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Transcription Factor σ^B of *Bacillus subtilis* Controls a Large Stationary-Phase Regulon

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Transcription factor σ^B of *Bacillus subtilis* is active during the stationary growth phase, but its physiological role remains unknown. Understanding the function and regulation of genes controlled by σ^B (*csb* genes) should provide important clues to σ^B function in stationary-phase cells. To this end, we used a genetic approach to identify six new *csb* genes. This strategy relies on two elements: (i) random transcriptional fusions between the *Escherichia coli lacZ* gene and genes on the *B. subtilis* chromosome, generated in vivo with transposon Tn*917lacZ*, and (ii) a plate transformation technique to introduce a null *sigB* mutation into the fusion-bearing recipients directly on indicator plates. This strategy allowed the comparison of fusion expression in strains that were isogenic save for the presence or absence of a functional σ^B protein. Beginning with 1,400 active fusions, we identified 11 that were wholly or partly controlled by σ^B. These fusions mapped to six different loci that exhibit substantial contrasts in their patterns of expression in the logarithmic and stationary growth phases, suggesting that they participate in diverse cellular functions. However, for all six loci, the σ^B-dependent component of their expression was manifest largely in the stationary phase. The high frequency of six independent *csb* loci detected in a random collection of 1,400 fusions screened, the fact that four of the six new loci were defined by a single fusion, and the absence of the previously identified *ctc* and *csbA* genes in the present collection strongly suggest that σ^B controls a large stationary-phase regulon.

Unraveling the mechanisms that regulate stationary-phase metabolism is an important step toward understanding the behavior of bacteria both in natural populations, where they commonly persist in a slowly growing or nongrowing state, and in industrial fermentations, where many products important for human health and welfare are produced during the stationary growth phase. The gram-positive bacterium *Bacillus subtilis* manifests diverse phenomena during the stationary phase, including expression of genes important for the transition from the growing to the nongrowing state, initiation of the sporulation process, development of motility and genetic competence, and production of extracellular enzymes and antibiotics (34). Much of the new gene expression required to meet these changing needs is controlled by the association of alternative σ factors with the catalytic core of RNA polymerase, thus reprogramming the promoter recognition specificity of the enzyme (18, 19, 26, 27).

In addition to its primary σ factor, σ^A, *B. subtilis* has eight alternative σ factors that have been well characterized genetically and biochemically (for reviews, see references 9, 19, 26, and 27). The alternative transcription factor σ^B, formerly called σ^{37}, was originally discovered biochemically through its association with a unique RNA polymerase activity present in early-stationary-phase cells (17). The available information regarding the regulation of σ^B synthesis and activity suggests that σ^B plays an important role in stationary-phase cells. Transcription of the *sigB* gene begins in the early stationary phase (25), and σ^B activity is controlled posttranslationally by a regulatory pathway responsive to stationary-phase signals (3, 5); the loss of a key element of this pathway is a lethal event in exponentially growing cells (5). However, null mutations in the σ^B structural gene (*sigB*) elicit no obvious growth or sporulation phenotype, and the physiological role of σ^B has yet to be identified (4, 12, 22, 25). Similarly, almost nothing is known regarding the function of the genes controlled by σ^B (*csb* genes) or the size of the σ^B regulon. The first *csb* genes discovered were *ctc*, a gene of unknown function (21, 22, 31), and *sigB* itself (25). Both *ctc* and *sigB* are highly expressed early in the stationary phase when cells are grown in rich medium supplemented with glucose and glutamine, conditions that inhibit both the sporulation process and the production of tricarboxylic acid cycle enzymes (23, 35).

One approach to understanding the physiological role of σ^B is to isolate additional *csb* genes, with the idea that the map locations and predicted products of such genes would provide important clues to the role of the σ^B regulon. To that end, we developed a genetic method that is generally useful for identifying genes controlled by nonessential regulatory elements. We tested this method by isolating and characterizing a new *csb* gene, *csbA*, which appears to be directly transcribed by σ^B in vivo (6). Like *ctc*, *csbA* is a gene of unknown function which is most highly expressed early in the stationary phase under conditions that inhibit both sporulation and production of tricarboxylic acid cycle enzymes.

