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Regulation of Synthesis of the *Bacillus subtilis* Transition-Phase, Spore-Associated Antibacterial Protein TasA

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Previously, we identified a novel component of *Bacillus subtilis* spores, called TasA, which possesses antibacterial activity. TasA is made early in spore formation, as cells enter stationary phase, and is secreted into the medium as well as deposited into the spore. Here, we show that *tasA* expression can occur as cells enter stationary phase even under sporulation-repressing conditions, indicating that TasA is a transition-phase protein. *tasA* and two upstream genes, *yqxM* and *sipW*, likely form an operon, transcription of which is under positive control by the transition-phase regulatory genes *spo0A* and *spo0H* and negative control by the transition phase regulatory gene *abrB*. These results are consistent with the suggestion that *yqxM*, *sipW*, and *tasA* constitute a transition phase operon that could play a protective role in a variety of cellular responses to stress during late-exponential-phase and early-stationary-phase growth in *B. subtilis*.

When confronted with nutrient deprivation, *B. subtilis* can choose from a large set of alternative responses such as the production of antibiotics and proteases, the uptake of exogenous DNA (the state of competence), and the formation of a dormant cell type called the spore (20). Previously, we identified a novel spore-associated protein, called TasA, which possesses an antibacterial activity and is also secreted into the culture supernatant at the beginning of sporulation (19). *tasA* is immediately downstream of *sipW* (Fig. 1A) (9), encoding a signal peptidase (22, 23) that is required for the maturation and secretion of TasA (19). *sipW* is downstream of and partially overlaps *yqxM*, whose function is unknown. The proximity of these three genes and the functional relationship between SipW and TasA raise the possibility of coordinate regulation of this gene cluster.

Previously, we found that the production of TasA requires Spo0A and σ^H, molecules that guide regulation at the juncture between exponential and stationary phases (5) (Fig. 2A), consistent with TasA synthesis early in sporulation (19). To examine the possibility that TasA is a transition-phase protein and to deepen our understanding of the regulatory controls on the expression of *tasA* and the adjacent genes, we have studied the regulation of TasA synthesis during a variety of growth conditions, defined 5′ sequences required for *tasA* expression, and identified some of the relevant regulatory factors.

MATERIALS AND METHODS

Strains, media, and recombinant DNA procedures. Strains, plasmids, and primers for PCR are listed in Tables 1 and 2. All recombinant DNA procedures were performed by using standard procedures (16), and manipulations in *B. subtilis* were carried out according to procedures in reference 2. Cells were grown in Difco sporulation medium (DSM) (2) unless otherwise indicated. 2×YT, Luria-Bertani (LB), and Terrific broth (TB) media (16) and King's B medium (8) were prepared as described elsewhere.

Transcriptional fusions to *lacZ*. First, we digested the *amyE* replacement vector pDG268 (6) with *Eco*RI and *Hin*dIII, purified the larger resulting fragment, and ligated it to the *Eco*RI-*Hin*dIII fragment of pSL1180 containing the multiple cloning site, creating pAGS38. Next, we amplified the *yqxM sipW tasA* locus by PCR using primers OL109 and OL110 and digested the fragment with *Nhe*I and *Not*I. We then cloned the fragment between the *Nhe*I and *Not*I sites of pAGS38, creating the *tasA-lacZ* transcriptional fusion plasmid pAGS42. To make the *yqxM-lacZ* transcriptional fusion (in pAGS43), we digested pAGS42 with *Nhe*I and *Hin*dIII and ligated the smaller resulting DNA fragment into similarly digested pAGS38. pAGS42 and pAGS43 were linearized with *Kpn*I and used to transform a variety of strains to chloramphenicol resistance (Table 1). We made pAGS51, pAGS53, pAGS54, pAGS55, pAGS56, pAGS57, and pAGS58 by essentially the same procedure but used oligonucleotides OL116 and OL110, OL131 and OL110, OL132 and OL110, OL133 and OL110, OL134 and OL110, OL135 and OL110, and OL136 and OL110, resulting in strains AGS340, AGS376, AGS377, AGS378, AGS379, AGS380, and AGS381, respectively. Strain 8G5::pLGW201, bearing *sipW-lacZ* as a single reciprocal (Campbell-type) integration at *sipW* (22), was a kind gift from J. M. van Dijl. We moved this fusion into PY79 by transformation, resulting in strain AGS307.

