Correlation between *Bacillus subtilis* scoC Phenotype and Gene Expression Determined Using Microarrays for Transcriptome Analysis

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The availability of the complete sequence of the *Bacillus subtilis* chromosome (F. Kunst et al., Nature 390:249–256, 1997) makes possible the construction of genome-wide DNA arrays and the study of this organism on a global scale. Because we have a long-standing interest in the effects of scoC on late-stage developmental phenomena as they relate to *aprE* expression, we studied the genome-wide effects of a scoC null mutant with the goal of furthering the understanding of the role of scoC in growth and developmental processes. In the present work we compared the expression patterns of isogenic *B. subtilis* strains, one of which carries a null mutation in the scoC locus (scoC4). The results obtained indicate that scoC regulates, either directly or indirectly, the expression of at least 560 genes in the *B. subtilis* genome. ScoC appeared to repress as well as activate gene expression. Changes in expression were observed in genes encoding transport and binding proteins, those involved in amino acid, carbohydrate, and nucleotide and/or nucleoside metabolism, and those associated with motility, sporulation, and adaptation to atypical conditions. Changes in gene expression were also observed for transcriptional regulators, along with sigma factors, regulatory phosphatases and kinases, and members of sensor regulator systems. In this report, we discuss some of the phenotypes associated with the scoC mutant in light of the transcriptome changes observed.

The transition state of *Bacillus subtilis*, which carries the cells from vegetative growth to stationary phase, is a stage in which the cell population is highly differentiated. In this stage, which is governed by the phosphorylation status of Spo0A, the cells can carry out a number of highly specialized functions (13). These functions include not only those controlling the initiation of the spore-forming process but also those responsible for competence and motility as well as the production of scavenging enzymes (e.g., AprE and NprE) and a host of secondary metabolites (33). By a not-yet-completely-elucidated mechanism, the fate of the individual cells within the population is determined by synthesis, secretion, processing, and uptake of signaling peptides used for cell-to-cell communication (29, 34). In addition, a number of other genes called transition state regulators play an important role in this differentiation process (28, 37). Hyperexpression and/or loss of function of any of these genes profoundly affects several aspects of the cell physiology.

One gene that belongs to this group of transition state regulators is scoC. Mutations in scoC have been isolated independently, by using different screening criteria, in at least three laboratories and are known as *hpr* (12), *catA* (14), and *scoC* (22). We suggest the adoption of the designation scoC for this gene and all its alleles to avoid confusion with the HPr system involved in sugar transport.

*scoC* (as the *hpr* allele) was first noted and defined by the study of *B. subtilis* mutants overproducing alkaline and neutral proteases as an approach to isolate sporulation-associated mutations. Other genetic lesions, *catA* (14) and *scoC* (22), which lead to a glucose-insensitive sporulation phenotype and exoprotease overproduction, were also mapped in the same chromosomal region. Later DNA sequencing showed that all of these mutants resulted from mutations in the same gene (27). Northern blots to quantify subtilisin-specific mRNA suggested that the activity of the scoC gene was exerted at the level of transcription of the *aprE* promoter (8). Sequencing of the scoC locus and of its alleles showed that the observed phenotypes were due to a loss-of-function mutation of ScoC (27), indicating that ScoC acts as a negative regulator of transcription of *aprE*. This study also showed that overproduction of the gene product reduced sporulation by 3 to 4 orders of magnitude. Transcription of the scoC locus became constitutive by null mutations in the *spo0A* gene, suggesting that Spo0A is a negative regulator of *scoC* transcription. Purification of the ScoC protein demonstrated that it is a DNA binding protein. A consensus binding sequence, RATANTATTY, was shown by footprint analysis to lie upstream of the *nprE*, *aprE*, and *sinI* genes (16).

It has been shown recently that scoC plays a direct role in the initiation of sporulation by acting as a repressor of the two major signaling peptide transport systems, *opp* and *app* (17). However, inactivation of the *napA* gene, which rescues the sporulation defect of an *opp* mutant, does not rescue the sporulation defect caused by the presence of scoC in a multi-copy plasmid (17). As pointed out by Koide et al. (17), this suggests the existence of other regulatory mechanisms by which the scoC gene controls sporulation. Furthermore, while scoC null mutations allow sporulation in the presence of glucose, they do not allow sporulation if both glucose and glutamine are present in the medium (33). In addition, the ob-
servation that socC mutants affect alkaline phosphatase expression (4), motility (16), oxidative stress response (5), and competence (S. Causer and E. Ferrari, unpublished data) suggests that the SocC gene product plays a major role in the physiology of B. subtilis. We have attempted to understand the effects of the socC mutation in B. subtilis by comparing the gene expression patterns of wild-type cells and those of the socC mutant using DNA microarrays.

MATERIALS AND METHODS

Strains and growth conditions. The strains used in this work are BG2815 socC4 ΔΔpepE22 and the isogenic strain BG2822 ΔΔpepE22. The presence of the socC4 mutation, a stop codon at position 32 (accession no. M20237), in strain BG2821 was verified by PCR and sequencing. The ΔΔpepE22 mutation has been described previously (41). Fresh cells were streaked onto Luria-Bertani medium—1.6% skim milk plates for overnight growth at 37°C. For each strain, single colonies were then inoculated into 10 ml of freshly prepared 2°1.6% skim milk plates for overnight growth at 37°C. Tenfold serial 1:10 dilutions were made, each into 10 ml of the same medium, for slow growth to detect alkaline phosphatase activity.

