shown (elu1-4). The peak fraction from a mock purification using *E. coli* cells harboring the empty expression plasmid is also shown (mock). (C) IVB cleaves itself in *cis*. Purified IVB-His_6 was incubated at room temperature for indicated times (in minutes). The reaction products were separated by SDS-PAGE and stained with Coomassie blue. (D) IVB cleaves itself in *trans*. Purified IVB-His_6 was mixed with the catalytic mutant IVB^{S378A} and incubated at room temperature for indicated times (in minutes). IVB^{S378A} was also incubated with the mock purification (mock) or buffer. Reactions were analyzed by SDS-PAGE and stained with Coomassie blue. The IVB^{S378A} cleavage product is indicated (arrow).

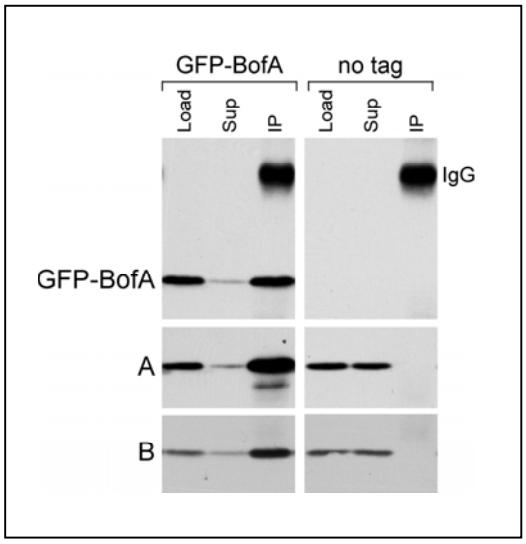

Figure S2. The majority of A, B and BofA reside in a complex after detergent-solubilization. Immunoblot analysis of co-immunoprecipitation assays. Detergent-solubilized membrane fractions from a strain harboring a GFP-BofA fusion (BDR653) and a strain lacking the GFP tag (RL831) were subjected to immunoprecipitation using anti-GFP antibody resin. The detergent-solubilized membrane extracts prior to immunoprecipitation (Load), the supernatants after immunoprecipitation (Sup) and immunoprecipitates (IP) were subjected to immunoblot analysis using anti-GFP, anti-A and anti-B antibodies. The heavy chain of the anti-GFP antibody that leached off the resin (recognized by the secondary antibody) is indicated (IgG). The immunoblots for the two strains were from the same gel (and the same films) but control lanes were removed for simplicity. Equivalent amounts of the Load and Supernatant were analyzed. The complex can be assessed by the immuno-depletion of A and B (compare Load and Sup).

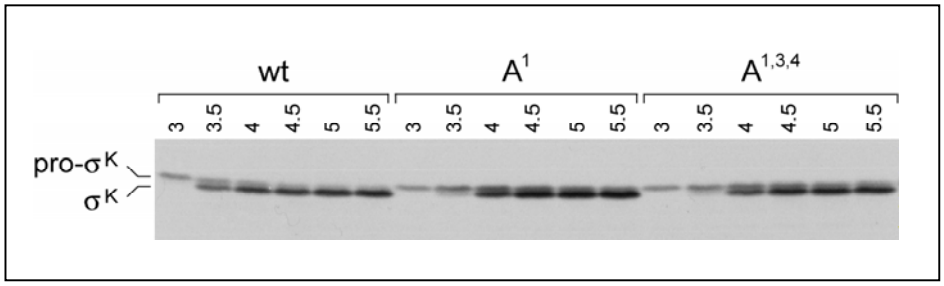

Figure S3. The delay in pro-σ^K processing when A cannot be cleaved at positions 1, 3 and 4 is similar to a cleavage site 1 mutant. A wild type strain (A^{wt}) (BNC243), a cleavage position 1 mutant (S155R) (A^1) (BNC681) and a cleavage positions 1, 3 and 4 mutant (S155R, S117R, A106R) ($A^{1,3,4}$) (BNC788) were induced to sporulate by resuspension. Whole cell extracts were prepared from samples collected at indicated times (in hours) after the initiation of sporulation. Pro-σ^K processing was monitored by immunoblot using anti-σ^K antibodies.

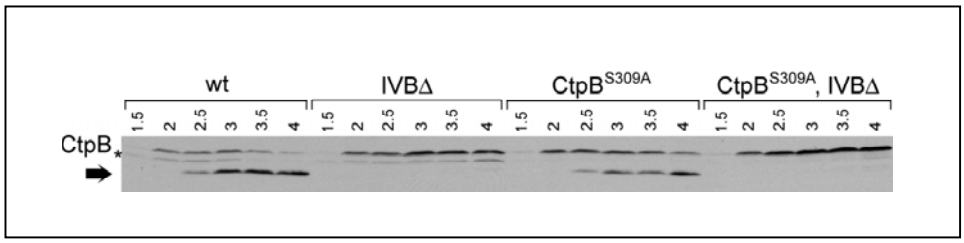

Figure S4. Analysis of CtpB proteolysis in a CtpB catalytic-site mutant. Wild type (BNC949), a IVB mutant (IVBΔ) (BNC953), a CtpB catalytic-site mutant ($CtpB^{S309A}$) (BNC951) and the double mutant (BNC955) were induced to sporulate by resuspension. At indicated times (in hours) after the initiation of sporulation, samples were collected. Whole cell extract were analyzed by immunoblot using anti-CtpB antibodies. The cleavage products derived from CtpB autoproteolysis (asterisk) and IVB-dependent cleavage (arrow) are indicated.

