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The σ^B-Dependent Promoter of the *Bacillus subtilis sigB* Operon Is Induced by Heat Shock

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σ^B, a secondary sigma factor of *Bacillus subtilis*, was found to increase 5- to 10-fold when cultures were shifted from 37 to 48°C. Western blot (immunoblot) analyses, in which monoclonal antibodies specific for the *sigB* operon products RsbV, RsbW, and σ^B were used to probe extracts from wild-type and mutant *B. subtilis* strains, revealed that all three proteins increased coordinately after heat shock and that this increase was dependent on σ^B but not RsbV, a positive regulator normally essential for σ^B-dependent *sigB* expression. Nuclease protection experiments of RNA synthesized after heat shock supported the notion that the shift to 48°C enhanced transcription from the *sigB* operon's σ^B-dependent promoter. The level of mRNA initiating at the σ^B-dependent *ctc* promoter was also seen to increase approximately 5- to 10-fold after heat shock. Pulse-labeling of the proteins synthesized after a shift to 48°C demonstrated that *sigB* wild-type and mutant strains produced the major heat-inducible proteins in similar amounts; however, at least seven additional proteins were present after the temperature shift in the wild-type strain but absent in the *sigB* null mutant. Thus, although σ^B is not required for the expression of essential heat shock genes, it is activated by heat shock to elevate its own synthesis and possibly the synthesis of several other heat-inducible proteins.

σ^B is a minor abundance sigma factor of *Bacillus subtilis* that is found associated with the RNA polymerase from vegetatively growing bacteria but which disappears from the extractable RNA polymerase population by the second hour after the onset of sporulation (12). Although σ^B was the first secondary sigma factor to be discovered in bacteria, its physiological role remains obscure (6, 9, 13). Null mutations in the σ^B structural gene (*sigB*) have no obvious effect on vegetative growth or sporulation (6, 9).

Even though the function of σ^B is not yet known, it is a potent regulatory protein with an elaborate mechanism for keeping its activity in check. *sigB* is the third gene in a four-gene operon with the other genes, *rsbV*, *rsbW*, and *rsbX* (formerly called *orfV*, *orfW*, and *orfX*, respectively), involved in controlling σ^B activity (3, 4, 7). Previous work suggested that the three gene products operate in a pathway of negative control, with RsbW being the primary inhibitor of σ^B activity and the other two gene products (RsbV and RsbX) functioning upstream of RsbW in the regulatory pathway (3, 7). Mutations which reduce RsbW's inhibitory effects severely impair the growth of strains which carry them and readily give rise to suppressor mutations which reduce or eliminate the activity of σ^B in these strains (2, 3, 7, 15). In addition to the *sigB* operon itself (3, 17), two genes (*ctc* and *csbA*) which depend on σ^B for a significant part of their expression have been identified (8, 14, 16). Both of these genes are maximally expressed at the end of the exponential phase of growth in media (Luria broth [LB] plus glucose and glutamine) that suppresses both sporulation and the enzymes of the tricarboxylic acid cycle (8, 14, 16). The roles of *ctc* and *csbA*, like that of σ^B itself, are undefined. Neither gene is essential for growth or sporulation (8, 28). The only phenotype observed after a loss of one of these genes is temperature-sensitive oligosporageny which occurred upon disruption of *ctc* by an integrating plasmid (pAK-6/p1949) (28). Placement of this *ctc* null mutation into a strain that carried a mutation in the closely linked but distinct *spoVC* gene (*spoVC285*) resulted in a growth as well as a sporulation defect at elevated temperatures. Growth of the *ctc*::pAK-6/p1949 *spoVC285* strain was transiently inhibited when exponential cultures were shifted from 37 to 48°C (28).

