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Stress-Induced Activation of the σ^B Transcription Factor of *Bacillus subtilis*

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The alternative transcription factor σ^B of *Bacillus subtilis* is activated during the stationary growth phase by a regulatory network responsive to stationary-phase signals. On the basis of the results reported here, we propose that σ^B controls a general stress regulon that is induced when cells encounter a variety of growth-limiting conditions. Expression of genes controlled by σ^B, including the *ctc* gene and the *sigB* operon that codes for σ^B and its associated regulatory proteins, was dramatically induced in both the exponential and stationary phases by environmental challenges known to elicit a general stress response. After cells were subjected to salt stress, the increased expression of *lacZ* transcriptional fusions to the *ctc* and *sigB* genes was entirely dependent on σ^B, and primer extension experiments confirmed that the σ^B-dependent transcriptional start site was used during salt induction of *sigB* operon expression. Western blotting (immunoblotting) experiments measuring the levels of σ^B protein indicated that ethanol addition and heat stress also induced σ^B activity during logarithmic growth. Salt and ethanol induction during logarithmic growth required RsbV, the positive regulator of σ^B activity that is normally necessary for activity in stationary-phase cells. However, heat induction of σ^B activity was largely independent of RsbV, indicating that there are two distinct pathways by which these environmental signals are conveyed to the transcriptional apparatus.

Sensing and adapting to fluctuations in environmental conditions are essential processes for the survival of all cells. In bacteria, alterations of gene expression in response to environmental change are often controlled by the association of alternative sigma (σ) factors with the catalytic core of RNA polymerase, thus reprogramming the promoter recognition specificity of the enzyme. Examples include the *Escherichia coli* heat shock regulon controlled by σ^{32} (47), the *E. coli* general stress regulon controlled by σ^S (19), and the sporulation process of *Bacillus subtilis*, controlled by a cascade of at least six different sigma factors (13, 36). σ^B is an alternative sigma factor of *B. subtilis* which is activated early in the stationary growth phase but is not required for sporulation (4, 12, 24, 31). The exact physiological role of σ^B and the environmental and cellular signals to which it responds remain to be established.

One approach to better understand the σ^B system is to identify and characterize genes that require σ^B for all or part of their expression (*csb* genes). The first *csb* gene discovered was *ctc*, a gene of unknown function whose transcription is strongly induced during stationary phase in a medium rich in glucose and glutamine, conditions that inhibit both the sporulation process and the formation of tricarboxylic acid cycle enzymes (23–25). We have uncovered eight additional *csb* genes, all of which manifest their σ^B-dependent activity in stationary phase under the same growth conditions that strongly induce *ctc* transcription (6, 8, 31, 50). One of these *csb* genes is *sigB* (31), the structural gene for σ^B itself, which lies in an operon with three other genes whose products regulate σ^B activity in response to stationary-phase signals (1, 7, 31). Significantly, the second *csb* gene whose function has been identified encodes UDP-glucose pyrophosphorylase (48). In *E. coli*, this enzyme forms part of the biosynthetic pathway for trehalose, a protective compound required for osmotolerance and thermotolerance in the stationary growth phase (14, 20).

Because these findings suggested that expression of some genes in the σ^B regulon could respond to environmental challenge, we tested the effects of various physiological stresses on σ^B activity. Here we report that, in addition to responding to the stress imposed by entry into the stationary phase, σ^B-dependent gene expression is also induced in both exponential- and stationary-phase cells by the introduction of salt into the growth medium. Heat stress and ethanol addition also significantly increase σ^B-dependent gene expression. We further show that the observed stress response of σ^B-dependent genes is a result of an increase in σ^B activity, controlled in part by products of the regulatory network previously found to be important for the transmission of stationary-phase signals to σ^B.

MATERIALS AND METHODS

Bacterial strains and genetic methods. *B. subtilis* strains used are shown in Table 1. For strain constructions, *B. subtilis* was made competent for natural transformation as described by Dubnau and Davidoff-Abelson (10). We converted the *cat* marker of strain PB198 to *kan* by transformation with pIK105 (27) and converted the *cat* insertion of strain PB153 to *spc* by transformation with pJL62 (34), kindly provided by John LeDeaux and Alan Grossman. Standard recombinant DNA methods were performed as described by Davis et al. (9), polymerase chain reactions were done according to published protocols (26), and DNA sequencing reactions were done by the dideoxynucleotide chain termination method as previously described (48).