Preparation of RNA. The following protocol was adapted from the work of Farrell (6). The frozen sample was rapidly transferred to a 50-ml Oakridge centrifuge tube with 5 ml of extraction buffer at room temperature (4 M guanidinium thiocyanate, 25 mM sodium citrate [pH 7], 0.5% sarcosyl, 100 mM β-mercaptoethanol). The sample was vortexed immediately for 45 s to ensure even mixing. One-tenth volume of 3 M sodium acetate (pH 5.5) was added and vortexed briefly. After centrifugation at 12,000 × g for 10 min at 4°C, the supernatant was transferred to a fresh ice-chilled tube. All remaining extraction steps were carried out on ice. An equal volume of 25:41 water-saturated phenol-chloroform-isomyl alcohol was added, mixed for 1 min, and incubated for 5 min. The mixture was centrifuged under the same conditions, and the aqueous phase was transferred to a new tube. The nucleic acid was precipitated in 0.75 volume of chilled isopropanol at −20°C for at least 1 h and then centrifuged at 20,000 × g for 20 min at 4°C. The isopropanol was removed completely, and the pellet was dissolved in 700 μl of extraction buffer. The sodium acetate addition, organic solvent extraction, and isopropanol precipitation were repeated. The second pellet was washed three times with 500 μl of ice-cold 70% ethanol (each for 10 min), followed by a final brief rinse with ice-cold 95% ethanol. The pellet was air dried for not more than 20 min and resuspended in 50 μl of diethyl pyrocarbonate (DEPC)-treated water with 1 U of RNase inhibitor (BM/Roche) per μl. The yield was determined by UV spectroscopy, and the crude RNA sample was stored at −80°C. An adequate amount of crude RNA (~250 μg) contaminated with genomic DNA was added to a 500-μl DNAase I reaction mixture (40 mM Tris-HCl [pH 7.6], 6 mM MgCl2, 2 mM CaCl2, 1 U of RNase inhibitor per μl, 150 U of DNAse I per 500 μl [BM/Roche]) and incubated for 30 min at 37°C. One-tenth volume of 3 M sodium acetate (pH 5.5) was added and mixed well, followed by organic solvent extraction, isopropanol precipitation, and ethanol washes as described above for the crude RNA preparation. After the pellet had been resuspended in DEPC-treated water with RNase inhibitor, the entire DNAse I treatment and its subsequent purifications were repeated to remove all traces of genomic DNA. After final resuspension in 50 μl of DEPC-treated water with 1 U of RNase inhibitor per μl, the yield was determined by UV spectroscopy (with a 260/280 ratio of at least 1.8). RNA quality was also verified by 3% agarose gel electrophoresis (i.e., 23S and 16S rRNA band intensities at a ratio of 1.3:1 to 1.6:1). The purified total Bacillus RNA sample was stored at −80°C.

IVT controls and RT-PCR preparation. Genes encoding Eryr (Staphylococcus aureus plasmid pE194, accession no. J01755-38), Blecr (S. aureus plasmid pUB110, accession no. M194065), Specr (Enterococcus faecalis, accession no. M69221), and green fluorescent protein (GFP) (plasmid pGFP, Clontech catalog no. 6096-1), start to stop codons inclusive, were PCR amplified from plasmid DNA using primers containing unique restriction sites for subsequent cloning into pBlueScript II KS (+) (Strategene). The resulting plasmids were linearized at a unique site downstream of the coding sequence, and runoff in vitro transcription (IVT) reaction products were prepared from the upstream T3 promoter of the plasmid using the MGAEscript T3 IVT kit (Ambion catalog no. 1338) following the supplier’s recommended protocol. Finally, the IVT product, 5 nM Bleor gene, 1 nM GFP gene, 200 pM Specr gene, and 50 pM Eryr gene. In addition, three transcripts (amyE, citC, and sbhA) were assayed both by the microarray assay and by a reverse transcription (RT)-PCR assay to quantify mRNA independently across two conditions (time or genotype). RT-PCR was performed with a Roche Molecular Biochemicals light cycler instrument (software version 3 and RNA amplification kit SYBR green I) according to the manufacturer’s instructions. Primer pairs were chosen which gave a good dose response on dilutions of genomic DNA, of IVT standards, and of total RNA preparations used in chip assays. For RT-PCR, primer sequences were as follows (F denotes forward or sense strand; R denotes reverse or antisense strand):

- 280-bp amyE product, 5' TGGAAAAGCTTCTTTGACAGACG-3' (F) and 5'-TGGGAGAATTGAGCGAGCAGG-3' (R);
- 227-bp cfr product, 5'-ATAAACAGAAGGTGAGGCTC-3' (F) and 5'-AAAGAGAATGACACGATATCAGG-3' (R);
- 234-bp sbhA product, 5'-CGTCACTACACTTAAGACCC-3' (F) and 5'-GGCAGGTTCGTTCTAC-3' (R);
- 248-bp Eryr product, 5'-TGGTCCGTACAGAGTACAGG-3' (F) and 5'-ACCACGCTACC-3' (R).

Products were verified by melting curve analysis on the light cycler, by size determination on agarose gels, and by DNA sequencing.

Microarray preparation. RNA harvest from a given Bacillus strain and at a given time point was reverse transcribed into biotin-labeled cDNA by the method of de Saizieu et al. (3). Total RNA (25 μg) and 5.5 μl of the staggered control IVT mixture were incubated at 37°C overnight in a 100-μl reaction mixture: 1× Gibco first-strand buffer (50 mM Tris-HCl [pH 8.3], 75 mM KCl, 3 mM MgCl2, 25.0 g of KCl, and 3.26 g of MgSO4 · 7H2O, 9.6 mg of FeSO4 · 7H2O, 25.0 g of Difco agarose, 50 ml of freshly prepared 2°1.6% skim milk plates for overnight growth at 37°C. Tenfold serial 1:3,000 dilutions were made, each into 10 ml of the same medium, for slow growth to detect alkaline phosphatase activity.

Time to logarithmic growth was used to inoculate the prewarmed (37°C) culture in a 500-ml beaker, allowing no more than 10 to 15 s to pass between pipetting and freezing. In the presence of liquid nitrogen, the filters were transferred to −80°C storage. Each filter with a cellular sample was ground up for 3 min in a standard hand-held coffee grinder with 45 ml of finely crushed dry ice and 10 g of glass beads (~110-μm Glasperlen; B. Braun). The powdered contents were then transferred via funnel to a 100-ml heavy-walled glass bottle (VWR Scientific Product Corporation). All equipment involved was prechilled with dry ice before use and maintained at dry-ice temperature throughout. The loosely capped bottle was then stored at −80°C overnight to allow the dry ice to sublime, leaving frozen cell powder and glass beads.