In *Escherichia coli*, proteins induced in response to thermal stress (heat shock) are encoded by a regulon that is transcribed by a form of RNA polymerase which contains a minor abundance secondary sigma factor (σ^{32}) (10, 11). The temperature-sensitive phenotype of the *ctc* mutant initially suggested that σ^B could be the *B. subtilis* counterpart of σ^{32}; however, additional experiments did not support this model. In contrast to *E. coli*, in which σ^{32} is critical to cell viability at elevated temperatures (30), *B. subtilis* strains with null mutations in *sigB* are no more temperature sensitive then their wild-type counterparts (28). The dispensability of σ^B to *B. subtilis* survival at elevated temperatures argues that σ^B isn't equivalent to σ^{32} and that if it plays a role during heat shock, this role is not critical. Recently, we generated a monoclonal antibody against σ^B and in this paper describe its use to monitor σ^B levels in response to temperature increase. Western blot (immunoblot) analyses revealed that σ^B is a heat-inducible protein, with its levels undergoing a rapid rise when *B. subtilis* cultures are shifted from 37 to 48°C. The rise in σ^B levels is due to enhanced transcription from the *sigB* operon's σ^B-dependent promoter, which appears to be activated during heat shock by a distinctly different mechanism from that which activates this promoter at the end of exponential growth. In addition, we discovered that although σ^B is not essential for the major elements of the *B. subtilis* heat shock response, it does contribute to the pattern of protein synthesis following heat shock. At least seven proteins, which are normally heat inducible, fail to become elevated in the absence of σ^B.

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MATERIALS AND METHODS

Bacterial strains and culture media. All *B. subtilis* strains used in this project were derived from PY22 that was originally obtained from P. Youngman (University of Georgia). Construction of BSA80 (Km^r *sigB314*) and BSA73 (Km^r *rsbV312*) has been previously described (3). Cells were grown in LB medium (20) with 0.5% NaCl for Western blot and S1 nuclease analysis and glucose minimum medium (SPI) (23) or Methionine Assay Medium (Difco Laboratories) for pulse-labeling of proteins.

Western blot analysis. Crude cell extracts were prepared from *B. subtilis* and fractionated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE; 12% acrylamide) as done previously (26). After electrophoretic transfer to nitrocellulose and blocking of the nitrocellulose with BLOTTO, the immobilized protein bands were probed with anti-σ^B, anti-RsbW, or anti-RsbV monoclonal antibodies. The anti-RsbV and anti-RsbW antibodies were prepared and analyzed for specificity as was previously described for the anti-σ^B monoclonal antibody (4). Bound antibody was visualized with an alkaline phosphatase-conjugated goat immunoglobulin against mouse immunoglobulin (Hyclone Laboratories, Inc.)

S1 nuclease analysis. RNA was prepared from logarithmically growing and heat-shocked *B. subtilis* by standard methods and stored at −70°C as an ethanol precipitate (27). RNA (50 μg) was hybridized to uniformly labeled single-stranded DNA probes which were synthesized with either an oligonucleotide (GTTTACTTGTATATC) that primed antisense DNA synthesis at nucleotide 316 of the σ^B-dependent *sigB* mRNA or an oligonucleotide (GCCTTCGTCCCAC AGCG) that primed DNA synthesis at nucleotide 348 of the σ^B-dependent *ctc* mRNA from DNA fragments cloned into M13mp18 (29). The hybridization conditions and nuclease treatment were as described by Konyecsni and Deretic (18). Protected, labeled DNA was denatured and analyzed on a DNA sequencing gel (8% acrylamide).

Dot blot analysis of RNA. Dot blot hybridizations were performed as previously described (27). Dilutions of RNA were spotted onto nitrocellulose. After heat fixing the RNA to the nitrocellulose and incubating the membrane in a blocking solution, the immobilized RNA was hybridized to the same probes that were used in the S1 analysis.

Heat shock pulse-labeling. *B. subtilis* cells were grown in SPI glucose minimal medium (23) supplemented with 20 mM $MgSO_4$ or Difco Methionine Assay Medium at 37°C to an A_{540} of 0.30. A portion of the culture was shifted to 48°C and pulse-labeled with EXPRE^{35}S^{35}S [^{35}S] (New England Nuclear/Dupont, Wilmington, Del.) protein labeling mix (0.5 μCi/ml, 1,151 Ci/mmol) for 5 min at different times after transfer. Samples were lysed as described by Arnosti et al. (1) and fractionated by SDS-PAGE. Labeled protein bands were visualized by fluorography with Amplify (Amersham Corp., Arlington Heights, Ill.) in accordance with the manufacturer's instructions.

DNA sequencing. DNA sequencing was performed by the Sanger method (21) with Sequenase reagents (U.S. Biochemical Corp.) and the protocol provided by the manufacturer.