Determination of relative TasA levels and β-galactosidase assays. Cells were harvested 5 h after the end of exponential phase, pelleted, and then lysed by resuspension in 25 mM Tris-HCl (pH 7.5)–50 mM glucose–10 mM EDTA–100 μg of lysozyme per ml followed by a 10-s burst of sonication (using a Fisher Dismembrator). We determined the concentration of total protein in each sample with a bicinchoninic acid protein assay reagent kit (Pierce) and then carried out electrophoresis on 12% polyacrylamide gels and Western blot analysis (19). We used a Hewlett-Packard ScanJet 6200C and AlphaImager 2000 version 4.03 software (Alpha Innotech Corporation) to determine the amounts of TasA relative to total protein in each strain and divided these ratios by the ratio of TasA to total protein in wild-type cells. We carried out β-galactosidase assays as described elsewhere (2) except that the reaction was performed at 37°C instead of 30°C.

RESULTS AND DISCUSSION

Medium requirements for TasA synthesis. We previously found that TasA synthesis began as cells entered stationary phase during growth in sporulation medium (DSM). To determine whether TasA could be made independently of the decision to sporulate, we used Western blot analysis to follow the steady-state accumulation of TasA in 2×YT medium (in which sporulation was decreased to 0.01% [data not shown]). We first detected TasA at the beginning of stationary phase and found that it was present for at least 7 h (Fig. 3 and data not shown). We also detected TasA in cells grown in King's B, LB, or TB medium, in which sporulation occurred in less than 0.01% of the cells (data not shown). Therefore, TasA is a transition-phase protein that first appears at the beginning of stationary phase.

Sequences required for *tasA* expression. The arrangement of *yqxM*, *sipW*, and *tasA* suggested they form an operon (Fig. 1A). As an initial test of this possibility, we compared the level of *tasA-lacZ* expression in a strain missing all of the sequences upstream of the *yqxM* open reading frame (AGS340) with the

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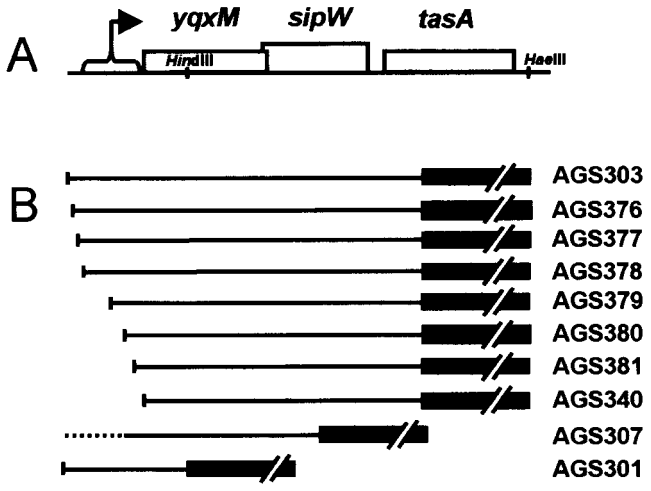

FIG. 1. The *yqxM sipW tasA* locus and DNA constructs. (A) The *yqxM sipW tasA* locus. Open boxes represent genes, and the region likely to contain the promoter(s) is indicated by a bracket. Restriction sites are indicated. (B) Transcriptional reporter fusion constructs. Black boxes represent the *lacZ* gene of *E. coli*. Names of strains bearing these fusions are indicated. Fusions were inserted by marker replacement at the *amyE* locus except for *sipW-lacZ* (a gift from J. M. van Dijl), which was integrated by single-reciprocal integration, indicated by the dotted line (to generate AGS307).

level in a strain in which 351 bp upstream of *yqxM* were retained (AGS303). In strain AGS303, *tasA-lacZ* expression began at about 15 min before the beginning of stationary phase and maintained a significant level for at least 7 h (Fig. 4A), similarly to the timing of appearance of TasA (Fig. 3 and reference 19). Consistent with this, we were able to readily detect TasA by Western blot analysis when only 351 bp of upstream sequence were present (data not shown). The removal of the upstream sequence reduced *tasA* expression almost to background levels (Fig. 4A). Likewise, we did not detect TasA by Western blot analysis when the upstream sequence was removed (data not shown).