Enzyme assays. We indirectly assayed σ^B activity at the *ctc* (24, 43) and *sigB* operon (31) promoters by employing transcriptional fusions to the *lacZ* reporter gene carried by the pDH32 integrational vector (22). The *ctc* promoter was contained on the 149-bp *Eco*RI-*Hin*dIII fragment described previously (7). The *sigB* promoter was contained on the 337-bp fragment that extends from the *Pst*I site 130 nucleotides upstream from the −35 region to the *Afl*II site within *rsbV* (31, 50). We inserted these constructions in single copy by a double

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TABLE 1. *B. subtilis* strains

Strain	Genotype	Reference or construction[a]
PB2	*trpc2*	Wild-type Marburg strain
PB153	*sigBΔ2::cat trpC2*	8
PB198	*amyE*::pDH32-*ctc trpC2*	7
PB205	*rsbVΔ1*	7
PB206	*amyE*::pDH32-*ctc rsbVΔ1*	7
PB217	*amyE*::pDH32-*ctc rsbVΔ1 rsbX::ery*	7
PB283	*amyE*::pDH32-*minCD trpC2*	35
PB286	*amyE*::pDH32-*sigB trpC2*	50
PB344	*sigBΔ3::spc trpC2*	pJL62 → PB153
PB345	*amyE*::pDH32-*ctc sigBΔ3::spc trpC2*	PB344 → PB198
PB346	*amyE*::pDH32-*sigB sigBΔ3::spc trpC2*	PB344 → PB286
PB347	*amyE*::pDH32-*ctc* (*kan*)[b] *trpC2*	pIK105 → PB198
PB348	*amyE*::pDH32-*ctc sigB4*	This study
PB349	*amyE*::pDH32-*ctc* (*kan*) *rsbVΔ1 sigB4*	PB217 + PB347 → PB348
PB354	*amyE*::pDH32-*sigB rsbVΔ1 trpC2*	PB205 + PB286 → PB2

[a] The arrow indicates transformation from donor to recipient.
[b] (*kan*) indicates that the *cat* marker of the pDH32 vector carried by strains PB198 and PB349 has been converted to *kan*.

crossover event between the *amy* front and back sequences of the pDH32 vector and the *amyE* locus in the *B. subtilis* chromosome. *B. subtilis* cells bearing these fusions were grown in Luria broth (LB) containing no salt and buffered to pH 6.9 with 21 mM K_2HPO_4 and 11 mM KH_2PO_4. Similarly buffered rich medium has been used in studies of *E. coli* osmoregulation (32). When cells reached mid-logarithmic growth they were diluted 1:25 into fresh medium. During either logarithmic growth or the early stationary phase, an environmental stress was imposed by adding to the culture medium either NaCl (0.3 M, final concentration), ethanol (4%, final concentration, vol/vol), or peroxide (0.005%, final concentration, vol/vol). Cell samples were treated essentially as described by Miller (40) for the assay of β-galactosidase activity. Cells were first washed with Z buffer and then permealized with sodium dodecyl sulfate and chloroform. Protein levels were determined on whole-cell samples with the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Richmond, Calif.). Activity was defined as $\Delta A_{420} \times 1{,}000$ per minute per milligram of protein.

Mapping the 5′ end of *sigB* message. RNA was prepared as previously described (48), with the following modifications. PB2 cells were grown in two parallel cultures containing buffered LB. During logarithmic growth NaCl was added to one culture at a final concentration of 0.3 M. Incubation was continued for another 15 min, after which cells from both cultures were harvested for RNA extraction. A 16-base oligomer previously used to map the 5′ end of *sigB* mRNA (31) was 5′ end labeled with [γ-^{32}P]dATP (3,000 Ci/mmol; Amersham) and T4 polynucleotide kinase (Promega Corp., Madison, Wis.). Primer annealing and extension were performed by using the Promega primer extension system essentially according to the manufacturer's instructions, except that 50 μg of RNA and 5 ng of end-labeled primer were used in a 40-μl reaction volume, denaturation was at 85°C for 5 min, and reannealing was at 42°C for 3 h.