FIG. 1. Growth curves for Bacillus strains BG2822 (wild type) (open diamonds) and BG2815 (socC4) (filled diamonds). t1, t2, t3, and t4, time points used for RNA isolation and array analysis (log phase, early transition, late transition, and stationary phase, respectively).

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MgCl2); 10 mM dithiothreitol; 40 μM random hexamer; 0.3 mM concentrations (each) of dCTP, dGTP and dTTP; 0.12 mM dATP; 0.3 mM biotin-dATP (NEN catalog no. NEL999); 2500 U of SuperScript II reverse transcriptase. To remove RNA, the reaction was brought to 0.25 M NaOH and incubated at 65°C for 30 min. The reaction mixture was neutralized with HCl, and the nucleic acid was precipitated at −20°C in ethanol with 2.5 M ammonium acetate. The pellet was washed, air dried, resuspended in water, and quantitated by UV spectrophotometry. The reaction yield was approximately 20 to 25 μg of biotin-labeled cDNA.

This cDNA (12 μg) was fragmented in 33 μl of 1× One-Phor-All buffer (Amersham-Pharmacia no. 27-0901-02) with 3.75 nM of Dnase I at 37°C for 10 min. After the Dnase had been heat killed, fragmentation was validated by running 2 μg of the fragmented cDNA on a 3% agarose gel. Biotin-containing cDNA routinely ranged in size from 25 to 125 nucleotides. The remaining 10 μg of cDNA was hybridized to an Affymetrix (Santa Clara, Calif.) Bacillus Gene-Chip array.

 array description. Probe sets on the custom B. subtilis expression array were designed from the wild-type (1168) B. subtilis sequence data of Kunst et al. (19) by Affymetrix and Genencor. The total of 4,107 open reading frames (ORF) were represented by the tiling of at least 20 probes pairs per ORF, each pair consisting of one perfectly matching complementary 25-mer and one control 25-mer with a centrally mismatched base. Genes longer than 1,500 bp were represented by additional probe sets, bringing the total number of ORF probe sets to 4,351. Probes sets for 40 tRNA genes were also tiled, as well as probe sets for over 40 control sequences. Affymetrix performed probe selection and array fabrication by published and proprietary methods (20, 40). The entire array of 454-by-454 25-μm features is bordered and interspersed by a standard oligonucleotide feature for the purposes of grid alignment and data analysis.

Hybridization, scanning, and data collection. Hybridizations were performed as described in the Affymetrix expression analysis technical manual (Affymetrix) using reagent suppliers as suggested. Fragmented biotin-labeled cDNA (10 μg) was added to a 220-μl hybridization cocktail containing 100 nM MES [N-morpholinoethanesulfonic acid], 1 mM EDTA, 0.01% Tween 20, 5 mg of total yeast RNA per ml, 0.5 mg of bovine serum albumin per ml, 0.1 mg of herring sperm DNA per ml, and 50 μM control oligonucleotide (AFFX-B1). The cocktails were heated to 95°C for 5 min, cooled to 40°C for 5 min, and briefly microcentrifuged to remove particulates, and 200 μl was injected into each prewarmed precriss (1× MES buffer plus 5 μg of yeast RNA per ml) cartridge. The arrays were rotated at 40°C overnight.

The samples were removed, and the arrays were filled with nonstringent wash buffer consisting of 6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH2PO4, and 1 mM EDTA [pH 7.7]) and 0.01% Tween 20, 5 mg of total yeast RNA per ml, 0.5 mg of bovine serum albumin per ml, 0.1 mg of herring sperm DNA per ml, and 50 μM control oligonucleotide (AFFX-B1). The cocktails were heated to 95°C for 5 min, cooled to 40°C for 5 min, and briefly microcentrifuged to remove particulates, and 200 μl was injected into each prewarmed precriss (1× MES buffer plus 5 μg of yeast RNA per ml) cartridge. The arrays were rotated at 40°C overnight.

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The arrays were stained in three steps: streptavidin, antistreptavidin antibody tagged with biotin, and streptavidin-phycoerythrin conjugate.

Signal on the arrays were detected with a Hewlett Packard gene array scanner using 570-nm laser light with 3-μm pixel resolution. The signal intensities (also known as average differences) of the 4,351 ORF probe sets were scaled and normalized to a target value of 1,000 as described in the Microarray Suite 4.0 user guide (Affymetrix). The absolute analysis values for average difference and absolute call were collected for all scans; the values for difference call and fold change were also collected for all comparative analysis scans (scoC versus wild-type cells at matched time points).

Data analysis. A replicate RNA sample was prepared for a given strain and time point. These two samples (R and R′) were prepared as identically as possible, followed by signal normalization in GeneChip 4.0. For each of the 4,351 ORF probe sets, we prepared a table of the average of the logs of the two replicates (ALS = 0.5 log R + 0.5 log R′) and the log ratio (LR = log R/R′). For this calculation, only the probe sets with two positive values were considered. The resulting table was sorted by the ALS. Then, in a series of sliding windows with a size of 201 ALS values, from one end of the table to the other, the mean ALS and the standard deviation for the corresponding LR (SDR,LR) values were determined. These values were saved as a look-up table listing SDR,LR in identical samples (i.e., scatter due to chance) as a function of ALS: in general, SDR,LR remained fairly constant when the ALS was >300 but increased as ALS decreased below 300. The plot of these look-up values is very consistent with a fitted equation based on a two-component error model for microarray-based data developed by Silicon Genetics, Redwood City, Calif. (B. Eynon and A. Conway, personal communication).