To study the timing of expression of the genes in this locus, we measured β-galactosidase activities of strains harboring transcriptional fusions of *yqxM*, *sipW*, and *tasA* to *lacZ* of *E. coli* (strains AGS301, AGS307, and AGS303, respectively [Fig.

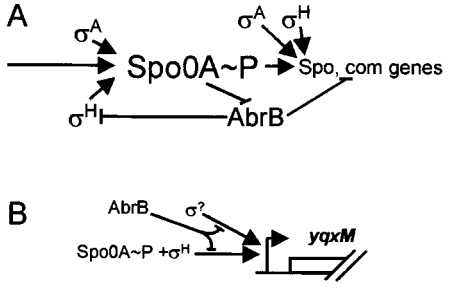

FIG. 2. Regulation of transition-phase gene expression and a model for the possible regulation of the *yqxM sipW tasA* operon. (A) Summary of the roles of Spo0A, σ^H, and AbrB on transition-phase gene expression. The long arrow on the left indicates the influence of factors controlling the cellular concentration of Spo0A~P, which in turn determines whether the cell will express sporulation (Spo) genes, competence (com) genes, or genes associated with another transition phase choice (on the right). Not all transition-phase genes are controlled directly by Spo0A. (B) A speculative model for transcriptional control of the operon. Spo0A and σ^H direct expression. Separately, an as yet unidentified additional sigma factor also directs expression. AbrB can repress both events. Although a single promoter is illustrated, multiple promoters may be present.

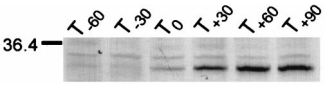

FIG. 3. Western blot analysis of TasA during growth in 2×YT. Extracts of cells grown in 2×YT were prepared at the indicated times before and after the onset of stationary phase (T_0). After fractionation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransfer to polyvinylidene difluoride membranes, TasA was detected with an anti-TasA antibody. Molecular mass is indicated in kilodaltons on the left.

1B]). We found that expression of all three genes initiated at the beginning of stationary-phase growth (Fig. 4A), consistent with the timing of TasA production (Fig. 3 and reference 19). The wild-type strain, without the *tasA-lacZ* fusion (PY79), showed background levels of β-galactosidase activity (and a white color on solid indicator medium [data not shown]). For unknown reasons, the levels of expression of the *yqxM-lacZ* and *sipW-lacZ* fusions were consistently lower than that of *tasA-lacZ*. To further define the upstream region needed for *tasA-lacZ* transcription, we generated a set of fusions missing progressively more of the 351-bp region (Fig. 1B). We found significant levels of *tasA-lacZ* expression when 233 bp or more upstream of *yqxM* were retained but detected no expression when 114 or fewer bp of upstream sequence were present (Fig. 4B). These results suggest that *yqxM*, *sipW*, and *tasA* constitute an operon and that the sequences required for *tasA* expression under these conditions of growth are confined to a region 233 bp upstream of *yqxM*. They also argue against the presence of strong promoters between the beginning of the *yqxM* open reading frame and the *tasA* open reading frame.

Roles of Spo0A, σ^H, and AbrB in *tasA* transcription. We found background levels of β-galactosidase activity in strains bearing mutations in *spo0A* (AGS313) and *spo0H* (AGS305) (Fig. 4C), suggesting that Spo0A and σ^H are required for *tasA* expression. We detected about 10-fold-higher levels of *tasA-lacZ* expression in a strain bearing an *abrB* mutation (AGS351) (Fig. 4D), indicating that AbrB represses *tasA* expression. We also found that TasA steady-state levels in two strains bearing null mutations in *abrB* (AGS347 and AGS348) were approximately sixfold higher than in the wild type (Table 3).