RESULTS

Elevation of σ^B levels in response to temperature. *B. subtilis* strains which lack σ^B are not temperature sensitive (2). Nevertheless, a plasmid insertion into the σ^B-dependent *ctc* gene confers a subtle temperature-dependent phenotype on strains which carry it (2, 28). This observation implies that although σ^B is not essential for cell viability during heat shock, at least a part of its regulon might still participate in the cell's response to thermal stress. If this is true, then the level of σ^B might fluctuate in response to temperature change. We tested this idea by incubating a wild-type *B. subtilis* strain in LB medium and shifting it to elevated temperatures during exponential growth. After 20 min of continued incubation, the cultures were harvested and analyzed by Western blot for the accumulation of σ^B protein. Figure 1 illustrates our results and demonstrates that the level of σ^B protein increases relative to total cell protein in the culture when it is shifted to 42 or 48°C over that seen in the culture when maintained at 37°C but decreases in the cultures shifted to 55 or 63°C. Maximum σ^B accumulation occurred in the culture moved to 48°C, in which, on the basis of the level of antibody bound, the abundance of σ^B rose approximately 10-fold over that seen in the culture maintained at 37°C. Thus, transfer of *B. subtilis* to a temperature which induces the heat shock response in this organism (1) elevates the level of σ^B.

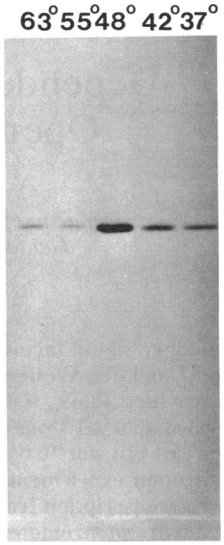

FIG. 1. Accumulation of σ^B after heat shock at various temperatures. Crude lysates were prepared from cultures of a wild-type (PY22) *B. subtilis* strain that had been grown in LB and shifted during exponential growth from 37°C to 42, 48, 55, or 63°C. Samples were harvested from the cultures after 20 min of exposure to the elevated temperature. Total proteins (100 μg) from the lysates were fractionated by SDS-PAGE, transferred to nitrocellulose, and probed with the anti-σ^B monoclonal antibody. Bound antibody was detected with alkaline phosphatase-conjugated goat anti-mouse immunoglobulin. The single band on the Western blot corresponds to σ^B.

Dependence of σ^B accumulation of *sigB* operon gene products. The principal promoter of the *sigB* operon is σ^B dependent and regulated by the products of the other genes (*rsbV*, *rsbW*, and *rsbX*) of the operon (3, 4, 7). Our previous data suggested that the *rsbW* gene product is the primary inhibitor of σ^B-dependent transcription, with *rsbV* gene product needed to counteract this inhibition (3). In the absence of RsbV, the *sigB* operon's σ^B-dependent promoter is not expressed because of RsbW inhibition. We were therefore interested in learning whether the heightened levels of σ^B seen during heat shock might be affected by these

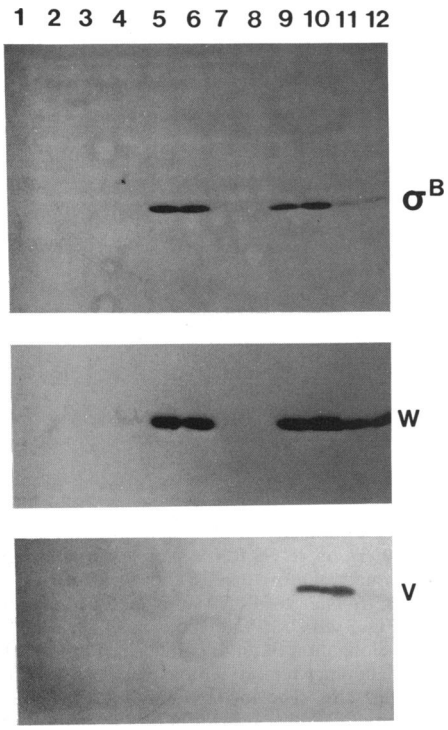

FIG. 2. Accumulation of σ^B, RsbV, and RsbW in wild-type and mutant strains of *B. subtilis* after heat shock. Strains PY22 (wild type, lanes 9 to 12), BSA73 (*rsbV312*, lanes 5 to 8), and BSA69 (*sigB314*, lanes 1 to 4) were grown in LB to mid-exponential phase and shifted to 48°C. Samples were harvested from the 37°C cultures at the time of the temperature shift (lanes 4, 8, and 12) and 1 h thereafter (lanes 3, 7, and 11), while samples derived from the heat-shocked cultures were harvested 30 min (lanes 2, 6, and 10) and 1 h (lanes 1, 5, and 9) after the temperature shift. Crude extracts were prepared, and 100 μg of total protein from each extract was fractionated by SDS-PAGE, transferred to nitrocellulose sheets, and probed with anti-σ^B, anti-RsbV, or anti-RsbW monoclonal antibodies as for Fig. 1. σ^B, W, and V, positions of σ^B, RsbW, and RsbV, respectively, on the Western blots.