Epitope tagging of the σ^B protein. We added the oligonucleotide 5′ GACTACAAGGACGACGATGACAAG 3′ between the codons specifying Pro-259 and Ser-260 of the *sigB* gene (12), using the *dut-ung E. coli* strain (33) as described in the Muta-Gene kit (Bio-Rad). This oligonucleotide encodes the FLAG epitope DYKDDDDK, recognized by the M2 anti-FLAG monoclonal antibody (International Biotechnologies, Inc., New Haven, Conn.). On the basis of the phenotypes caused by C-terminal truncations of the similar σ^F protein (42), the in-frame insertion of the FLAG epitope at this C-terminal position of σ^B was not expected to interfere with function. This position was also chosen to have minimal influence on expression of the *rsbX* gene, which lies immediately downstream of *sigB* and which encodes a negative regulator of σ^B activity (12, 24, 31). Consistent with these expectations, after the chromosomal copy of the wild-type *sigB* allele was replaced with the epitope-tagged *sigB4* allele, the resulting strain PB348 was indistinguishable from the wild type with regard to regulation of *csb* gene expression.

RESULTS

σ^B activity increases after salt addition to stationary-phase and logarithmically growing cells. The σ^B-dependent expression of all known *csb* genes is primarily manifest in the stationary growth phase (6, 8, 25, 31). The discovery that one *csb* gene encodes UDP-glucose pyrophosphorylase suggested that expression of some of these genes might respond to other kinds of environmental stresses in addition to the stress imposed by entry into the stationary phase (48).

Consistent with this notion, our initial experiments indicated that most of the nine available *csb-lacZ* transcriptional fusions showed a dramatic increase in expression when the strains harboring them were grown on solid medium containing 0.3 M NaCl (5). Figure 1A shows that the level of stationary-phase expression from the well-characterized *ctc* promoter increased about 25-fold with the addition of salt to cells growing in buffered LB medium and that this increase in expression was completely dependent on σ^B. Similar results were seen after salt addition to cells growing in a defined minimal glucose medium (data not shown).

The observation that the expression of most known *csb* genes was salt inducible suggested that σ^B itself might be central to this response. Because the *sigB* operon is autogenously regulated by σ^B (31), we wished to determine whether salt caused an increase in stationary-phase transcription from the σ^B-dependent *sigB* promoter. As shown in Fig. 1B, addition of salt elicited a 15-fold increase in σ^B activity measured at this promoter, compared to the activity seen in an identical culture that did not receive salt. The *minCD* promoter is also expressed in the stationary phase but is under control of σ^H rather than σ^B (35). Expression from this promoter was