Here we report the use of a modification of our original method to identify 11 operon fusions that are directly or indirectly controlled by σ^B. Our initial characterization of these fusions shows that they define six new *csb* loci whose stationary-phase expression is either completely or partly dependent on σ^B. In the accompanying article, Varón et al. (36) demonstrate that one such fusion, *csb42*, is transcribed from a σ^B-dependent promoter in vivo and is an allele of *gtaB*, which encodes a predicted product similar to UDP-glucose pyrophosphorylases of other bacteria. Together these results suggest that σ^B controls a large stationary-phase regulon and that the products of some genes in this regulon might counter environmental stresses imposed on nongrowing cells.

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TABLE 1. *B. subtilis* strains

Strain	Genotype	Description, reference, or construction[a]
PB2	*trpC2*	Wild-type Marburg strain (32)
PB3	*purA16 cysA14 trpC2*	QB944 (10)
PB5	*tre12 metC3 glyB133 trpC2*	QB934 (10)
PB9	*leuA8 aroG932 ald-1 trpC2*	QB936 (10)
PB10	*hisA1 thr-5 trpC2*	QB917 (10)
PB11	*sacA321 ctrA trpC2*	QB123 (10)
PB153	*sigBΔ2::cat trpC2*	6
PB155	*csbA*::Tn*917lacZ trpC2*	6
PB184	*uvr*::pMT2 *trpC2*	6
PB262	*csb22*::Tn*917lacZ socB1 pheA1 trpC2*	This study
PB263	*csb22*::Tn*917lacZ trpC2*	PB262→PB2
PB264	*csb22*::Tn*917lacZ sigBΔ2::cat trpC2*	PB153→PB263
PB265	*csb34*::Tn*917lacZ socB1 pheA1 trpC2*	This study
PB266	*csb34*::Tn*917lacZ trpC2*	PB265→PB2
PB267	*csb34*::Tn*917lacZ sigBΔ2::cat trpC2*	PB153→PB266
PB268	*csb40*::Tn*917lacZ socB1 pheA1 trpC2*	This study
PB269	*csb40*::Tn*917lacZ trpC2*	PB268→PB2
PB270	*csb40*::Tn*917lacZ sigBΔ2::cat trpC2*	PB153→PB269
PB271	*csb42*::Tn*917lacZ socB1 pheA1 trpC2*	This study
PB272	*csb42*::Tn*917lacZ trpC2*	PB271→PB2
PB273	*csb42*::Tn*917lacZ sigBΔ2::cat trpC2*	PB153→PB272
PB274	*csb76*::Tn*917lacZ socB1 pheA1 trpC2*	This study
PB275	*csb76*::Tn*917lacZ trpC2*	PB274→PB2
PB276	*csb76*::Tn*917lacZ sigBΔ2::cat trpC2*	PB153→PB275
PB277	*csb105*::Tn*917lacZ socB1 pheA1 trpC2*	This study
PB278	*csb105*::Tn*917lacZ trpC2*	PB277→PB2
PB279	*csb105*::Tn*917lacZ sigBΔ2::cat trpC2*	PB153→PB278
EU100	*socB1 pheA1 trpC2*	22
435	*sspE::lacZ-cat*	29
SF1210	*ΔhutPH::neo*	13

[a] Arrows indicate transformation from donor to recipient.

MATERIALS AND METHODS

Bacteria, phage, and genetic methods. DNA comprising the Tn*917lacZ* fusion library of Love et al. (28) was kindly provided by Ron Yasbin. The *B. subtilis* strains used are shown in Table 1. For strain constructions and transformational crosses, *B. subtilis* PB2 and its derivatives were made competent for natural transformation in liquid medium as described by Dubnau and Davidoff-Abelson (11). The same liquid transformation method was used to introduce the Tn*917lacZ* fusion library of Love et al. into the EU100 (*socB1*) genetic background. Plate transformations to inactivate the *sigB* gene were done as described by Hahn et al. (15), using chromosomal DNA from strain PB153 (*sigBΔ2::cat*). We mapped the chromosomal locations of the identified *csb* insertions by means of PBS1 transductional crosses, performed as previously described (32). Selections for drug resistance were performed on tryptose blood agar base plates (Difco Laboratories) containing chloramphenicol (5 μg/ml), neomycin (10 μg/ml), or a combination of erythromycin (0.5 μg/ml) and lincomycin (12.5 μg/ml). Selection for auxotrophic markers was done on the minimal medium of Anagnostopoulos and Spizizen (2), supplemented for the unselected auxotrophic requirements of the recipient (10). Polymerase chain reactions (PCRs) were done by standard protocols (24).