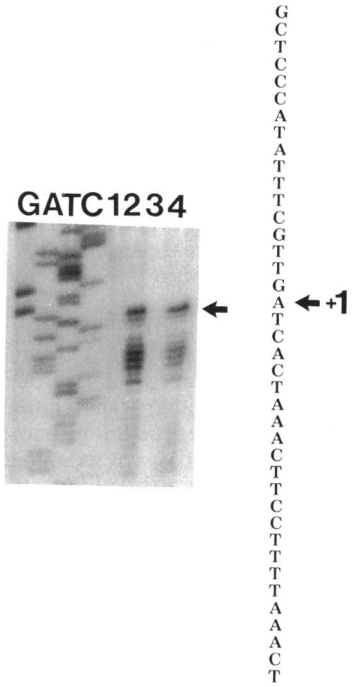

FIG. 3. S1 nuclease analysis of transcription at the σ^B-dependent promoter of the *sigB* operon during growth at 37°C and after heat shock at 48°C. RNA samples were prepared from PY22 (wild type) and BSA73 (*rsbV312*) during exponential growth at 37°C and 20 min after shifting the cultures to 48°C. Total RNA (50 μg), as calculated by spectrophotometry at 260 nm, was hybridized at 67°C to a uniformly labeled, single-stranded DNA probe that was prepared by elongation of an oligonucleotide primer (GTTTACTTGTATATC) which hybridizes to the *sigB* operon transcript at +69 bases. After 1 h of hybridization, unhybridized RNA and DNA were degraded by the addition of 1,000 U of S1 nuclease. The protected fragments were then resolved on an 8% denaturing polyacrylamide sequencing gel alongside a sequencing ladder generated with the same oligonucleotide on an M13 clone carrying a fragment of the *sigB* operon. The sequence shown to the right is that of the noncoding strand, with +1 depicting the σ^B-dependent transcriptional start site (16) which corresponds to the start site of the largest protected fragment on the gel (arrow). Lane 1, PY22, 37°C; lane 2, PY22, 48°C; lane 3, BSA73, 37°C; lane 4, BSA73, 48°C.

regulators. Wild-type or mutant *B. subtilis* strains containing null alleles of either *sigB* (*sigB314*) or *rsbV* (*rsbV312*) were grown at 37°C and then shifted to 48°C and probed in a Western blot analysis for three of the *sigB* operon gene products (σ^B, *RsbV*, and *RsbW*). Figure 2 depicts our findings in three Western blots of identical extract samples probed with anti-σ^B, anti-RsbW, or anti-RsbV antibodies. In a wild-type *B. subtilis* strain, the levels of all three proteins increased in response to the shift to 48°C (Fig. 2, lanes 9 and 10 versus 11 and 12). The coordinate increase in all three proteins is consistent with the heat-induced elevation of σ^B being due to increased expression of the *sigB* operon. The *sigB314* and *rsbV312* alleles do not encode products that can be detected in Western blot analyses (3). Extracts prepared from a *sigB314* strain would therefore be missing σ^B; however, if the *sigB* operon is activated by heat shock in the absence of σ^B, the *rsbV* and *rsbW* products should still be formed. A Western blot analysis of such a strain revealed neither RsbV nor RsbW at 37 or 48°C (Fig. 2, lanes 1 to 4). The absence of these proteins indicates that the heat shock-dependent induction of *sigB* relies on σ^B.

Western blot analysis was next performed on an *rsbV312* strain (Fig. 2, lanes 5 to 8). This strain displays the expected absence of σ^B and RsbW as well as RsbV under normal growth conditions (i.e., 37°C in LB) (Fig. 2, lanes 7 and 8) but unexpectedly shows a heat shock-dependent induction of σ^B and RsbW (Fig. 2, lanes 5 and 6). The appearance of σ^B and RsbW in the *rsbV* null mutant after heat shock when they were not detectable before the temperature increase argues that the heat shock-dependent elevation of the *sigB* operon products reflects new synthesis and not a stabilization of preexisting proteins. In addition, the dependence of the induction on σ^B but not RsbV implies that heat shock can relieve RsbW inhibition of σ^B by an RsbV-independent mechanism.