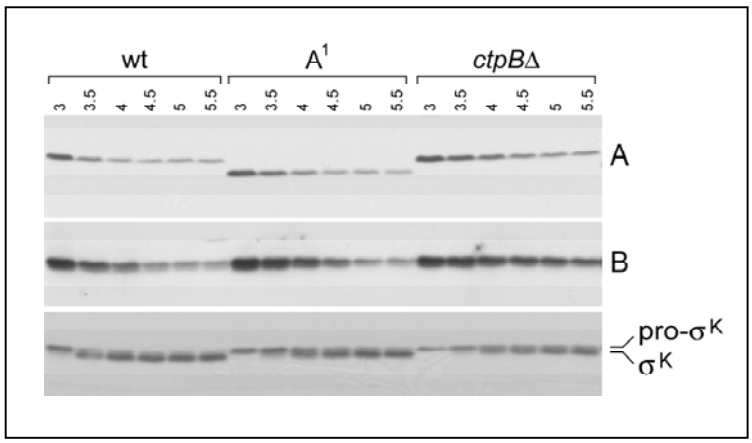

Figure S5. The A protein is stabilized in the absence of CtpB. A wild type strain (wt) (BNC243), a IVB cleavage position 1 mutant (S155R) (A^1) (BNC681) and a *ctpB* null strain (BNC734) were induced to sporulate by resuspension. Whole cell extracts were prepared and analyzed by immunoblot using anti-A, anti-B and anti-σ^K antibodies. The change in mobility of the A^1 mutant was due to the S155R substitution.

Table S1. Strains Used in This Study

strain	genotype	reference
RL831	*spoIIIG::neo*	Margolis et al. 1991
BDR653	*amyE::P_{spoIVF}-gfp-Δ(27)bofA (spec), bofA::cat, IVB::erm*	This work
BNC243	*spoIVF::cat, lacA::P_{spoIVF}-B (erm), amyE::P_{spoIVF}-A (spec)*	This work
BNC635	*spoIIIGΔ1, spoIVF::cat, lacA::P_{spoIVF}-B-gfp (erm), amyE::P_{spoIVF}-A (spec)*	This work
BNC639	*spoIIIGΔ1, spoIVF::cat, lacA::P_{spoIVF}-B-gfp (erm), amyE::P_{spoIVF}-A^{S155R} (spec)*	This work
BNC681	*spoIVF::cat, lacA::P_{spoIVF}-B (erm), amyE::P_{spoIVF}-A^{S155R} (spec)*	This work
BNC689	*IVB::phleo*	This work
BNC692	*IVB::phleo, spoIVF::cat, lacA::P_{spoIVF}-B (erm), amyE::P_{spoIVF}-A (spec)*	This work
BNC694	*IVB::phleo, spoIVF::cat, lacA::P_{spoIVF}-B (erm), amyE::P_{spoIVF}-gfp-A (spec)*	This work
BNC734	*spoIVF::cat, lacA::P_{spoIVF}-B (erm), amyE::P_{spoIVF}-A (spec), ctpB::tet*	This work
BNC788	*spoIVF::cat, lacA::P_{spoIVF}-B (erm), amyE::P_{spoIVF}-$A^{A106R, S117R, S155R}$ (spec)*	This work
BNC848	*spoIVF::cat, lacA::P_{spoIVF}-B (erm), amyE::P_{spoIVF}-$A^{V146F, G147Q}$ (spec)*	This work
BNC850	*spoIVF::cat, lacA::P_{spoIVF}-B (erm), amyE::P_{spoIVF}-$A^{A106R, S117R, V146F, G147Q, S155R}$ (spec)*	This work
BNC861	*IVB::phleo, spoIVF::cat, lacA::P_{spoIVF}-B (erm), amyE::P_{spoIVF}-$A^{V146F, G147Q}$ (spec)*	This work
BNC867	*spoIVF::cat, lacA::P_{spoIVF}-B (erm), amyE::P_{spoIVF}-$A^{A106R, S117R, V146F, G147Q, S155R}$ (spec), ctpB::tet*	This work
BNC887	*IVB::phleo, spoIVF::cat, lacA::P_{spoIVF}-B (erm), amyE::P_{spoIVF}-gfp-A^{S155R} (spec)*	This work
BNC889	*IVB::phleo, spoIVF::cat, lacA::P_{spoIVF}-B (erm), amyE::P_{spoIVF}-gfp-A (spec), bofA::tet*	This work
BNC910	*spoIVF::cat, lacA::P_{spoIVF}-B (erm), amyE::P_{spoIVF}-$A^{A106R, S117R, A131R, V146F, G147Q, S155R}$ (spec)*	This work
BNC949	*ctpB::tet, amyE::ctpB (spec)*	This work
BNC951	*ctpB::tet, amyE::ctpB^{S309A} (spec)*	This work
BNC953	*IVB::phleo, ctpB::tet, amyE::ctpB (spec)*	This work
BNC955	*IVB::phleo, ctpB::tet, amyE::ctpB^{S309A} (spec)*	This work