There were four experimental time points for both the scoC and the wild-type shake flask cultures (80, 130, 190, and 310 min after inoculation [t1 through t4, respectively]). For each time point, the scoC array was treated as the experimen- tary array and the wild-type array was treated as the baseline array: GeneChip 4.0 software (Affymetrix) calculated fold change and difference change values in these comparative analyses (GeneChip software user guide). A gene was considered significantly changed between scoC and wild-type cells if for at least one of the four time points three conditions were met: (i) the difference change value was not “no change”; (ii) the absolute call for the gene in the strain in the higher expression level was listed as “present”; (iii) the fold change calculated by GeneChip 4.0 software was at least 4.37 times higher than SDR,LR for the ALS of the gene on scoC and wild type. A Z score of 4.37 corresponds to a Bonferroni-corrected confidence level of one in 80,000, or 0.05 divided by 4,000 genes. For large genes with multiple probe sets, at least one of the probe sets needed to be significant by the above criteria.

RESULTS

Quantitating transcriptional differences. To determine the sensitivity and reproducibility of the microarray assay, several controls were performed and other indicators were monitored throughout the study. All RNA samples were spiked with a mixture of four in vitro transcripts at various concentrations to monitor the sensitivity and consistency of signal varying as a function of target concentration. Figure 2 shows the linearity of the IVT detection over several orders of magnitude in a complex mixture of total Bacillus RNA. To determine if the changes in expression assayed by the array were similar to those in other established methods, total RNA from two samples varying in growth phase or in genotype was quantitated by both RT-PCR and array hybridization (Table 1). For transcripts increasing, decreasing, and remaining unchanged under the conditions compared, the two assay methods were in close agreement.

To determine array reproducibility, the intensities of all gene transcripts assayed across two samples, prepared as identically as possible, were compared. A Pearson correlation coefficient of 0.953 between replicates indicates good agreement. This value is slightly lower than that calculated for nylon membrane arrays reported recently (7). This is likely due to the

FIG. 2. Spiked control IVT signal as a function of concentration. Final concentrations of the four control in vitro transcripts in the 200-μl hybridization cocktail (assuming an equimolar reverse trans-
higher signal values acquired from detection of radiolabeled targets on membranes relative to fluorescence-detected signals on glass microarrays used in this study (R. Caldwell, unpublished observation).

The percentage of ORF probe sets receiving an absolute call of "present" ranged from 67 to 74% on each array, with slightly more genes showing presence in the stationary than log phase. The percentage of ORF probe sets showing presence for at least one of the four time points in either scoC or the wild type is larger (>85%), indicating that a vast majority of transcripts are detectable at some point during growth by the GeneChip assay, though generally not at all time points.

**General overview.** Across the four time points at which scoC cells were compared to wild-type cells, mRNAs transcribed from 560 genes (representing 14% of the Bacillus genome) met the significant-change criteria defined in Materials and Methods. These 560 most stringently selected genes fall into nearly all functional-class categories (Table 2), from genes coding for enzyme catalysis to those coding for proteins with a regulatory role.

For genes that have been assigned known or presumed functions, the largest group with altered transcriptional levels in the scoC mutant is a group of 76 genes coding for transport proteins, binding proteins, and lipoproteins, all of which are associated with the cell membrane. Forty-seven affected genes belong to the group involved in amino acid (and related molecule) metabolism, notably arginine, histidine, leucine, isoleucine, valine, and threonine biosynthetic genes. As one might expect, we identified changes in expression levels for several genes associated with sporulation, belonging to the SubtiList functional class 1.8 (25, 26) (http://genolist.pasteur.fr/SubtiList/). These changes in GeneChip genes are summarized in Table 3. Other large functional groups affected by scoC include 29 genes associated with motility, 38 associated with carbohydrate metabolism (notably myoinositol and acetoin metabolism), 27 associated with metabolism of nucleotides and nucleosides (purine and pyrimidine biosynthetic genes), and 19 associated with adaptation to atypical conditions and detoxification. Thirty-five ribosomal proteins also showed a change in gene expression. The relevance of 35 out of 55 ribosomal proteins being transcribed at a higher level in the scoC mutant at time point t3 is not immediately obvious. There are 166 genes with unknown function or having no similarity to any protein in existing databases.

Biochemical and genetic data are available for a number of genes for which scoC-specific expression changes are known, as determined by earlier studies. Table 4 shows that there is a substantial agreement between our observations and previously reported findings.

**Regulatory genes affected by ScoC.** The functional category of regulatory proteins shows a relatively large number of scoC-affected genes. Tables 5 and 6 list these genes and their
TABLE 3. Sporulation genes affected by the scoC4 mutation