Effect of heat shock on *sigB* transcription. The dependence of σ^B accumulation after a shift to 48°C on σ^B itself suggests that this increase is due to induced transcription from the *sigB* operon's σ^B-dependent promoter. In order to examine this possibility, we performed an S1 nuclease analysis of the RNA being synthesized from the σ^B-dependent *sigB* promoter either during growth at 37°C or after heat shock. The results of this study are presented as Fig. 3. The σ^B-

dependent promoter of σ^B had been previously shown to direct the initiation of transcription at the nucleotide depicted as 1+ in the figure (16). The largest *sigB*-specific RNA that was present in heat-shocked cells initiated at this site (Fig. 3, lane 2) and was approximately an order of magnitude more abundant than the transcript initiating at this nucleotide during vegetative growth at 37°C (Fig. 3, lane 1). This result is consistent with the notion that the increase in σ^B that we observed as a consequence of a shift form 37 to 48°C is at least partly due to increased *sigB* RNA levels and that this RNA originates at the *sigB* operon's normal σ^B-dependent transcriptional start site.

Although the *sigB* operon's σ^B-dependent promoter normally requires a functional *rsbV* gene product for activity, RsbV is dispensable for σ^B accumulation during heat shock (Fig. 2). In order to ask whether transcription was initiating at the *sigB* operon's σ^B-dependent promoter during heat shock in the absence of RsbV, we examined the levels of RNA initiating at this site in a *B. subtilis* strain carrying an *rsbV* null allele (*rsbV312*). Figure 3 illustrates that although no detectable RNA synthesis initiates from this site in the *rsbV312* strain at 37°C (lane 3), amounts similar to these seen in the strain with the wild-type *rsbV* allele are present when the mutant culture is shifted to 48°C (lane 4). This result, when taken with the observation that heat shock induction of *sigB* requires σ^B (Fig. 2), demonstrates that the shift to 48°C activates transcription of *sigB* at its principal promoter by σ^B-containing RNA polymerase through a mechanism that bypasses the need for using RsbV.

***ctc* transcription during heat shock.** Both *sigB* and *ctc* contain σ^B-dependent promoters (13, 15). Thus, it seemed possible that *ctc*, like *sigB*, might also be inducible by heat shock. Our initial experiments did not support such a view. β-Galactosidase assays of *B. subtilis* extracts that had been prepared from cells in which the *E. coli lacZ* reporter gene was translationally coupled to *ctc* showed a drop in β-galactosidase activity rather than an increase when cultures carrying the fusion were shifted to 48°C (2). Unexpectedly, a transcriptional fusion of *lacZ* to the *sigB* operon displayed a similar drop in β-galactosidase after a shift to 48°C (2). This conflicted with the Western blot and nuclease protection experiments, which clearly indicated heightened activity at *sigB* in response to the temperature shift (Fig. 2 and 3). We therefore considered the possibility that *E. coli* β-galactosidase may not be stable in *B. subtilis* at 48°C and probed the heat-shocked extracts with anti-β-galactosidase antibody in a Western blot analysis. The antibody detected appreciable but highly variable amounts of β-galactosidase in the heat-shocked extracts, with only a poor correlation between the amount of antigen detected and the amount of β-galactosidase activity present in the extract (2). This implied that a significant portion of the enzyme was inactive and unstable in *B. subtilis* at 48°C. We concluded that *lacZ* is an inappropriate reporter system in *B. subtilis* under heat shock conditions and repeated the analysis of the *ctc* response to heat shock with a nuclease protection experiment to identify changes in the *ctc*-specific mRNA originating from its σ^B-dependent promoter. Figure 4 illustrates the results of this experiment, which show a dramatic increase in *ctc*-specific RNA of a size anticipated for that originating from the σ^B-dependent promoter after a shift of the culture from 37 to 48°C (Fig. 4, lower arrow). In order to roughly quantitate the increase in RNA abundance of both the *ctc* and *sigB* messages, we spotted serial dilutions of RNA from cells either growing at 37°C or harvested at different times after transfer to 48°C onto nitrocellulose and probed this RNA