Table S2. Oligonucleotides Used in This Study

Oligonucleotides used for PCR

primer	sequence*
oDR67	GGCGAATTCGGAATGATGGCTAAGATTAAG
oDR68	GGCAAGCTTGCATTCGTTGGGTTTGTAC
oDR69	GGCGAATTCGTCAGTCAGATTAAACCCG
oDR303	CAGGCCATGGGAGTATTTGAAACCCAAACACAAGCG
oDR307	GGCAAGCTTGCTTGCTTTTTCTTTTCCATAAATATC
oDR400	GTGCCCATATGGGAATCAACCTGCTTGAGATG
oDR401	GACCGTCGACATTGACAAATAATGATTTCAATGCTG
oNC3	CGCGGATCCGATAAGGTCGAGCCATTTAT
oNC5	CCCAAGCTTACAAAGGATGATGGCAATGAATAAATGGCTCGACCTTATC
oNC11	CGGCTCGAGGTTGACGAAATCACAAACGAAAC
oNC12	GGCGAATTCTACTTCGCTTCAGCTACTTTCAG
oNC13	GCCGGATCCCTGTTAAATTTGTGGCAGGTGC
oNC14	GCCTCTAGACAGCGACGTATATTAGAATAACAC
oNC39	GGCCTGCAGCTTTCGAAAGTCGCTTCAGGT
oNC40	CGCGTCGACACTACTTCACTCTCCTCGCT
oNC41	CGGAATTCTGACTGCCGGAGTTTC
oNC44	CAGCCTCTCTAAAACCGCAAGT

* restriction endonuclease sites are underlined

Oligonucleotides used for site-directed mutagenesis

primer	sequence	mutation
oNC31	GATCTGATCGCGCCTGCACGCGGGAAAGTACAGCAGGATT	A^{S155R}
oNC47	CAGATTAAACCCGCCGTACGCAAAACCTTTGAAACTGAATTT	A^{A106R}
oNC49	GAAACTGAATTTCAATTTGCTAGAGCAAGCCATTGGTTCGAA	A^{S117R}
oNC53	CAAAAATAAGGAACAGCAGATTGAATTTCAGAAAGATCTGATCGCGCCTGC	$A^{V146F, G147Q}$
oNC73	GAAACCAAATTCGGAAATCCGCTTCGTTTCCTGGCTCCTGAACACAAAAA	A^{A131R}

Table S3. Plasmids Used in This Study

plasmid	description	source or reference
pET24b(+)	vector for His-tagged protein synthesis in *E. coli*	Novagen
pET28a (+)	vector for His-tagged protein synthesis in *E. coli*	Novagen
pKM35	*Δss-IVB-his*$_6$	This work
pQP51-1	*Δss-IVB*^{S378A}*-his*$_6$	This work
pDT73	*His*$_6$*-A*$_{EC}$	This work
pNC62	*His*$_6$*-A*$_{EC}^{S155R}$	This work
pNC80	*His*$_6$*-A*$_{EC}^{A106R, S117R, S155R}$	This work
pNC98	*His*$_6$*-A*$_{EC}^{V146F, G147Q}$	This work
pDR211	*Δss-ctpB-his*$_6$	This work
pDR212	*Δss-ctpB*^{S309A}*-his*$_6$	This work
pDR104	*amyE::P*$_{spoIVF}$*-gfp-A (spec)*	Rudner and Losick 2002
pNC1	*amyE::P*$_{spoIVF}$*-A (spec)*	This work
pNC8	*lacA::P*$_{spoIVF}$*-B (erm)*	This work
pNC12	*bofA ::tet*	This work
pNC29	*amyE::P*$_{spoIVF}$*-A*^{S155R} *(spec)*	This work
pNC40	*amyE::P*$_{spoIVF}$*-gfp-A*^{S155R} *(spec)*	This work
pNC52	*lacA::P*$_{spoIVF}$*-B-gfp (erm)*	This work
pNC59	*IVB::phleo*	This work
pNC77	*amyE::P*$_{spoIVF}$*-A*$^{V146F, G147Q}$ *(spec)*	This work
pNC84	*amyE::P*$_{spoIVF}$*-A*$^{A106R, S117R, S155R}$ *(spec)*	This work
pNC88	*amyE::P*$_{spoIVF}$*-A*$^{V146F, G147Q, S155R}$ *(spec)*	This work
pNC90	*amyE::P*$_{spoIVF}$*-A*$^{A106R, S117R, V146F, G147Q, S155R}$ *(spec)*	This work
pNC92	*amyE::P*$_{spoIVF}$*-gfp-A*$^{V146F, G147Q}$ *(spec)*	This work
pNC110	*amyE::P*$_{spoIVF}$*-A*$^{A106R, S117R, A131R, V146F, G147Q, S155R}$ *(spec)*	This work