<table>
<thead>
<tr>
<th>Gene</th>
<th>Change (fold)</th>
<th>Time point</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>bofA</td>
<td>4.4</td>
<td>t4</td>
<td>Inhibition of the pro-sigma-K processing machinery</td>
</tr>
<tr>
<td>coeE</td>
<td>3.7</td>
<td>t4</td>
<td>Spore coat protein (outer)</td>
</tr>
<tr>
<td>coeA</td>
<td>4.6</td>
<td>t4</td>
<td>Polypeptide composition of the spore coat</td>
</tr>
<tr>
<td>coeC</td>
<td>4.1</td>
<td>t4</td>
<td>Polypeptide composition of the spore coat</td>
</tr>
<tr>
<td>coeF</td>
<td>4.4</td>
<td>t4</td>
<td>Polypeptide composition of the spore coat</td>
</tr>
<tr>
<td>cotY</td>
<td>7.8</td>
<td>t4</td>
<td>Spore coat protein (insoluble fraction)</td>
</tr>
<tr>
<td>dufC</td>
<td>6.1</td>
<td>t4</td>
<td>Penicillin-binding protein (putative Ala-Ala carboxypeptidase)</td>
</tr>
<tr>
<td>phaA</td>
<td>−3.0</td>
<td>t2</td>
<td>Phosphatase (RapA) inhibitor</td>
</tr>
<tr>
<td>rpaA</td>
<td>−3.5</td>
<td>t1</td>
<td>Response regulator aspartate phosphatase</td>
</tr>
<tr>
<td>sinI</td>
<td>2.3</td>
<td>t2</td>
<td>Antagonist of SinR</td>
</tr>
<tr>
<td>spmA</td>
<td>4.8</td>
<td>t4</td>
<td>Spore maturation protein (spore core dehydration)</td>
</tr>
<tr>
<td>spmA</td>
<td>4.7</td>
<td>t4</td>
<td>Spore maturation protein (spore core dehydration)</td>
</tr>
<tr>
<td>spoID</td>
<td>3.9</td>
<td>t4</td>
<td>Required for complete dissolution of the asymmetric septum</td>
</tr>
<tr>
<td>spoIIIAB</td>
<td>5.7</td>
<td>t4</td>
<td>Mutants block sporulation after engulfment</td>
</tr>
<tr>
<td>spoIIIAC</td>
<td>3.6</td>
<td>t4</td>
<td>Mutants block sporulation after engulfment</td>
</tr>
<tr>
<td>spoIIIAD</td>
<td>3.3</td>
<td>t4</td>
<td>Mutants block sporulation after engulfment</td>
</tr>
<tr>
<td>spoIIIAF</td>
<td>3.9</td>
<td>t4</td>
<td>Mutants block sporulation after engulfment</td>
</tr>
<tr>
<td>spoIIIAH</td>
<td>3.1</td>
<td>t4</td>
<td>Mutants block sporulation after engulfment</td>
</tr>
<tr>
<td>spoIIIAH</td>
<td>3.3</td>
<td>t4</td>
<td>Mutants block sporulation after engulfment</td>
</tr>
<tr>
<td>spoIIIIP</td>
<td>4.7</td>
<td>t4</td>
<td>Required for dissolution of the septal cell wall</td>
</tr>
<tr>
<td>spoIVQ</td>
<td>2.9</td>
<td>t4</td>
<td>Required for completion of engulfment</td>
</tr>
<tr>
<td>spoIWA</td>
<td>4.1</td>
<td>t4</td>
<td>Required for proper spore cortex formation and coat assembly</td>
</tr>
<tr>
<td>spoIVFA</td>
<td>3.8</td>
<td>t4</td>
<td>Inhibition of spoIVFB</td>
</tr>
<tr>
<td>spoIVFB</td>
<td>4.3</td>
<td>t4</td>
<td>Required for the processing of pro-sigma-K to active sigma-K</td>
</tr>
<tr>
<td>spoIVB</td>
<td>4.9</td>
<td>t4</td>
<td>Involved in spore cortex synthesis</td>
</tr>
<tr>
<td>spoIVD</td>
<td>6.1</td>
<td>t4</td>
<td>Required for assembly of the spore coat</td>
</tr>
<tr>
<td>spoIVK</td>
<td>4.8</td>
<td>t4</td>
<td>Disruption leads to the production of immature spores</td>
</tr>
<tr>
<td>spoVM</td>
<td>4.5</td>
<td>t4</td>
<td>Required for normal spore cortex and coat synthesis</td>
</tr>
<tr>
<td>spoVR</td>
<td>8.4</td>
<td>t4</td>
<td>Involved in spore cortex synthesis</td>
</tr>
<tr>
<td>spsA</td>
<td>3.5</td>
<td>t4</td>
<td>Required for the processing of pro-sigma-K to activate sigma-K</td>
</tr>
<tr>
<td>spsC</td>
<td>6.7</td>
<td>t4</td>
<td>Small acid-soluble spore protein (major alpha-type SASP)</td>
</tr>
<tr>
<td>spsE</td>
<td>2.9</td>
<td>t4</td>
<td>Small acid-soluble spore protein (minor alpha-beta-type SASP)</td>
</tr>
<tr>
<td>tlp</td>
<td>7.3</td>
<td>t4</td>
<td>Small acid-soluble spore protein (thioredoxin-like protein)</td>
</tr>
<tr>
<td>usd</td>
<td>8.3</td>
<td>t4</td>
<td>Required for translation of spoIHD</td>
</tr>
<tr>
<td>ykaT</td>
<td>7.1</td>
<td>t4</td>
<td>Unknown; sporulation protein sigma-E-controlled</td>
</tr>
<tr>
<td>ykvU</td>
<td>5.4</td>
<td>t4</td>
<td>Unknown; similar to spore cortex membrane protein</td>
</tr>
<tr>
<td>yrbA</td>
<td>3.8</td>
<td>t4</td>
<td>Unknown; similar to spore coat protein</td>
</tr>
</tbody>
</table>

* A value greater than 0 indicates higher scoC mutant expression relative to wild type.

* See Fig. 1.

TABLE 4. Correlation of findings in this study with findings in relevant literature

<table>
<thead>
<tr>
<th>Gene monitored</th>
<th>Change in scoC-specific expressiona</th>
<th>This study</th>
<th>Literature (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hag</td>
<td>↓</td>
<td>Slight ↓b</td>
<td>(23)</td>
</tr>
<tr>
<td>oppA</td>
<td>−1.5×↑</td>
<td>2×↑b</td>
<td>(17)</td>
</tr>
<tr>
<td>oppBCDF</td>
<td>2×↑</td>
<td>2×↑</td>
<td>(17)</td>
</tr>
<tr>
<td>appA</td>
<td>2×↑</td>
<td>2×↑</td>
<td>(17)</td>
</tr>
<tr>
<td>appBCDF</td>
<td>2×↑</td>
<td>2×↑</td>
<td>(17)</td>
</tr>
<tr>
<td>npE and aprE</td>
<td>2×↑</td>
<td>2–3×↑c</td>
<td>(4)</td>
</tr>
<tr>
<td>npE</td>
<td>3×↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aprE</td>
<td>3×↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>5–10×↑</td>
<td>↑e</td>
<td>(15)</td>
</tr>
<tr>
<td>phoB</td>
<td>1.5×↑</td>
<td>5×↑</td>
<td>(33)</td>
</tr>
</tbody>
</table>

* Relative to the wild type. ↑ and ↓, increase and decrease.