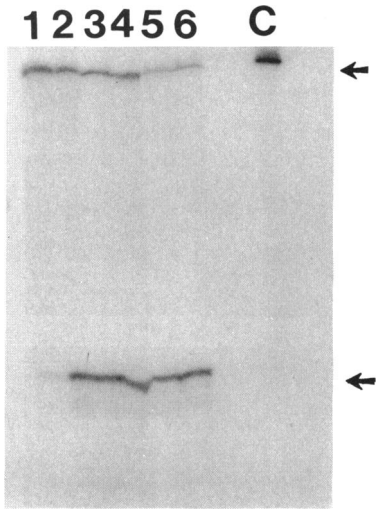

FIG. 4. S1 nuclease analysis of transcription at the *ctc* operon during growth at 37 or 48°C. RNA samples were prepared from PY22 during exponential growth at 37°C (lane 1) and at 5, 10, 20, 30, and 45 min after transfer to 48°C (lanes 2 to 6, respectively) and analyzed as for Fig. 3 with a ^{32}P-labeled, single-stranded DNA probe prepared by the elongation of an oligonucleotide probe that hybridizes to the *ctc* coding region 99 bases downstream from the σ^B-dependent start site. The lower arrow indicates the estimated position of the anticipated *ctc* transcript on the basis of the mobility of denatured, end-labeled, *Hae*III-digested fragments of pUC19 DNA. Lane C, control lane in which probe but no RNA was added to the reaction system. The upper arrow indicates the position of undigested probe DNA.

with a ^{32}P-labeled antisense single-stranded DNA specific for either the *ctc* or *sigB* message. On the basis of the autoradiograms of the bound probes (Fig. 5), it appears that both *ctc* (Fig. 5A) and *sigB* (Fig. 5B) mRNAs increase 5- to 10-fold by 5 to 10 min after the shift to 48°C and remain elevated for 30 to 45 min after the temperature shift. We conclude that the σ^B-dependent promoters of both *sigB* and *ctc* are transiently activated by a shift to 48°C.

Induction of heat shock proteins in a mutant lacking σ^B. The finding that the *sigB* operon is induced by heat shock and that this induction is σ^B dependent implies that σ^B might be a component of the heat shock response in *B. subtilis*. Arnosti et al. characterized the pattern of heat shock-induced protein synthesis in *B. subtilis* and noted the induction of at least 26 distinct heat-inducible proteins when a *B. subtilis* culture was shifted from 37 to 50°C (1). We repeated their experiments with strains of *B. subtilis* that carried either a functional or a mutant *sigB* gene. The cells were grown in minimal glucose medium at 37°C and then transferred to 48°C and pulse-labeled for 5 min with [^{35}S]Met and [^{35}S]Cys at various times after transfer. As depicted in the autoradiogram of the labeled proteins in Fig. 6, the profiles of proteins synthesized at elevated temperatures in both the *sigB* (BSA70)- and *sigB314* (BSA80)-containing strains are similar, including the induction of several prominent protein bands whose mobility is consistent with known *B. subtilis* heat shock gene products (1).

Although the induction of the major heat shock proteins appears to be equivalent in the *sigB* wild-type and mutant strains, longer exposures of the autoradiogram indicated the presence of lower-molecular-weight proteins in the heat-shocked wild-type strain that were absent from the *sigB*

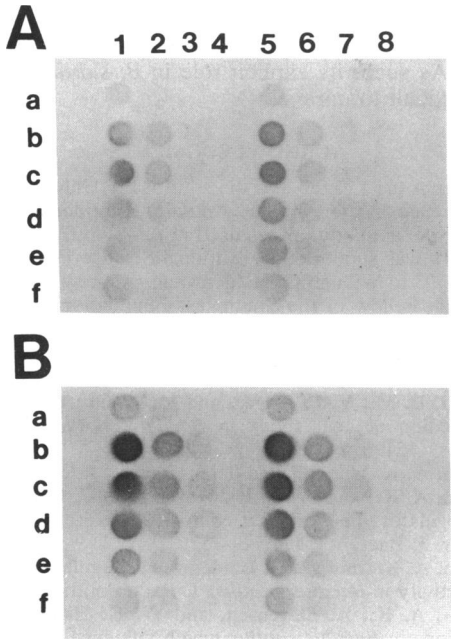

FIG. 5. Dot blot analysis of *sigB* and *ctc* mRNAs from *B. subtilis* at 37 and 48°C. RNA purified as for Fig. 4 was spotted horizontally in decreasing amounts (2.0, 0.67, 0.22, and 0.07 μg) in 2 μl of 0.3 M NaCl–30 mM trisodium citrate (pH 7.5) across nitrocellulose sheets. The RNA was spotted in duplicate, with lanes 1 to 4 equivalent to lanes 5 to 8, respectively. The nitrocellulose sheets were processed as previously described (26) and then hybridized to either the ^{32}P-labeled *sigB* (A)- or *ctc* (B)-specific DNA probes used in the nuclease protection experiments (Fig. 3 and 4). Bound radioactive probe was visualized by autoradiography. Row a, RNA extracted from cells growing at 37°C; rows b to f, RNA extracted from cells harvested 5, 10, 20, 30, and 45 min, respectively, after transfer to 48°C.