Enzyme assays. *B. subtilis* strains were grown in Luria broth (LB) (8) to the late logarithmic stage and diluted 1:25 into fresh medium. Samples taken in both the logarithmic and stationary phases of growth were assayed for β-galactosidase activity by the method of Miller (30), using sodium dodecyl sulfate and chloroform to permeabilize the cells. Activity was expressed in Miller units, defined as $\Delta A_{420} \times 1{,}000$ per minute per milliliter per optical density at 600 nm.

RESULTS

Identification of *lacZ* transcriptional fusions that have decreased expression in the absence of the *sigB* gene product. We previously developed a genetic approach to identify genes that are controlled by σ^B in vivo, based on the assumption that the expression of such genes would be decreased in the absence of σ^B (6). Our approach relies on two elements: (i) random transcriptional fusions between the *Escherichia coli lacZ* gene and genes on the *B. subtilis* chromosome, generated in vivo with transposon Tn*917lacZ* (37), and (ii) a plate transformation technique (15) to introduce a null *sigB* mutation into the fusion-bearing recipients directly on indicator plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal). This procedure allowed us to monitor gene expression in fusion-bearing strains that were isogenic save for the presence or absence of a functional *sigB* product. The *csbA* gene identified in our first screen was strongly, but not exclusively, dependent on σ^B. *csbA* was shown to have tandem promoters, one dependent on σ^B and a second, weaker, σ^A-like promoter (6).

We reasoned that if many *csb* genes were subject to dual control like *csbA*, our initial screen would have overlooked fusions whose expression had a substantial contribution from a second, σ^B-independent promoter. We therefore sought to enhance the sensitivity of the screen by increasing the signal from σ^B-dependent promoters. To this end, we repeated the screen of the Tn*917lacZ* library in a *socB1*

genetic background. *socB1* is a frameshift mutation in *rsbX*, a gene which lies immediately downstream of *sigB* and which encodes a negative regulator of σ^B (3, 5, 12, 22, 25). Because expression of σ^B-dependent genes is substantially increased in a *socB1* background (22, 25), any differences in *csb*::Tn*917lacZ* expression in the presence and absence of *sigB* would be enhanced. Therefore, this strategy should enable us to identify new *csb* fusions that are not predominantly dependent on σ^B as well as additional *csb* fusions that are only weakly expressed overall.

To test the modified method, we introduced the fusion library of Love et al. (28) into the EU100 (*socB1*) background and screened 1,400 colonies whose blue color on X-Gal plates indicated that the transposon had inserted downstream from an active promoter. Upon inactivation of *sigB* by plate transformation, 11 prospective *csb* fusions were identified among the 1,400 colonies. The expression of two of these fusions appeared to be completely dependent on σ^B. On X-Gal indicator plates, the colony color of the *socB1* parental strains containing the *csb22* and *csb34* fusions changed from light blue to white when their *sigB* genes were converted to *sigBΔ2::cat*. The expression of the remaining nine fusions appeared partly dependent on σ^B, with the colony color shifting from a darker to a lighter blue upon inactivation of *sigB*.

Before proceeding with further characterization, we wanted to ensure that each strain studied carried only a single fusion in a wild-type (*socB*$^+$) genetic background. Each of the 11 fusions was moved by transformation into strain PB2, which had not been subjected to Tn*917lacZ* mutagenesis and which is wild type at the *socB* (*rsbX*) locus. For each of the resulting fusion-bearing strains, we also made a corresponding strain that was isogenic except for the *sigB* locus, at which *sigB* had been converted to *sigBΔ2::cat* by transformations with donor DNA from PB153. None of the new *csb* insertions caused an obvious growth or sporulation phenotype in either the *sigB*$^+$ or *sigBΔ2::cat* background.

Chromosomal locations of the *csb* fusions. We sorted the 11 *csb* fusions by mapping their chromosomal locations and found that they represent at least six new loci. We consider a locus to be defined by either a genetically distinct fusion or a cluster of fusions that have a common expression pattern on indicator plates, that is, similar strengths of expression and similar degrees of σ^B dependence. We performed PBS1 transductional crosses with the fusion-bearing strains as donors and the mapping kit strains of Dedonder et al. (10) as recipients. Other markers, such as drug resistance elements within *dinA*, *hut*, or *sspE*, were also used as appropriate.