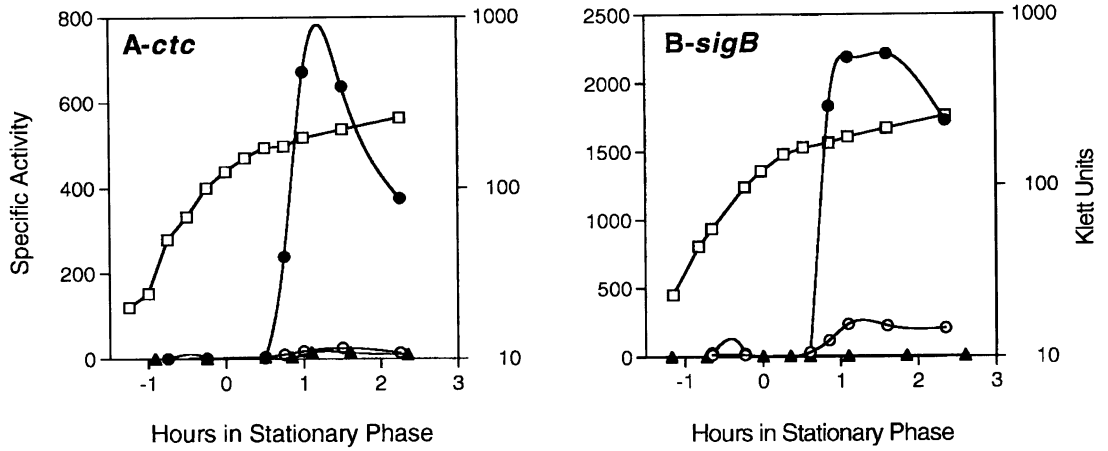

FIG. 1. σ^B-dependent activity of *lacZ* fusions after salt addition to stationary-phase cells. σ^B activity was measured by monitoring β-galactosidase production from single-copy transcriptional fusions to either the *ctc* promoter (A) or the *sigB* operon promoter (B). *B. subtilis* strains harboring these fusions were grown in buffered LB medium, and 0.3 M NaCl was added to one of two parallel cultures as the cells entered stationary phase. Samples were removed at the indicated times and assayed for β-galactosidase activity. (A) PB198 (*amyE*::pDH32-*ctc*) with (●) and without (○) added NaCl and PB345 (*amyE*::pDH32-*ctc sigBΔ3*) with added NaCl (▲). (B) PB286 (*amyE*::pDH32-*sigB*) with (●) and without (○) added NaCl and PB346 (*amyE*::pDH32-*sigB sigBΔ3*) with added NaCl (▲). Growth of the unstressed control strains is indicated (□) in each panel.

unaffected by salt addition (data not shown), indicating that the increased expression of σ^B-dependent genes was not due to a general effect of salt on stationary-phase promoters.

Salt induction of σ^B activity was even more pronounced during logarithmic growth, when σ^B is normally inactive (1, 6–8, 25, 31). As shown in Fig. 2, a rapid induction was observed with both the *ctc* and *sigB* promoters, with maximum β-galactosidase accumulation occurring between 15 and 30 min after salt addition. This induction was completely dependent on σ^B. Transcription of promoters that are σ^B independent, such as the *minCD* promoter, was insensitive to salt addition during logarithmic growth (data not shown).

Levels of *sigB* operon message increase during salt stress. We inferred from the transcriptional fusion data that salt addition increased the activity of σ^B. To directly examine the effect of salt on σ^B activity in logarithmically growing cells, we mapped the 5′ end of *sigB* operon message by primer extension. As shown in Fig. 3, the levels of *sigB* operon message increased upon salt addition, paralleling the salt induction of σ^B activity measured by β-galactosidase accumulation (Fig. 2). Furthermore, the sole site of transcriptional initiation found in these salt-induced, logarithmically growing cells corresponded exactly to the previously identified σ^B-dependent site that is used when cells enter the stationary growth phase (31).

Levels of the σ^B protein increase in response to salt, heat, or ethanol stress. The increased transcription of the autogenously regulated *sigB* operon suggested that one consequence of salt-induced activation of σ^B might be an increased amount of σ^B protein in the cell. For these experiments, σ^B was detected on Western blots (immunoblots) by means of an epitope tag engineered within the carboxyl terminus of the protein. As shown in Fig. 4, the addition of salt to exponentially growing