* Measured as a LacZ fusion.

* Enzyme assay.

* Alkaline phosphatase gene not known.

* Qualitative assay, probably not a secreted enzyme (see Discussion).
another additional ScoC-binding consensus was identified at −289.

**Sporulation and catabolite repression.** In agreement with the sporulation control phenotype of scoC, several additional sporulation control genes are affected in the scoC4 null mutant (Table 7). These regulatory genes, which exert their effects through nontranscriptional means (e.g., bofA, spoIVFAB, spo0M, and usd), were not previously known to lie under ScoC control. It is important to note that the absence of ScoC lowers the level of napA phosphatase transcription and that of its inhibitor, pheA, more than threefold compared to levels in the wild-type strain (Table 3). Recent studies on whole-genome analysis of catabolite repression in *B. subtilis* describe the down-regulatory effect of glucose on the expression of a large number of genes (24, 42). The expression of some of these genes, such as gapB (−5-fold), pckA (−2-fold), cstA (−2-fold), mstX (−2-to −3-fold), acoA (−30-fold) and yesLM (−2-to −4-fold) are also down-regulated in the scoC mutant in this study. However, the transcription of some other genes repressed in presence of glucose, such as those of the iol and the opp operons, is increased in scoC-deficient strains.

A comparison of the transcriptional behavior of sporulation-associated genes in the scoC mutant with the membrane array results obtained by Fawcett et al. (7) suggests both a synergistic and an antagonistic effect between spo0A and scoC. In the present study, in at least one time point, the absence of ScoC lowered the transcriptional levels of the dpp, hut, and ybcPQST-ybdAB operons. These genes have been shown to depend on intact Spo0A for efficient transcription in the published membrane study (7). Similarly, the transcription of the yxbA, yxbB, and yxnB genes appears to require both Spo0A and ScoC (as determined from both studies).

Most of the scoC effects, however, suggest controlling effects in opposition between ScoC and Spo0A. Several of the genes reviewed by Stragier and Losick (36) that have a known or putative role in sporulation show a higher level of expression in the scoC4 strain than in the wild type (Table 3). Furthermore, more than 50% of the genes with unknown functions listed by Fawcett et al. (7), genes whose transcriptions are both Spo0A and α-dependent, are transcribed at a higher level in the scoC4 mutant (Table 7). This list includes the yabP and yabQ genes, which have been identified as essential for sporulation (1, 7).

It is interesting that not all the degradative enzymes whose expression is associated with the stationary phase and which also appear to be under spo0A control (7) are repressed by ScoC. As an example, while the transcriptions of aprE, nprE, and nprB are elevated in scoC, the transcriptions of vpr, csn, and pel are not affected.

---

**TABLE 5. Genes encoding transcriptional regulatory proteins (SubtiList functional class 3.5.2) whose expression is significantly changed in the scoC4 strain**

<table>
<thead>
<tr>
<th>Regulator</th>
<th>Change (fold)</th>
<th>Time point</th>
<th>Description</th>
<th>Gene(s) controlled</th>
<th>Potential ScoC site(s)*</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>comKb</td>
<td>−2.5</td>
<td>t3</td>
<td>Competence transcription factor</td>
<td>Competence and sigD-controlled genes</td>
<td>GATTTTATC</td>
<td>−96</td>
</tr>
<tr>
<td>ykoM</td>
<td>−3.7</td>
<td>t4</td>
<td>Unknown; MarR family</td>
<td>?</td>
<td>AAAAAATTAC</td>
<td>−155</td>
</tr>
<tr>
<td>ykvE</td>
<td>−2.8</td>
<td>t4</td>
<td>Unknown; MarR family</td>
<td>?</td>
<td>AAGAGTATC</td>
<td>−360</td>
</tr>
<tr>
<td>pprR</td>
<td>−3.6</td>
<td>t1</td>
<td>Transcriptional attenuation of the pyrimidine operon</td>
<td>pprPBCADFE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cheY</td>
<td>−3.8</td>
<td>t4</td>
<td>Two-component response regulator</td>
<td>Flagellar, chemotaxis genes</td>
<td>AGTATTATC</td>
<td>−184</td>
</tr>
<tr>
<td>cheB</td>
<td>−4.6</td>
<td>t4</td>
<td>MCP-glutamate methyltransferase</td>
<td>Chemotaxis genes</td>
<td>GAAATTATC</td>
<td>−35</td>
</tr>
<tr>
<td>yzR</td>
<td>−2.7</td>
<td>t3</td>
<td>Unknown; DecR family</td>
<td>?</td>
<td>AATAATTTAC</td>
<td>−232</td>
</tr>
<tr>
<td>spoIVD</td>
<td>5.6</td>
<td>t4</td>
<td>Transcriptional regulator of sigma-E- and sigma-K-dependent genes</td>
<td>Sporulation genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hutP</td>
<td>−4.0</td>
<td>t2</td>
<td>Transcriptional activator of the histidine utilization operon</td>
<td>hutPHUIGM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yesN</td>
<td>−2.6</td>
<td>t4</td>
<td>Unknown; similar to two-component response regulator</td>
<td>?</td>
<td>AATTTATTC</td>
<td>−418</td>
</tr>
<tr>
<td>ywrC</td>
<td>−3.1</td>
<td>t1</td>
<td>Unknown; Lrp/AsnC family</td>
<td>?</td>
<td>AATATTATTC</td>
<td>−69</td>
</tr>
<tr>
<td>spoIIP</td>
<td>−4.0</td>
<td>t2</td>
<td>Transcriptional activator of the histidine utilization operon</td>
<td>hutPHUIGM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yesN</td>
<td>−2.6</td>
<td>t4</td>
<td>Unknown; similar to two-component response regulator</td>
<td>?</td>
<td>AATTTATTC</td>
<td>−418</td>
</tr>
<tr>
<td>egrR</td>
<td>−3.4</td>
<td>t3</td>
<td>Transcriptional repressor of gapA</td>
<td>gapA</td>
<td>AATATTGTC</td>
<td>−159</td>
</tr>
</tbody>
</table>