From the transductional linkage data shown in Table 2 and additional mapping data described in the following section, we placed 10 of the 11 *csb* fusions into five loci, as shown in Fig. 1. However, we were unable to make an effective PBS1 lysate with the donor strain bearing the *csb42* insertion and were therefore unable to find the *csb42* locus by transductional mapping. This unique phenotype, together with the distinct expression pattern of *csb42* (described in the following section), led us to provisionally assign *csb42* to a sixth, indeterminate locus. In the accompanying article, Varón et al. demonstrate the distinct location of *csb42* by a physical mapping method and confirm this location by transformational crosses (36). This locus for *csb42* is included in Fig. 1. Of the six new *csb* loci, four are represented by a single insertion and two are defined by multiple insertions.

Expression patterns of the *csb* fusions. In order to determine whether the six loci had different patterns of expression, we measured the β-galactosidase activity directed by representative fusions during the logarithmic and stationary growth phases in both wild-type and *sigB* mutant backgrounds. Cells containing the fusions were grown both in LB and in LB supplemented with 5% glucose and 0.2% glutamine, a medium that induces high levels of expression for the σ^B-dependent *csbA*, *ctc*, and *sigB* genes (6, 23, 35). As shown in Fig. 2, 3, and 4, the six fusions displayed five distinct patterns of overall expression; these patterns are described in the following sections. However, in all cases, σ^B-dependent expression was largely confined to the stationary growth phase.

TABLE 2. Two-factor transductional crosses to map the chromosomal loci of *csb* fusions

Fusion	Selected marker(s)[a]
csb22	*ctrA* (68), *hisA* (10)
csb34	*purA* (59), *hutPH* (38)
csb40	*aroG* (98), *leu* (31)
csb10	*aroG* (78), *leu* (25)
csb15	*aroG* (72), *leu* (33)
csb58	*aroG* (98), *leu* (22)
csb76	*hisA* (47)
csb54	*hisA* (54)
csb105	*sspE* (87), *glyB* (25)

[a] Numbers in parentheses indicate the percent cotransduction of the macrolide-lincosamide-streptogramin B resistance of the *csb*::Tn*917lacZ* insertions with the indicated marker.

***csb22* and *csb34*.** The *csb22* and *csb34* fusions define two independent loci, with *csb22* located at about 320° on the map of Anagnostopoulos et al. (1) and *csb34* mapping at 346°. As shown in Fig. 2, under the conditions tested both *csb22* and *csb34* were totally dependent on σ^B for their expression. In the *sigB* null background, the β-galactosidase activities for both fusions were as low as the residual β-galactosidase level found in the fusionless control strain (data not shown). Moreover, in both growth media tested,

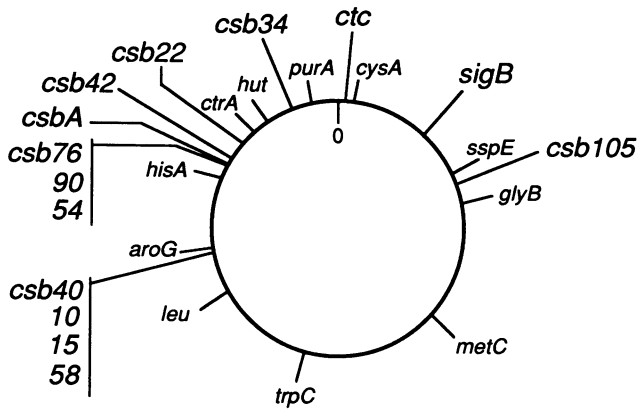

FIG. 1. Locations of *csb* genes on the *B. subtilis* chromosome. Ten of the 11 newly identified *csb* fusions were placed into five loci by their transductional and transformational linkage to known markers and, in the case of *csb54*, *csb76*, and *csb90*, by PCR analysis (see text). A sixth locus, that of the *csb42* (*gtaB*) fusion, was determined by a physical mapping method as described in the accompanying article (36). The locations of the σ^B-dependent *csbA* (6), *ctc* (16), and *sigB* (4, 12) genes are from the literature. The *B. subtilis* genetic map and the locations of the markers used in the mapping experiments are from reference 1. The 0 at the top of the map indicates both 0° and the origin of chromosomal replication.