The effect of AbrB on *tasA* expression could be indirect, via repression of *spo0H* (25) (Fig. 2A). If this is true, then *tasA-lacZ* expression should be at background levels in an *abrB spo0H* double mutant. In such a strain (AGS352), expression of *tasA-lacZ* occurred about 2 h earlier than in the wild type and ultimately was three- to fivefold higher than in the wild type, similarly to an *abrB* strain (AGS351) (Fig. 4D). TasA levels in strains bearing *abrB* and *spo0H* mutations (AGS349 and AGS350) were somewhat higher than in the wild type (Table 3). From these data, we infer that AbrB does not repress *tasA* expression solely by inhibition of *spo0H* expression. These results also suggest that AbrB represses a σ^H-independent mechanism of *tasA* expression that acts prior to the onset of stationary phase. In contrast to the *abrB* mutant, in the double mutant *tasA* expression did not remain high but rather decreased over time. The difference in *tasA* expression between the two strains further argues that AbrB also represses a post-exponential σ^H-dependent phase of expression. Apparently, when *abrB* is intact, this phase of σ^H-directed expression is not completely repressed, accounting for the wild-type pattern of *tasA* expression.

One interpretation of these findings is the presence of two promoters upstream of *yqxM*, one of which is σ^H and Spo0A dependent and other of which utilizes a second sigma factor and is Spo0A independent. To determine whether a σ^H-independent promoter might reside within the already identified

TABLE 1. Strains and plasmids used

Strain or plasmid	Genotype or description	Reference, source, or construction
B. subtilis		
PY79	Prototrophic	26
JH642	*trpC2 pheA1*	Lab stock
HB4035	*sigD*Δ::Kan^r	J. Helmann
RL102	*spo0H*Δ*Hin*dIII-*Eco*RI::*cat trpC2*	R. Losick
PM126	*sigB*Δ::*cat*	R. Losick
AG518	*abr*::Tn*917 trpC2 pheA1*	A. D. Grossman
AG839	Δ*abr*::*cat trpC2 pheA1*	A. D. Grossman
AG1242	*spo0A*ΔPs *trpC2 pheA1*	A. D. Grossman
AGS301	*amyE*::*yqxM-lacZ*	PY79 × pAGS43
AGS303	*amyE*::*tasA-lacZ*	PY79 × pAGS42
AGS305	*spo0H*Δ*Hin*dIII-*Eco*RI::*cat*::Spec^r *amyE*::*tasA-lacZ*	AGS232 × pAGS42
AGS306	*sigD*Δ::Kan^r	PY79 × DNA HB4035
AGS307	*sipW*ΩpLGW201 (*sipW-lacZ*)	PY79 × 8G5::pLGW201 (22)
AGS313	*spo0A*Δ::Erm^r *amyE*::*tasA-lacZ*	AGS303 × DNA RL891 (10)
AGS340	*amyE*::pAGS51	PY79 × pAGS51
AGS347	*abrB*::Tn*917*	PY79 × DNA AG518
AGS348	Δ*abrB*::*cat*	PY79 × DNA AG839
AGS349	*spo0H*Δ*Hin*dIII-*Eco*RI::*cat*::Spec^r *abrB*::Tn*917*	AGS232 × DNA AG518
AGS350	*spo0H*Δ*Hin*dIII-*Eco*RI::*cat*::Spec^r Δ*abrB*::*cat*	AGS232 × DNA AG839
AGS351	*abrB*::Tn*917 amyE*::*tasA-lacZ*	AGS347 × DNA AGS303
AGS352	*abrB*::Tn*917 spo0H*Δ*Hin*dIII-*Eco*RI::*cat*::Spec^r *amyE*::*tasA-lacZ*	AGS349 × DNA AGS303
AGS361	*spo0A*::pSPC101 *amyE*::*tasA-lacZ*	AGS303 × DNA JKH75 (4)
AGS362	*spo0A*::pSPC101-S233P *amyE*::*tasA-lacZ*	AGS303 × DNA JKH104 (4)
AGS363	*spo0A*::pSPC101-G227R *amyE*::*tasA-lacZ*	AGS303 × DNA JKH74 (4)
AGS364	*spo0A*::pSPC101-F236S *amyE*::*tasA-lacZ*	AGS303 × DNA JKH105 (4)
AGS365	*spo0A*::pSPC101-V240A *amyE*::*tasA-lacZ*	AGS303 × DNA JKH106 (4)
AGS366	*spo0A*::pSPC101-V240G K265R *amyE*::*tasA-lacZ*	AGS303 × DNA JKH107 (4)
AGS367	*spo0A*ΔPs *amyE*::*tasA-lacZ trpC2 pheA*	AG1242 × DNA AGS303
AGS368	Δ*sigB*::*cat*	PY79 × DNA PM126
AGS376	*amyE*::pAGS53	PY79 × pAGS53
AGS377	*amyE*::pAGS54	PY79 × pAGS54
AGS378	*amyE*::pAGS55	PY79 × pAGS55
AGS379	*amyE*::pAGS56	PY79 × pAGS56
AGS380	*amyE*::pAGS57	PY79 × pAGS57
AGS381	*amyE*::pAGS58	PY79 × pAGS58
AGS387	*amyE*::*tasA-lacZ trpC2 pheA1*	AGS351 × DNA AGS303
AGS392	*abrB*::Tn*917 spo0A*Δ::pSPC101 *amyE*::*tasA-lacZ*	AGS351 × DNA JKH75 (4)
E. coli DH5α	Cloning host	Lab stock
Plasmid		
pAGS42	351 bp upstream of *yqxM sipW tasA-lacZ*	This study
pAGS43	*yqxM-lacZ*	This study
pAGS51	Promoterless *yqxM sipW tasA-lacZ*	This study
pAGS53	281 bp upstream of *yqxM sipW tasA-lacZ*	This study
pAGS54	264 bp upstream of *yqxM sipW tasA-lacZ*	This study
pAGS55	233 bp upstream of *yqxM sipW tasA-lacZ*	This study
pAGS56	114 bp upstream of *yqxM sipW tasA-lacZ*	This study
pAGS57	40 bp upstream of *yqxM sipW tasA-lacZ*	This study
pAGS58	351 bp upstream of *yqxM sipW tasA-lacZ*	This study
pDG268	Permits marker replacement of *lacZ* fusion at *amy*	6
pDG364	Permits marker replacement at *amy*	7
pSL1180	Harbors extensive multiple cloning site	Pharmacia