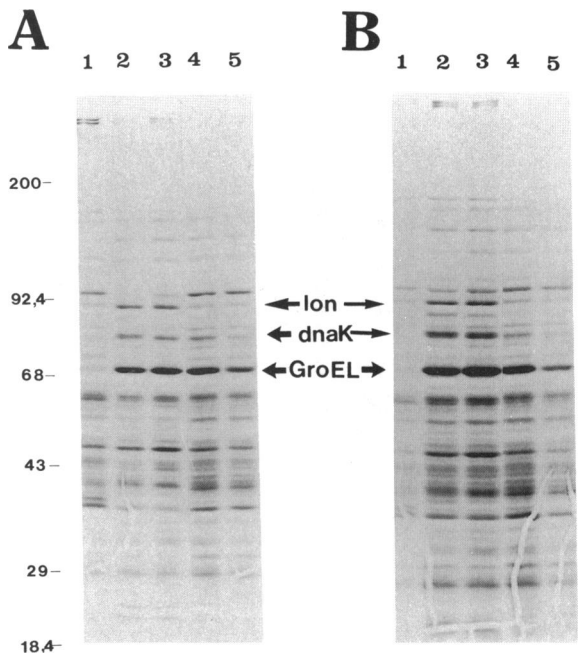

FIG. 6. Pulse labeling of proteins in *sigB* and *sigB314* strains after transfer to 48°C. *B. subtilis* strains BSA70 (*aph3'5"/sigB*) (A) and BSA80 (*aph3'5"/sigB314*) (B) were grown in Spizizen minimum medium (22), labeled for 5 min with [^{35}S]methionine and [^{35}S]cystine, and processed as described in Materials and Methods. Total cell lysates were fractionated by SDS-PAGE (6 to 15% acrylamide) with labeled protein bands visualized by fluorography. Lanes 1, cells labeled at 37°C; lanes 2 to 5, cells labeled at 5, 10, 20, and 30 min, respectively, after transfer to 48°C. The arrows labeled lon, dnaK, and GroEL represent the positions to which *B. subtilis* proteins with the molecular weights of these heat-inducible proteins (1) would migrate in our gel system. The numbers to the left of the autoradiograms depict the migration positions of protein size markers (Bethesda Research Laboratories high-range standards).

mutant (data not shown). We therefore repeated the experiment, separating the labeled proteins on a gel of 15% acrylamide to better visualize these faster-migrating proteins. As can be seen in Fig. 7, this gel system resolved at least seven proteins whose appearance after transfer to 48°C is dependent on σ^B. The largest band could be σ^B itself, which migrates with an apparent size of 37,000 Da (13). The other products of the *sigB* operon, RsbW (18,000 Da), RsbV (12,000 Da), and RsbX (22,000 Da) (17), as well as the predicted *ctc* product (22,000 Da) (14), may be among the other σ^B-dependent protein bands. We conclude that σ^B does make a contribution to the pattern of protein induction after heat shock; however, the significance of this contribution to the heat shock response is unclear.

DISCUSSION

The role of σ^B in *B. subtilis* physiology remains undefined. Null mutations in *sigB* confer no obvious phenotype on either vegetatively growing or sporulating *B. subtilis* (6, 9). A limited number of σ^B-dependent promoters have been isolated and share the characteristic of increased activity when *B. subtilis* enters the stationary phase of growth (8, 16). These results suggested that the σ^B regulon provides a nonessential function associated with postexponential gene expression. The present study adds another possible role to the σ^B regulon by showing that the level of σ^B can be raised during heat shock in a process that is dependent on σ^B itself. In addition, several other *B. subtilis* proteins, normally present after heat shock, fail to appear in the absence of σ^B. σ^B is therefore both a heat-inducible protein and a heat shock σ factor.