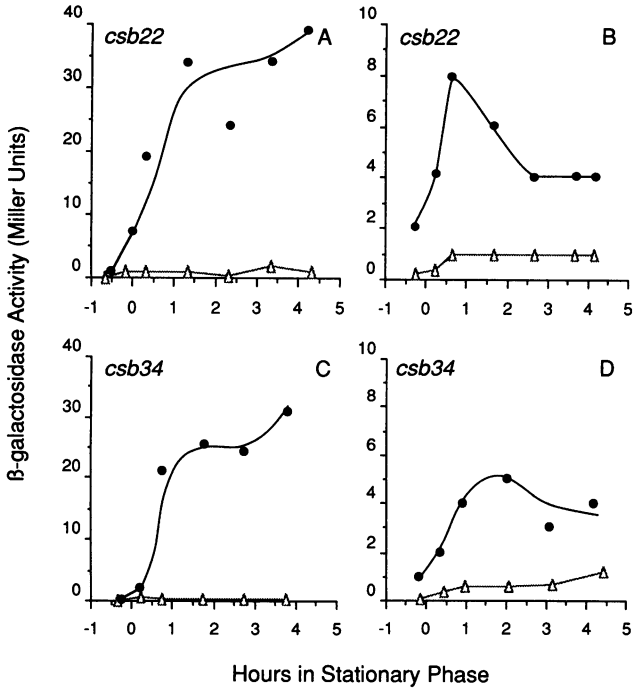

FIG. 2. Expression of fusions completely dependent on σ^B, *csb22* and *csb34*. In panels A and B, the β-galactosidase activity of strain PB263 (*csb22 sigB*$^+$) is indicated by circles and that of strain PB264 (*csb22 sigBΔ2::cat*) is indicated by triangles. In panels C and D, the β-galactosidase activity of strain PB266 (*csb34 sigB*$^+$) is indicated by circles and that of strain PB267 (*csb34 sigBΔ2::cat*) is indicated by triangles. In the experiments shown in panels A and C, cells were grown in LB supplemented with 5% glucose and 0.2% glutamine; the β-galactosidase activity of fusionless control strain PB2 never exceeded 1 Miller unit in this medium, and this background activity has not been subtracted from the activities indicated. For the experiments shown in panels B and D, cells were grown in unsupplemented LB; the β-galactosidase activity of the fusionless control never exceeded 2 Miller units. Please note the expanded *y* axes in panels B and D relative to those in panels A and C. Zero hour on the *x* axis indicates the end of logarithmic growth.

the majority of fusion expression occurred after the end of logarithmic growth. As was the case for the *ctc* and *csbA* fusions described previously (6, 23), peak expression of the *csb22* and *csb34* fusions was about five- to sixfold higher in supplemented LB than in unsupplemented LB.

csb40. The *csb40* fusion represents a cluster of phenotypically similar insertions located at about 264° on the *B. subtilis* chromosome. The cluster includes *csb10*, *csb15*, *csb40*, and *csb58*, all of which were 53 to 66% linked to *aroG* by transformation. We do not know how many different genes these fusions represent, but given their similar timings and levels of expression in three different genetic backgrounds (wild type, *socB1*, and *sigBΔ2::cat*), we presume that they lie within a single transcriptional unit. As shown in Fig. 3, about 60 to 80% of *csb40* fusion activity is dependent on σ^B, and there is little expression until after the end of logarithmic growth. In contrast to many *csb* fusions, *csb40* appears to have significant σ^B-dependent expression when cells are grown in unsupplemented LB. The expression of *csb40* also increases less dramatically than that of the other fusions when cells are grown in LB supplemented with glucose and glutamine.

csb42. The *csb42* locus is defined by a single fusion whose activity is 30 to 40% dependent on σ^B when cells are grown in LB containing glucose and glutamine (Fig. 3). All of this σ^B-dependent activity arises after the end of logarithmic growth and only in supplemented LB. *csb42* also has substantial σ^B-independent expression during logarithmic growth. In the accompanying article, Varón et al. (36) show that *csb42* is an allele of *gtaB*, that *gtaB* encodes UDP-glucose pyrophosphorylase, and that *gtaB* is expressed from tandem promoters, one of which is σ^B dependent.

csb76. The *csb76* fusion represents a cluster of phenotypically similar fusions, *csb54*, *csb76*, and *csb90*, located at about 305° on the *B. subtilis* chromosome. The previously isolated *csbA* gene maps in this same region, immediately upstream from the *dinA* (*uvr*) gene (6, 7). We therefore performed transformational crosses to determine the linkage between the newly isolated fusions and the *cat* marker in the *dinA* (*uvr*) reading frame of strain PB184. The *erm* markers of the strains bearing the *csb54*, *csb76*, and *csbA* fusions were all found to be 52 to 66% linked to the *cat* marker of PB184. These results indicated that the *csb54* and *csb76* fusions lie within a few kilobases of *dinA* (*uvr*) and suggested that they might be alleles of *csbA*.