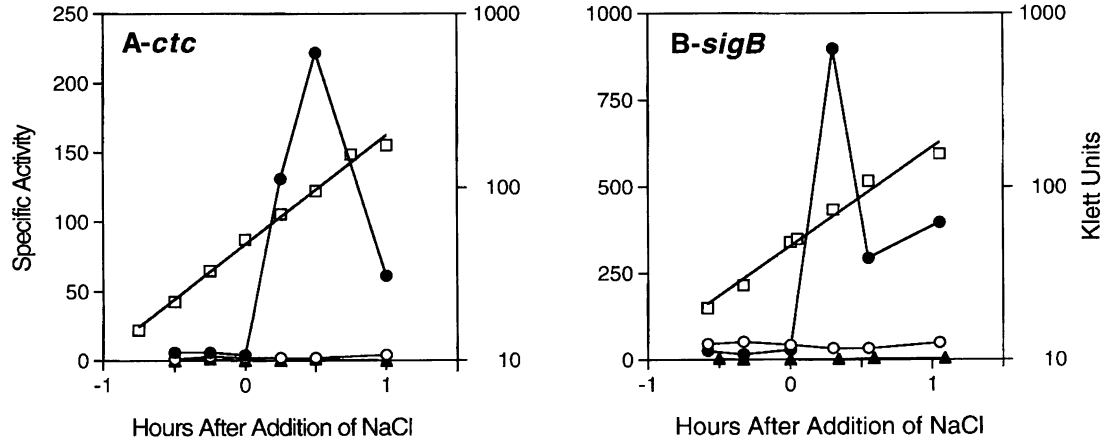

FIG. 2. σ^B-dependent activity of *lacZ* fusions after salt addition to logarithmically growing cells. σ^B activity was measured as described in the legend to Fig. 1, but 0.3 M NaCl was added to one of two parallel cultures in mid-logarithmic growth (time zero). (A) PB198 (*amyE*::pDH32-*ctc*) with (●) and without (○) added NaCl and PB345 (*amyE*::pDH32-*ctc sigBΔ3*) with added NaCl (▲). (B) PB286 (*amyE*::pDH32-*sigB*) with (●) and without (○) added NaCl and PB346 (*amyE*::pDH32-*sigB sigBΔ3*) with added NaCl (▲). Growth of the unstressed control strains is indicated (□) in each panel.

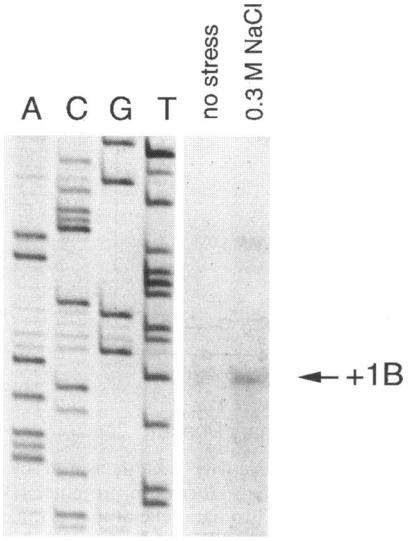

FIG. 3. Primer extension analysis of *sigB* operon message after salt induction. Two parallel cultures of wild-type strain PB2 were grown in buffered LB medium, and 0.3 M NaCl was added to one during mid-logarithmic growth. Cells from both cultures were harvested 15 min after NaCl addition, and the RNA was extracted. Primer extensions were done with a molar excess of a 16-nucleotide primer complementary to a region downstream from the site of transcriptional initiation for the *sigB* operon (31). Samples containing 25 μg of RNA were loaded onto adjacent lanes of a sequencing gel. Lanes A, C, G, and T comprise a dideoxynucleotide sequencing ladder run in parallel, with the same 16-nucleotide primer. +1B, the σ^B-dependent 5′ end of *sigB* operon message (31).

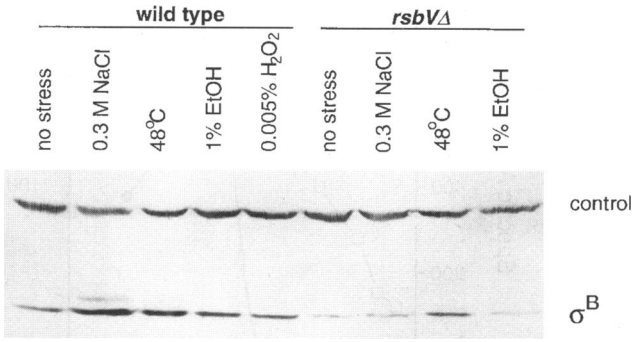

FIG. 4. Western blot of σ^B levels after induction with salt, heat, ethanol (EtOH), or oxidative stress. Parallel cultures of PB348 (*sigB4*) were grown in buffered LB medium at 37°C, and the indicated stresses were imposed during mid-logarithmic growth. After 15 min the cells were harvested, resuspended in buffer A (10 mM Tris [pH 7.4], 5 mM $MgCl_2$, 100 mM EDTA, and 100 μg of phenylmethylsulfonyl fluoride per ml), and broken by sonication. Cell debris was removed from the crude extracts by centrifugation. Samples containing 100 μg of cellular protein were separated on a sodium dodecyl sulfate–10% polyacrylamide gel and transferred to a Zeta Probe blotting membrane (Bio-Rad). σ^B protein was detected with a monoclonal antibody that recognizes the FLAG epitope; this epitope was added within the carboxyl-terminal portion of the protein encoded by the *sigB4* allele. Bound antibody was visualized with alkaline phosphatase-conjugated rabbit anti-mouse antibody (Sigma Chemical Co., St. Louis, Mo.). As a control, a constant amount of epitope-tagged bacterial alkaline phosphatase (Immunex Corp.) was added to each extract before gel separation and blotting. To determine the role of the RsbV positive regulator in these responses, the indicated stresses were also imposed on PB349 (*rsbVΔ1 sigB4*). No bands were detected in an extract of the wild-type strain PB2, which lacks the epitope tag (not shown).