* Sequences upstream of affected genes (−10 to −500 bp) were searched for the presence of the canonical ScoC binding site RATANTATY (R = A or G, Y = C or T, N = any base), allowing at most one mismatch, using the Find Regulatory Sequence function of the GeneSpring software (Silicon Genetics).

b Both comK and yesN miss the strict 4.37σ significance cutoff (comK Z score = 4.31, yesN Z score = 4.15); however, the gene encoding the cognate histidine kinase of YesN (yesM) does pass the 4.37σ threshold.
Nitrogen metabolism. Microarray analysis reveals a number of changes in the transcriptional profile of several genes coding for enzymes involved in nitrogen utilization. The transcription of all the genes in the *hut* operon is decreased in the *scoC4* mutant between 3- and 20-fold compared to that in the wild-type strain (Fig. 3A). Similarly, the *sigL*-specific transcription of the genes involved in the degradation of isoleucine and valine (2) is down-regulated in *scoC4*, as is the transcription of the *ureABC* genes (Figs. 3B and C). A striking observation is that these three *scoC*-affected operons are also regulated by *codY* (9, 32). Moreover, the transcriptional profile of these three operons demonstrates the typically tight clustering of transcriptional profiles expected for genes lying within operons, giving further confidence in the robustness of the expression assay.

In addition, with further implications for the influence of *scoC* on genes involved in nitrogen metabolism, transcription levels of the operon *glnQHMP* (coding for the ABC glutamine transporter) are four- to sixfold higher in the mutant than in the wild type.

Motility and chemotaxis. To ascertain the motility of the cells under our growth conditions, we looked at samples from cultures of both strains at the four time points. Wild-type strain motility ranged from approximately 60% at t1 to more than 90% at t4. In the *scoC4* mutant culture, however, most of the cells were filamentous and only 5% (t1) to 20% (t4) of the cells were motile.

Figure 4 illustrates the transcriptional profiles for all the genes from *flgB* to *cheD*, in which a large number of motility and chemotaxis genes appear to be expressed at a lower level at the three later time points. Expression values were highest in mid-log phase (t1) and decreased progressively to the last time point analyzed in stationary phase (t4), while *sinI* transcription progressively increased with a steeper slope in the mutant than in the wild type. The transcription of the *hag* gene, which codes for flagellin, is also decreased in the *scoC* mutant. It is interesting that, as in the case of the *hut*, *bdk*, and *ure* operons, both *codY* and *scoC* are known to play a role in the expression of *hag* (23).

It is also interesting that the gene coding for McpC, a methyl-accepting protein mediating the carbohydrate chemotaxis in synergy with the phosphoenolpyruvate-dependent phosphotransferase system, is also down-regulated in *scoC*, although obviously down-regulated in *scoC* in our assay, failed to meet the most stringent requirements set by our statistical analysis (Z scores of only 4.1).

### TABLE 6. Genes encoding regulatory proteins not acting through transcription (nonmembers of SubtiList functional class 3.5.2) whose expression is significantly changed in the *scoC4* strain

<table>
<thead>
<tr>
<th>Regulator</th>
<th>Change (fold)</th>
<th>Time point</th>
<th>Description</th>
<th>Potential ScoC site(s)</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>bofA</em></td>
<td>4.4</td>
<td>t4</td>
<td>With SpoIVFA, inhibits processing of pro-α^K^ by SpoIVFB</td>
<td>α^K^ GATAGAATC</td>
<td>−147</td>
</tr>
<tr>
<td><em>spoIVFA</em></td>
<td>3.8</td>
<td>t4</td>
<td>With BofA, inhibits processing of pro-α^K^ by SpoIVFB</td>
<td>α^K^ GAAAATATT</td>
<td>−468</td>
</tr>
<tr>
<td><em>spoIVFB</em></td>
<td>4.3</td>
<td>t4</td>
<td>Processing of pro-α^K^</td>
<td>α^K^</td>
<td></td>
</tr>
<tr>
<td><em>rapA</em></td>
<td>−3.5</td>
<td>t1</td>
<td>Response regulator aspartate phosphatase; dephosphorylates SpolF-P</td>
<td>SpolF</td>
<td></td>
</tr>
<tr>
<td><em>phrA</em></td>
<td>−3.6</td>
<td>t1</td>
<td>Inhibitor of the activity of phosphatase RapA</td>
<td>RapA GAAAGTATC</td>
<td>−282</td>
</tr>
<tr>
<td><em>spo0M</em></td>
<td>2.3</td>
<td>t3</td>
<td>Sporulation control gene; expression controlled by sigma-H</td>
<td>? AATATTATT</td>
<td>−125</td>
</tr>
<tr>
<td><em>sinI</em></td>
<td>2.3</td>
<td>t2</td>
<td>Antagonist of SinR, transcriptional regulator of post-exponential-phase response genes</td>
<td>SinR AATACGATT</td>
<td>−142</td>
</tr>
<tr>
<td><em>udas</em></td>
<td>8.3</td>
<td>t4</td>
<td>Required for translation of spoIID</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td><em>yesM</em></td>
<td>−3.9</td>
<td>t4</td>
<td>Two-component sensor histidine kinase</td>
<td>YesN</td>
<td></td>
</tr>
<tr>
<td><em>yvdD</em></td>
<td>−3.5</td>
<td>t1</td>
<td>Similar to α^34^ modulating factor from gram-negative organisms</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td><em>rsbV</em></td>
<td>−3.8</td>
<td>t2</td>
<td>Positive regulator of α^B^ activity (anti-anti-sigma factor)</td>
<td>α^B^ GATATGATT</td>
<td>−271</td>
</tr>
<tr>
<td><em>rsbW</em></td>
<td>−3.5</td>
<td>t2</td>
<td>Negative regulator of α^B^ activity (anti-sigma factor)</td>
<td>α^B^ GATTCTATT</td>
<td>−387</td>
</tr>
</tbody>
</table>

* Sequences upstream of affected genes (~10 to ~500 bp) were searched for the presence of the canonical ScoC binding site RATANTATY (R = A or G, Y = C or T, N = any base), allowing at most one mismatch, using the Find Regulatory Sequence function of the GeneSpring software (Silicon Genetics).
levels in scoC4 cells than in the isogenic wild-type cells. The Z
scores associated with these measurements are greater than
9.0, indicating a high degree of significance in the difference
between the two strains.