TABLE 2. Primers used

Primer	Sequence[a]	Enzyme(s) cut	Region bound[b]
OL99	5′AAAAACTCGAGCATATGTTTCGATTGTTTCAC3′	*Xho*I and *Nde*I	1 to 18
OL109	5′AAAAAAGCTAGCAGCGGTGTTTCTTCTGCGTG3′	*Nhe*I	−332 to −351
OL110	5′AAAAAAGCGGCCGCCCTCCAACTAAAGCTAATCCTAGTG3′	*Not*I	1424 to 1448
OL116	5′AAAAAAGCTAGCATGTTTCGATTGTTTCAC3′	*Nhe*I	1 to 18
OL131	5′AAAAAAAGCTAGCGATCATCATGCTGTCACCCT3′	*Nhe*I	−261 to −280
OL132	5′AAAAAAAGCTAGCCTTTCTTTGTTTATTATTACC3′	*Nhe*I	−242 to −262
OL133	5′AAAAAAAGCTAGCGGGATATGCATTTAAATTCTCAC3′	*Nhe*I	−209 to −233
OL134	5′AAAAAAAGCTAGCATGAGCGATTTCGGTGTTTTT3′	*Nhe*I	−94 to −114
OL135	5′AAAAAAAGCTAGCATTGTCATATCAAGT3′	*Nhe*I	−26 to −42
OL136	5′AAAAAAAGCTAGCTCAAGTTACAGTGTTTTACAGGAG3′	*Nhe*I	−7 to −32

[a] The restriction endonuclease site(s) in each oligonucleotide is underlined. For OL99, the *Xho*I site is singly underlined and the *Nde*I site is doubly underlined.
[b] Region of the chromosome bound by the oligonucleotide, indicated as nucleotide positions relative to the start of the *yqxM* open reading frame.