To address this possibility, PCR analysis was used to determine the physical distance between the *csb54* and *csb76* transposon insertions and the previously characterized *csbA* gene. We included the *csb90* fusion in this PCR analysis because of its phenotypic similarity to *csb54* and *csb76*. For

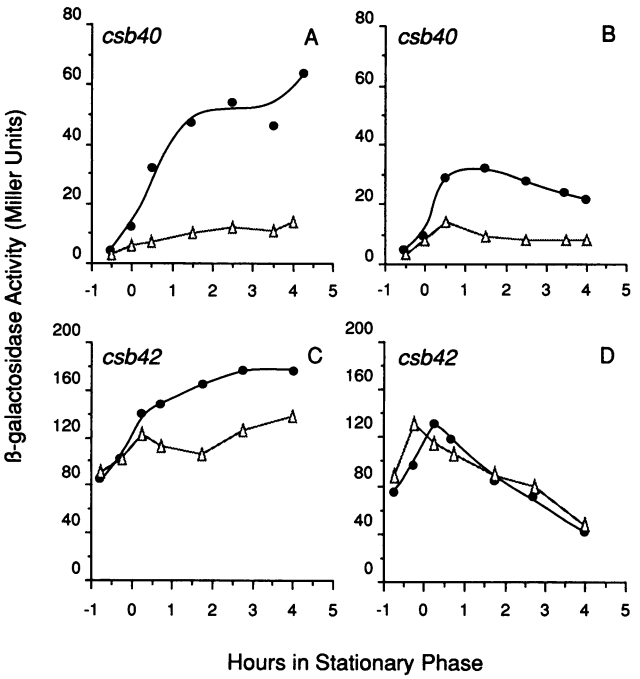

FIG. 3. Expression of fusions partly dependent on σ^B: *csb40* and *csb42*. In panels A and B, the β-galactosidase activity of strain PB269 (*csb40 sigB*$^+$) is indicated by circles and that of strain PB270 (*csb40 sigBΔ2::cat*) is indicated by triangles. In panels C and D, the β-galactosidase activity of strain PB272 (*csb42 sigB*$^+$) is indicated by circles and that of strain PB273 (*csb42 sigBΔ2::cat*) is indicated by triangles. In the experiments shown in panels A and C, cells were grown in LB supplemented with 5% glucose and 0.2% glutamine, whereas for those shown in panels B and D, cells were grown in unsupplemented LB. Zero hour indicates the end of logarithmic growth.

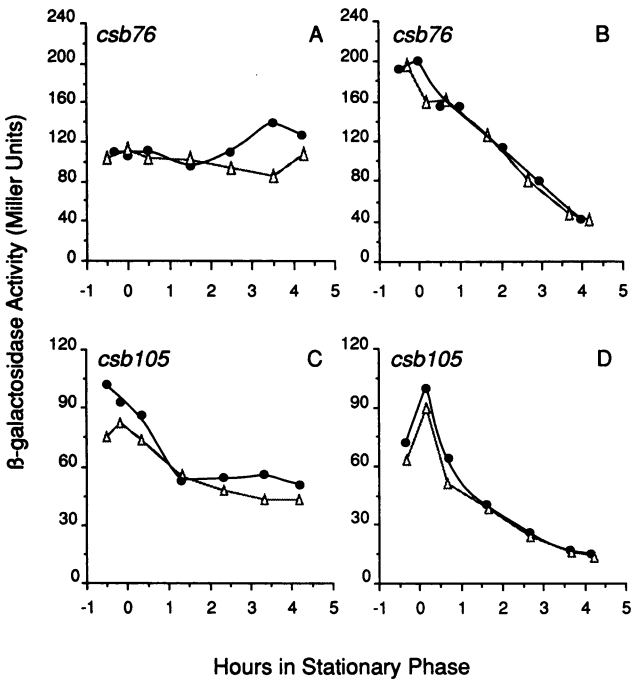

FIG. 4. Expression of fusions weakly dependent on σ^B: *csb76* and *csb105*. In panels A and B, the β-galactosidase activity of strain PB275 (*csb76 sigB*$^+$) is indicated by circles and that of strain PB276 (*csb76 sigBΔ2::cat*) is indicated by triangles. In panels C and D, the β-galactosidase activity of strain PB278 (*csb105 sigB*$^+$) is indicated by circles and that of strain PB279 (*csb105 sigBΔ2::cat*) is indicated by triangles. In the experiments shown in panels A and C, cells were grown in LB supplemented with 5% glucose and 0.2% glutamine, whereas for those shown in panels B and D, cells were grown in unsupplemented LB. Zero hour indicates the end of logarithmic growth.