In *E. coli* the heat shock response is controlled by a secondary sigma factor (σ^{32}) which increases in abundance after a rise in temperature to direct RNA polymerase to the promoters of the principal heat shock genes (10, 11, 24, 25). σ^B does not seem to be the *B. subtilis* counterpart of σ^{32}. Unlike σ^{32}-deficient strains of *E. coli* (11), σ^B-deficient strains of *B. subtilis* neither are overtly temperature sensitive (30) nor do they fail to synthesize the principal heat shock proteins (Fig. 6). Recent evidence suggests that the principal *B. subtilis* sigma subunit (σ^A), in concert with regulatory factors, rather than a secondary sigma factor, activates the major heat shock genes in this organism (19, 22). If the heat inducibility of the *sigB* operon is physiologically relevant, its role in aiding *B. subtilis* to withstand thermal stress is either subtle or not essential under the laboratory conditions we employed.

In this report we have shown that σ^B levels are elevated during heat shock through an increase in the abundance of mRNA derived from the *sigB* operon's σ^B-dependent promoter. During growth at 37°C, this mRNA depends not only on σ^B but also on RsbV. In our previous studies, null

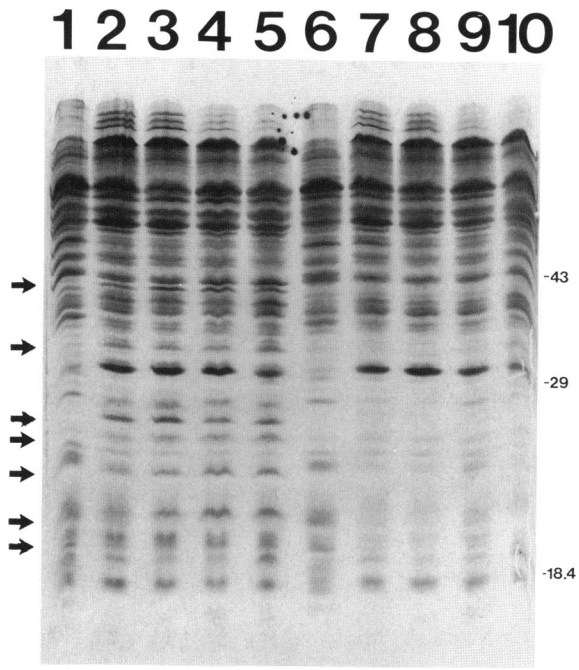

FIG. 7. Pulse-labeling of σ^B-dependent heat-inducible proteins. *B. subtilis* strains BSA70 (*aph3'5"/sigB*) (lanes 1 to 5) and BSA80 (*aph3'5"/sigB314* (lanes 6 to 10) were grown in methionine assay medium (Difco), labeled, and analyzed as for Fig. 6, with the exception that the lysates were fractionated on a gel containing 15% acrylamide. Lanes 1 and 5, cells labeled at 37°C; lanes 2 and 6, 3 and 7, 4 and 8, 5 and 10, cells labeled 5, 10, 20, and 30 min, respectively, after transfer to 48°C. The arrows indicate heat-inducible proteins present in the BSA70 extract that are not induced in the BSA80 strain. The numbers to the right of the autoradiogram depict the migration positions of protein size markers (Bethesda Research Laboratories high range standards).

mutations in *rsbV* appeared to totally block induction of σ^B-dependent promoters unless RsbW, the primary inhibitor of σ^B, was also absent from the cell (3). Therefore, the observation that the temperature-dependent induction of *sigB* does not require a functional *rsbV* gene product was unexpected. This is the first instance in which we can detect substantial expression of a σ^B-dependent promoter in the absence of RsbV. The induction of the *sigB* operon's σ^B-dependent promoter in the *rsbV312* strain suggests that either the *rsbW* product itself is thermolabile or there is an additional mechanism to inactivate RsbW that is induced by heat shock. If this latter possibility is true, then RsbW could be a common target for distinct σ^B activator systems, with RsbV being just one of these activators.

σ^A, σ^D, and σ^B are all vegetative cell sigma factors. However, σ^A and σ^D levels have been reported to decline during heat shock (1), while, as we report here, σ^B levels increase. Thus, it appears that σ^B participates in the response of *B. subtilis* to at least two different types of environmental stimuli (entry into stationary phase and elevated temperature); however, the nonessential feature of σ^B's role in these responses leaves its function obscure. The few operons that are currently known to be transcribed by σ^B-containing RNA polymerase contain additional promoters that are recognized by other RNA polymerase holoenzymes (5, 8). This promoter redundancy may be the basis for the cryptic nature of σ^B function. If the bulk of the σ^B regulon can be transcribed by alternative means, σ^B may enhance some stress responses without being critical to any of them. As such, its explicit role in *B. subtilis* physiology will be difficult to unravel.

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