wild-type cells caused a rapid increase in the level of σ^B protein relative to the level of total cell protein. Thus, the activity of σ^B, the level of transcript from the *sigB* operon promoter, and the amount of σ^B protein all increase in response to salt.

We took advantage of this assay system to further suggest that heat stress and ethanol addition increase σ^B levels (Fig. 4). In contrast, oxidative stress brought about by the addition of peroxide did not produce a significant change in σ^B levels. We confirmed the induction by ethanol stress as well as the lack of induction by oxidative stress with β-galactosidase assays of the *sigB-lacZ* transcriptional fusion, shown in Fig. 5. The increase in σ^B levels elicited by heat stress was paralleled by an increase in *sigB* operon message (not shown), corroborating the findings of Benson and Haldenwang (3). Together these results indicate that σ^B provides one route through which signals of salt, heat, and ethanol stress are conveyed to the transcription apparatus.

Induction of σ^B activity by salt and ethanol stress requires the RsbV positive regulator. Previous genetic analysis identified a regulatory network that modulates σ^B activity posttranslationally in response to stationary-phase signals (1, 7). This analysis indicated that the RsbW protein is a key regulator of σ^B activity and that RsbW is responsible for rendering σ^B inactive during logarithmic growth (7). Subsequent biochemical analysis has shown that RsbW is an anti-σ factor that binds directly to σ^B and negatively controls its activity through an as yet unknown mechanism (2). The RsbV protein counters the effect of RsbW and thus acts as a positive regulator that is normally required for σ^B activity to appear in the stationary phase (1, 7).

In order to assess the role of this network in transmitting environmental signals to σ^B, we subjected a strain carrying an in-frame deletion of the *rsbV* gene to the same stresses that increased σ^B levels in wild-type cells and then assayed σ^B levels by Western blotting. As shown in Fig. 4, the *rsbV* deletion eliminated the inducing effects of salt and ethanol addition, suggesting that these stresses require an intact RsbV protein in order to activate σ^B. In contrast and as predicted by the results of Benson and Haldenwang (3), the *rsbV* deletion had a much less pronounced effect on heat induction. On the basis of the level of bound antibody in Fig. 4, both the uninduced level of σ^B and the level following heat induction were much lower in the mutant than in wild-type cells, yet the induction ratio appeared similar in both strains. Confirming the results of the Western blot experiment, the fusion assay results in Fig. 5 clearly show that both the salt induction and ethanol induction of σ^B activity require the *rsbV* product. From the results shown in Fig. 4 and 5, we conclude that the signaling pathway for heat stress is not entirely congruent with that for salt and ethanol.

DISCUSSION

Bacteria undergo elaborate adaptive responses to starvation (13, 19, 30, 36, 38, 46). In *Bacillus subtilis*, study of this adaptive response has largely focused on the process of endospore formation and on the signaling networks that prevent its inappropriate triggering (see references 13 and 36 for reviews). Our results suggest that the alternate transcription factor σ^B of *B. subtilis*, together with its associated regulatory network, may provide a less extreme alternative to sporulation under growth-limiting conditions. This alternative might become of critical importance under environmental conditions that do not support the sporulation process.