this experiment, we used one primer corresponding to the chromosomal sequence just upstream from the σ^B-dependent promoter of *csbA* and a second primer complementary to the left end of the Tn*917* element, proximal to the *erm* gene. In contrast to the 300-bp PCR fragment we obtained with chromosomal DNA from the *csbA* fusion strain as a template, we found fragments of 1,900 bp with chromosomal templates from the *csb54*, *csb76*, and *csb90* strains, suggesting that the latter three fusions are siblings arising from a single transposition event. In addition, these results establish that the Tn*917* elements of *csb54*, *csb76*, and *csb90* do not lie within *csbA* but instead have their points of insertion midway through the *dinA* (*uvr*) gene, downstream from the *csbA* transcription terminator (6). In our hands, strains carrying null *dinA* (*uvr*) mutations were only slightly more sensitive than the wild type to DNA damage induced by mitomycin, and the *csb76* fusion strain had a phenotype similar these null mutants. Null *sigB* mutations likewise had little effect on the mitomycin sensitivity of logarithmically growing cells.

The *csb76* fusion retained only a very weak σ^B-dependent component of its expression in the wild-type background, and this was only evident 2 to 3 h into the stationary phase when cells were grown in supplemented LB (Fig. 4). In contrast to its weak σ^B dependence in the wild-type background, *csb76* exhibited marked dependence in the *socB1* genetic background, in which it was originally identified (data not shown).

csb105. The *csb105* locus is identified by a single fusion mapping at 63° on the chromosome. Like the *csb76* fusion, *csb105* had clear σ^B dependence in the *socB1* background (data not shown) but only weak dependence in the wild-type background (Fig. 4). However, in contrast to the *csb76* fusion, after 2 days of incubation on tryptose blood agar base plates containing X-Gal, *csb105* showed consistently higher expression in the wild-type background than in the null *sigB* strain. The lack of correlation between stationary-phase expression patterns in cells grown on solid and liquid media has been noted by others (33) and is not unexpected given the different growth environments. From the σ^B dependence of the *csb105* fusion observed on plates, we imagine that the expression of the gene it identifies might require σ^B under physiological conditions that we have not yet reproduced in liquid medium.

DISCUSSION

In order to determine the physiological role of the σ^B transcription factor, we have adopted a global approach to identify genes that require σ^B for all or part of their expression (*csb* genes). Other than the condition that the transposon insertion not inactivate an essential gene, this approach requires no initial bias regarding gene function. Therefore, our expectation is that the map locations and predicted products of the *csb* genes will provide clues to the range of cellular activities influenced by σ^B.

Our original genetic method was designed to identify genes dependent on nonessential regulatory elements (6). Here we describe a modification of the general method that specifically increases its sensitivity to σ^B-dependent genes. We used this modified method to identify six new *csb* loci whose expression patterns range from weakly dependent to fully dependent on σ^B. Because the σ^B-dependent expression of these new fusions was greatly reduced in the wild-type background compared with the *socB1* genetic background used for this screening, most would have escaped detection by the original procedure.

On the basis of the results of our initial characterization of the new *csb* genes, we conclude that σ^B controls a large stationary-phase regulon. The high frequency of independent *csb* loci detected in the present screen, the fact that four of the six new *csb* loci were defined by a single fusion, and the absence of the previously identified *ctc* and *csbA* genes in the present collection all strongly suggest that the search for *csb* genes has not yet approached saturation of the existing loci. Furthermore, both the present and previous studies (6, 23) indicate that under the growth conditions tested, σ^B-dependent expression of all known *csb* genes is manifest primarily in the stationary phase.