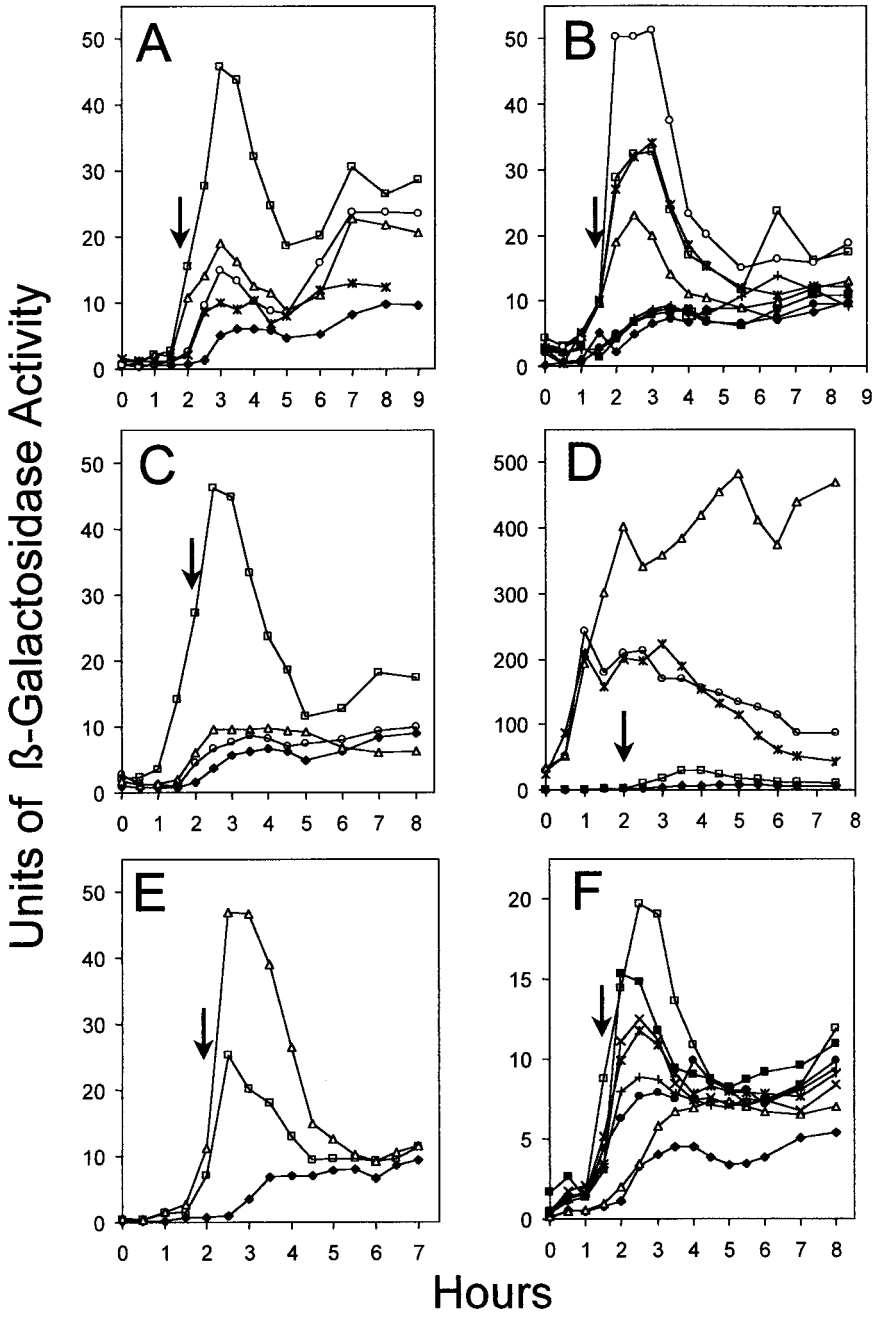

FIG. 4. β-galactosidase activity in cells bearing *yqxM*-, *sipW*-, or *tasA-lacZ*. All strains harbor either *sipW-lacZ* (AGS301), *yqxM-lacZ* (AGS307), no fusion (PY79 and JH642), or *tasA-lacZ* (all remaining strains). In each plot, the onset of stationary phase (time zero) is indicated by an arrow. (A) Fusions to *tasA*, *sipW*, or *yqxM* and a *tasA-lacZ* fusion in which the 351 bp upstream of *yqxM* have been deleted. Strains tested: AGS301 (*yqxM-lacZ*) (△), AGS307 (*sipW-lacZ*) (○), AGS303 (*tasA-lacZ*) (□), AGS340 (*yqxM* upstream sequences deleted, *tasA-lacZ*) (✻), and PY79 (◆). (B) *tasA-lacZ* fusions retaining various regions upstream of *yqxM*. Strains tested: AGS303 (retaining 351 bp) (□), AGS376 (281 bp) (△), AGS377 (264 bp) (○), AGS378 (233 bp) (✻), AGS379 (114 bp) (●), AGS380 (40 bp) (+), AGS381 (30 bp) (■), and PY79 (◆). (C) Effects of *spo0A* and *spo0H*. Strains tested: AGS303 (wild type) (□), AGS305 (*spo0H*Δ*Hin*dIII-*Eco*RI::*cat*::Spec^r) (△), AGS313 (*spo0A*Δ::Erm^r) (○), and PY79 (◆). (D) Effects of *abrB*. Strains tested: AGS303 (wild type) (□), AGS351 (Δ*abrB*::Tn*917*) (△), AGS352 (Δ*abrB*::*cat spo0H*Δ*Hin*dIII-*Eco*RI::*cat*::Spec^r) (○), AGS392 (*abrB*::Tn*917*::pSpc101 *spo0A*Δ::Erm^r) (✻), and PY79 (◆). (E) Effect of deletion of the σ^H-driven promoter of *spo0A*. Strains tested: AGS387 (wild type) (□), AGS367 (*spo0A*ΔPs) (△), and JH642 (◆). (F) Effects of alleles of *spo0A* deficient in σ^A-dependent gene expression. Strains tested: AGS303 (wild type) (□), AGS361 (*spo0A*::pSPC101) (△), AGS362 (*spo0A*::pSPC101-S233P) (×), AGS363 (*spo0A*::pSPC101-G227R) (✻), AGS364 (*spo0A*::pSPC101-F236S) (●), AGS365 (*spo0A*::pSPC101-V240A) (+), AGS366 (*spo0A*::pSPC101-V240G K265R) (■), and PY79 (◆). Units of β-galactosidase activity are defined in Materials and Methods. Scales along the *y* axis vary from panel to panel; in particular, the scale in panel D is compressed.

351-bp region upstream of the *yqxM* open reading frame, we measured β-galactosidase activities in *spo0H abrB tasA-lacZ* strains missing various portions of this region. We detected *tasA-lacZ* expression when 233 bp or more were present but not when only 114 bp or fewer remained (data not shown). Therefore, if a second promoter exists, its activity requires sequences within the same 233-bp region required by the σ^H-dependent promoter.