In *E. coli* the response to stationary phase involves major

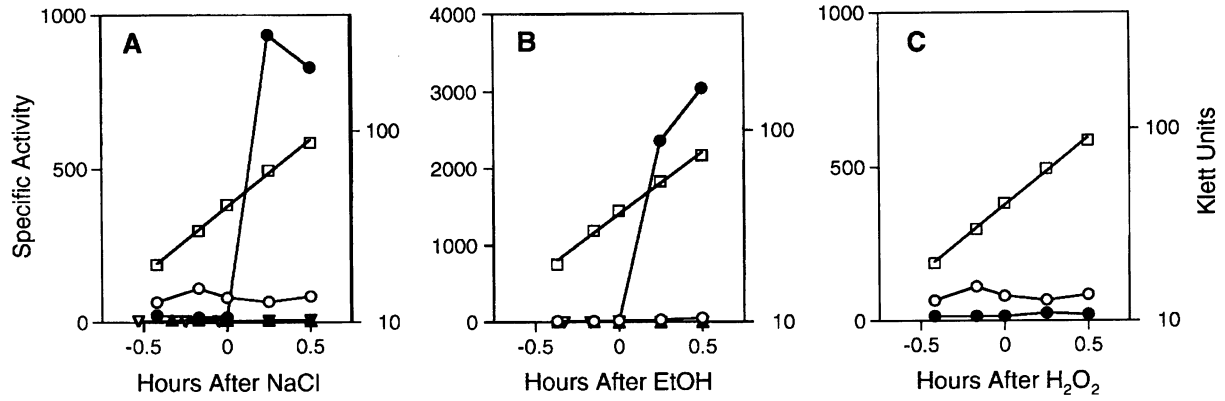

FIG. 5. σ^B-dependent activity of *lacZ* fusions in logarithmically growing cells subjected to environmental stress. σ^B activity was measured by monitoring β-galactosidase production from a single-copy transcriptional fusion to the *sigB* operon promoter. Strains were grown in buffered LB medium, and either 0.3 M NaCl (A), 4% ethanol (EtOH) (B), or 0.005% peroxide (C) was added to parallel cultures in mid-logarithmic growth (time zero). Samples were removed at the indicated times and assayed for β-galactosidase activity. Strains subjected to stress were PB286 (*amyE*::pDH32-*sigB*; ●), PB346 (*amyE*::pDH32-*sigB sigBΔ3*; ▲), and PB354 (*amyE*::pDH32-*sigB rsbVΔ1*; ▽). Following salt stress (A) or ethanol stress (B), the β-galactosidase activities of strains PB346 (*sigBΔ3*) and PB354 (*rsbVΔ1*) were equally low and indistinguishable from the background activity of a *B. subtilis* strain bearing no fusion. β-Galactosidase activity (○) and growth (□) of the unstressed PB286 control are also shown in each panel.

changes in cell physiology, in the cell envelope, and in membrane composition (46). Significantly, a distinct set of proteins is induced in stationary-phase *E. coli* cells (15). Because such cells are more resistant to osmotic stress, heat shock, and oxidative stress than are logarithmically growing cells, this set of proteins is thought to provide general stress protection (28, 29, 38). Several genes important for this starvation-induced general stress protection are under control of the stationary-phase σ factor encoded by *rpoS* (see reference 19 for a review).

The products of some σ^S-dependent genes also play a protective role when logarithmically growing *E. coli* cells are challenged by osmotic or thermal shock, producing a state of general stress resistance approaching that seen in stationary-phase cells (20, 21, 39). This state is also characterized by cross-resistance, in which cells challenged first by a tolerable level of one stress become resistant to a normally lethal challenge by another. One attractive explanation for the general stress response is that however the growth-limiting condition is imposed, whether by entry into stationary phase or by environmental challenge in logarithmic growth, the cell must prepare in advance to counter stresses to which it has not yet been exposed, with the aim of establishing a more resistant state before resources become unduly limiting (38, 46).

Like σ^S of *E. coli*, *B. subtilis* σ^B also controls a large, stationary-phase regulon (6), and we suggested previously that some genes in the σ^B regulon might play a role in countering environmental stresses imposed on nongrowing cells (48). Consistent with this notion, we show here that σ^B activity increases in response to salt, heat, and ethanol shock. Furthermore, we observed this stress response in the logarithmic as well as the stationary growth phase. Because σ^B activity is induced both upon entry into the stationary phase and by environmental stress during logarithmic growth, the attractive possibility arises that σ^B controls a regulon important for general stress resistance in *B. subtilis*.