Although *csb* genes all share the property that at least part of their stationary-phase expression is controlled by σ^B, the differences in their σ^B dependence and in their overall expression patterns suggest that they participate in diverse cellular functions. For example, the *csb22* and *csb34* fusions are completely dependent on σ^B under the conditions tested and have little or no expression during logarithmic growth. The *csb40* fusion is chiefly dependent on σ^B and likewise has minimal expression during logarithmic growth. In contrast, the *csb42*, *csb76*, and *csb105* fusions are only partly or weakly dependent on σ^B, and each has substantial logarithmic expression. For the latter three fusions, the experiments with cells grown in LB supplemented with glucose and glutamine suggest that each has a different expression pattern. The *csb42* fusion is active during logarithmic growth,

and this activity increases upon entry into the stationary phase. In contrast, the *csb76* fusion maintains relatively constant β-galactosidase accumulation throughout the logarithmic and stationary growth phases, whereas the accumulation in the *csb105* fusion declines in the stationary phase. We therefore conclude that the six new *csb* loci manifest at least five different patterns of expression. These results suggest that some *csb* genes may function primarily in the stationary phase, whereas others appear to have a role during both the logarithmic and stationary growth phases.

We presume that *csb* genes strongly dependent on σ^B (such as *csb22* and *csb34*) are likely to have promoters directly recognized by a σ^B-containing holoenzyme. However, it remains possible that such genes are indirectly controlled by σ^B, with their expression absolutely dependent on another direct regulator whose synthesis originates from a σ^B-dependent promoter. The *csb* genes that are partly dependent on σ^B (such as *csb40* and *csb42*) might also be directly or indirectly controlled by σ^B. Additionally, these partially dependent genes could have multiple promoters, one controlled by σ^B and at least one that is active in the absence of σ^B. This latter possibility has been shown to be the case for *csb42*, which is under the control of dual promoters, one of which appears to be directly dependent on σ^B (36). These results provide evidence that at least one of the new *csb* genes is directly transcribed by σ^B-containing holoenzyme in vivo.

But what of the *csb76* and *csb105* fusions that are at best only weakly dependent on σ^B in the wild-type background? The cluster of fusions represented by *csb76* map within the *dinA* (*uvr*) reading frame, which encodes a homolog of *E. coli* UvrB (6, 7). *dinA* lies immediately downstream from the σ^B-dependent *csbA* gene. Although both *csbA* and *dinA* are transcribed in the same direction, the available evidence indicates that they lie in independent transcriptional units. The *dinA* coding region is separated from the σ^B-dependent *csbA* promoter by a sequence resembling a factor-independent transcriptional terminator, and this sequence has been shown to have terminator activity in vivo (6). Furthermore, *dinA* expression is controlled in response to DNA damage by a σ^A-like promoter which lies in the intercistronic region between the *csbA* terminator and the *dinA* coding region (7). Because there is no other recognizable σ^B-dependent promoter upstream from *dinA*, we consider it likely that the σ^B-dependent component of *csb76* (*dinA*) expression originates from the *csbA* promoter and enters *dinA* as a result of read-through transcription.

If this is the case, then the question arises whether the weakly dependent *csb76* (*dinA*) fusion and the *csb105* fusion are artifacts generated by the modified screening method. It is possible that the enhanced σ^B activity in the *socB1* genetic background, while facilitating the detection of weakly expressed *csb* fusions, also causes some fusions not normally dependent on σ^B to appear as such because of read-through transcription from upstream. In this view, such fusions are useful for identifying an upstream *csb* gene but do not themselves constitute part of the σ^B regulon. On the other hand, the increased σ^B activity seen in the *socB1* strain might mimic a wild-type response to an environmental condition that we have not yet duplicated under laboratory conditions. If this is the case, then the σ^B-dependent expression of *csb76* and *csb105* might have physiological importance under some growth conditions not yet tested.

Whatever the ultimate explanation for the phenotype of these weakly dependent fusions may be, the striking conclusion remains that the σ^B-dependent expression of all the newly isolated *csb* genes appears primarily in the stationary growth phase and is enhanced when cells are grown in rich medium containing high levels of glucose and glutamine. This timing of expression and dependence on medium closely resemble those of the previously isolated *csbA* and *ctc* genes (6, 23) and those of the *sigB* gene itself (35). Thus, σ^B may be important in stationary-phase metabolism under growth conditions that inhibit the sporulation process and the formation of tricarboxylic acid cycle enzymes.

The question of the role of σ^B in stationary-phase cells can now be addressed by characterizing the new *csb* genes, and, in the accompanying article, Varón et al. (36) report that one of these genes, *csb42*, encodes UDP-glucose pyrophosphorylase. Because the UDP-glucose pyrophosphorylase of enteric bacteria is critical for stationary-phase tolerance of high osmolarity and elevated temperature (14, 20), the intriguing possibility arises that *B. subtilis* σ^B controls at least some genes important to surviving stationary-phase stress under environmental conditions that are inimical to sporulation.

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