TABLE 3. Effects of transcription factor gene mutations on TasA levels

Strain	Relevant genotype	Fold change in TasA level
AG1242	*spo0A*ΔPs	4.3
AGS347	*abrB*	6.3
AGS348	*abrB*	6.4
AGS349	*spo0H abrB*	1.7
AGS350	*spo0H abrB*	1.6
AGS362	*spo0A* S233P	0.31
AGS363	*spo0A* G227R	0.31
AGS364	*spo0A* F236S	0.11
AGS365	*spo0A* V240A	0.13
AGS366	*spo0A* V240G K265R	0.46

Spo0A could affect *tasA* expression indirectly, via repression of *abrB* (Fig. 2A). If this is so, then *tasA* expression in the *abrB spo0A* strain would appear similar to what was seen in the strain bearing only the *abrB* mutation. If, on the other hand, Spo0A directly activated *tasA* expression, then *tasA* expression in the *abrB spo0A* strain would resemble expression in the *abrB spo0H* strain. We addressed this issue by measuring *tasA-lacZ* expression in an *abrB spo0A* strain (AGS392). We found that the pattern of expression resembled that of the *abrB spo0H* strain (AGS352) (Fig. 4D). These data indicate that Spo0A does not act exclusively through AbrB to activate *tasA* expression and are consistent with the possibility that Spo0A binds the *tasA* promoter. A possible Spo0A-binding site is present between nucleotides −32 and −38 relative to the beginning of the *yqxM* open reading frame.

The finding that *tasA* expression depends on σ^H raised the possibility that *tasA* expression requires σ^H-directed expression of *spo0A* (1, 15). To test this, we measured *tasA-lacZ* expression and TasA steady-state levels in a strain in which *spo0A* is missing its σ^H-dependent promoter (*spo0A*ΔPs), placing *spo0A* expression under the control solely of σ^A (18). We compared β-galactosidase activity in a *spo0A*ΔPs *tasA-lacZ*-bearing strain (AGS367) to the activity in congenic strains harboring either the *tasA-lacZ* fusion (AGS387) or no fusion (JH642). We found that the levels of *tasA-lacZ* expression (Fig. 4E) and TasA synthesis (Table 3) were significantly above background, suggesting that the absolute requirement for σ^H is not due to its action at the *spo0A* promoter. Because the *spo0A*ΔPs strain (AG1242) cannot sporulate (18), this result also supports our view that TasA is not exclusively a sporulation protein.