The work of Hecker and his colleagues (16–18, 44) has identified such a general stress regulon in *B. subtilis*. On two-dimensional gels, a common set of proteins was found to be induced in response to salt, heat, ethanol, or hydrogen peroxide stress or by amino acid, carbon, or oxygen limitation. Furthermore, the salt and heat responses in *B. subtilis* provide a cross-resistance similar to that seen in the *E. coli* system (49). Although we think it likely that the synthesis of at least some of these proteins is directly controlled by σ^B, the fact that hydrogen peroxide stress does not induce σ^B activity (but does induce the general stress regulon) indicates that there must be additional regulation on this system. Furthermore, we have not yet found an environmental condition in which a *sigB* null mutant is at a survival disadvantage compared to the wild type, including osmotic shock or heat shock of logarithmic- or stationary-phase cells or extreme desiccation (5). Although it is possible that we have not yet created a laboratory condition that mimics the challenges encountered by *B. subtilis* in its soil environment, it is also possible that any general stress proteins controlled by σ^B compose part of a redundant system.

We have identified three new environmental signals that activate σ^B, and we have presented evidence demonstrating that the regulatory network known to control σ^B activity in response to stationary-phase signals is also required for σ^B induction following salt or ethanol stress. In contrast, the signal for heat induction at least partially bypasses this network, a result initially discovered by Benson and Haldenwang (3) and confirmed by our work. In Fig. 6 we present one model for how these environmental signals are conveyed to σ^B. Because the RsbW anti-σ factor is a central regulator of σ^B activity that responds to stationary-phase signals (1, 2, 7), the simplest interpretation suggests that RsbW also serves to transmit other signals of environmental challenge to the transcriptional apparatus.

The activity of another stationary-phase transcription factor, σ^F, is controlled in part by the same molecular mechanism as σ^B, the details of which are under active investigation (11, 41, 45). Evidence indicates that σ^F is responsible for initiating gene expression in the forespore compartment of the developing sporangium and therefore controls a critical juncture of the sporulation program (37). Both σ^B and σ^F are present at low but detectable levels in logarithmically growing cells, well before the induction of σ^B activity by stress or the induction of σ^F activity by formation of the asymmetric sporulation septum. Because σ^F and σ^B are rendered inactive during logarithmic growth by their cognate anti-σ factors, it is attractive to consider that each might be poised to counter adverse envi-

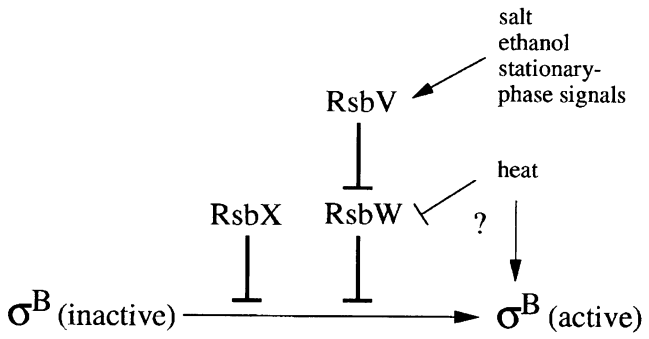

FIG. 6. Model of σ^B activation in response to environmental stress. The gene products of the *sigB* operon form an ordered regulatory pathway, with the RsbW anti-σ factor acting as the central negative regulator of σ^B activity and the RsbV protein countering the effects of RsbW when cells enter stationary phase (1, 2, 7). As shown in Fig. 4 and 5, the pathways for transduction of the salt and ethanol signals also require RsbV. In contrast, heat stress activates σ^B through an RsbV-independent mechanism, either by directly inactivating RsbW (light T-shaped line) or by means of another signaling pathway which remains to be identified (light arrow). The inactivation of RsbW and its subsequent σ^B-dependent resynthesis could provide a homeostatic mechanism for precise control of σ^B activity.

ronmental conditions, either by irrevocably launching the sporulation process in the case of σ^F or by inducing a general stress regulon in the case of σ^B. Given the different roles of these two σ factors in stationary-phase physiology and their similar modes of regulation, it will be intriguing to compare the environmental and cellular signals that lead to their activation.

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