Role of σ^A in *tasA* transcription. It is possible that *tasA* expression requires Spo0A-dependent σ^A-directed gene expression. To test this, we made use of strains carrying special alleles of *spo0A* (*spo0A*::pSPC101-G227R, *spo0A*::pSPC101-S233P, *spo0A*::pSPC101-F236S, *spo0A*::pSPC101-V240A, or *spo0A*::pSPC101-V240G K265R [4]), encoding versions of Spo0A that are unable to activate the σ^A-dependent genes *spoIIE* and *spoIIG* but are able to direct expression of at least one σ^H-dependent gene (*spoIIA*) to different degrees. We compared *tasA-lacZ* expression and TasA steady-state levels in the wild-type strain (AGS303) and the *spo0A* null mutant strain (AGS361) with strains bearing these altered *spo0A* alleles (AGS362, AGS363, AGS364, AGS365, and AGS366). The levels of *tasA-lacZ* expression (Fig. 4F) and of TasA (Table 3) varied in strains bearing the altered *spo0A* alleles but were significantly above background in all cases. These data are consistent with the possibility that *tasA* expression largely relies on Spo0A acting together with σ^H.

To determine whether the specialized sigma factors σ^B or σ^D (3) are needed for TasA synthesis, we used Western blot analysis to determine if TasA was present in extracts of a *sigB*Δ::*cat* strain (AGS368) or a *sigD*Δ::Kan^r strain (AGS306). In both strains, TasA levels were similar to those in wild type (data not shown), indicating that neither σ^B nor σ^D is absolutely required for TasA production.

Our results suggest that Spo0A and σ^H, directly or indirectly, activate expression of the operon (Fig. 2B). The involvement of σ^H in *tasA* expression is supported by the results of Serrano et al. (17), who showed that *tasA* was expressed during vegetative growth when *spo0H* expression was activated prematurely, using an inducible promoter. The expression of *tasA* in a strain missing the σ^H-directed *spo0A* promoter (*spo0A*ΔPs) argues against the possibility that the major role of σ^H in *tasA* expression is at the level of *spo0A* expression and further implies that high levels of Spo0A are not required for *tasA* expression (18). AbrB is likely to repress both σ^H-dependent and σ^H-independent phases of *tasA* expression, possibly originating from two promoters. Therefore, AbrB appears to serve at least two roles in *tasA* expression. First, AbrB prevents inappropriate *tasA* expression during exponential phase, as it does for many other genes (20). Second, it reduces *tasA* expression during post-exponential-phase growth.

We have not yet identified the start site(s) of transcription of the operon. As yet, primer extension and 5′RACE (rapid amplification of 5′ cDNA ends) methods have yielded ambiguous results. This could be due to a low level of message and/or secondary structure at the 5′ end of the message.

We do not know the significance of the coordinate regulation of *yqxM*, *sipW*, and *tasA*. However, the clustering and coregulation of a specialized signal peptidase gene with one or more substrate-encoding genes may occur more widely than is usually appreciated. Interesting examples of this occur in *Bacillus subtilis* (natto), which is well known to harbor a variety of cryptic plasmids (21, 24). Two such plasmids, pTA1015 and pTA1040, encode proteins very similar to the chromosomally encoded *B. subtilis* signal peptidase SipS (11, 12). Intriguingly, in each of these plasmids, an open reading frame of unknown function, encoding a plausible signal peptide, sits immediately upstream of the signal peptidase gene. Apparently, these pairs of genes form operons. The potentially secreted gene products could be substrates for the encoded signal peptidases. The additional presence of genes encoding response regulator aspartate phosphatases (13, 14) in some of these plasmids suggests a role in post-exponential-phase responses. Possibly, the coordinate expression of a signal peptidase gene with one or more genes encoding substrates provides an important level of control over secretion during early-stationary-phase growth.

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