DISCUSSION

It has been recognized that, “[a]lthough transient in labora-
tory cultures, the transition state is probably the predominant
metabolically active state of Bacillus in the natural environ-
ment of the soil where nutrients are usually limited” (38). In
order to maximize the utilization of available resources, bacilli
have developed complex regulatory circuits to allow functional
diversification of the population and to fine tune and quickly
adapt their metabolism to a constant state of nutrient
flux. This

communal organization ensures that a fraction of the popula-
tion survives, either by the resumption of vegetative growth by
exploiting new nutrient sources made available by the secretion
of degradative enzymes, or by the entrance into sporulation,
the ultimate survival strategy in a nutrient-depleted environ-
ment. To this end, while Spo0A acts as the master switch
determining whether the cell continues vegetative proliferation
or carries out stationary-phase functions, Bacillus has evolved
a number of other regulators, called transition state regulators
(38), with partially overlapping control functions. ScoC belongs
to this group of regulators. However, the complete role of
ScoC in the life of bacilli has not been fully elucidated.

In the present work, we have expanded the scope of our
knowledge of scoC’s overall regulatory role. To do so, we
analyzed the B. subtilis transcriptome under defined laboratory
conditions with the aid of species-specific oligonucleotide mi-
croarrays. The scoC4 mutant used in this experiment reveals
transcriptional changes from isogenic wild-type cells in approx-
Therefore, a smaller change occurring at low signal levels, the error of measurement tends to be dominated by a small fraction of the measurement. In contrast, at high signal levels, the scatter of the error of measurement is a better measure of significance than the gene expression change alone.

The reliability of the results obtained using this approach is supported by their agreement with data previously reported in the literature and summarized in Table 4. For example, the expression patterns for the opp operon genes in our experiments are similar to the results recently reported for an oppA-lacZ fusion analysis by Koide et al. (17). Furthermore, the scoC4-transcriptional level of aprE was substantially increased in the present study, as has been reported elsewhere (8). Also, the increase in the level of sinI-specific mRNA in scoC cells is in agreement with previously unpublished data (33). ScoC has been shown to also affect a previously unidentified alkaline phosphatase. From our expression data, it appears that this measured activity is due to PhoB (4).

A major role attributed to scoC has been its effect on the sporulation process, coupled to catabolite repression (4, 14) by a not-yet-clarified mechanism. It has been reported that scoC controls the activity of SinR by transcriptionally modulating the gene expression of the SinR antagonist, sinI (15, 32). In addition, ScoC has been described as controlling the expression of the app and opp operons, whose gene products are responsible for the import of quorum-sensing signaling peptides (17). At first glance, while these are very important roles, it may not be sufficient to explain all the phenotypes associated with scoC. Data presented here show that the level of transcription of rapA and phrA in a scoC null mutant is diminished more than threefold. This is likely to affect the level of Spo0A phosphorylation, which, in turn, will affect the level of spo0A mRNA due to the fact that phosphorylated Spo0A activates its own transcription (13). Although our statistical analysis does not indicate a significant change in the absolute level of spo0A transcript between the wild type and mutant, there is a slight but noticeable difference in the level of spo0A transcript in the scoC strain at t2 relative to the level of spo0F transcription. While in all the strains we have tested so far spo0A mRNA rises sharply around Tm, it has already increased considerably just before transition relative to the spo0F mRNA in the scoC mutant (Fig. 5). We also find that the transcription of bofA, spoIVFA and spoIVFb, involved in the processing of σK, increases in the scoC strain after t3 (fourfold higher at t4).

However, while we find the level of rapA and phrA significantly reduced in the scoC strain, the expression levels of the other members of the Rap/Phr family, several of which are under Spo0A control (7), do not appear to be significantly affected. The expression of several of the other genes known to play an important role in the initiation of sporulation, such as spo0B, spo0F, abrB, kinA, kinB, kinC, or those encoding the sporulation-specific sigma factors, is likewise not significantly affected by scoC4.

In all, the results show that ScoC affects the level of expression of 38 sporulation genes as tabulated by Stragier and Losick (36). In addition, about 50% of the genes with unknown functions, identified by Fawcett et al. (7) as requiring both Spo0A and σK for transcription, show positive shifts in expression in the scoC null mutation (Table 7). We show that ScoC has both negative (pckA and gapB) and positive (iol operon)
effects on the expression of a number of genes involved in carbon metabolism (24, 42). This balancing act of both down- and up-regulation of genes may partially explain how scoC null mutations relieve the sporulation repression exerted by glucose. While these findings do not lead to a complete understanding of the mechanism by which scoC plays a role in sporulation, it certainly indicates a wider role for scoC in this process than previously appreciated.

*B. subtilis* has a varied repertoire of genes regulating nitrogen metabolism. The main regulatory genes identified to date are codY, tranA, glnR, and glnA (9). In the absence of ScoC, it was a surprise to observe a very strong downward effect on the transcription of genes involved in the utilization of amino acids as a nitrogen source, such as the *hut* and *kbd* operons. The fact that scoC also causes a decrease in the transcriptional level of the *ureABC* operon seems to suggest that there is at least a partial overlap in the regulation of this nitrogen-related group of genes by both CodY and ScoC. The possibility of a coregulatory function is strengthened by the down-regulation effect we have observed in scoC4 for other CodY-regulated genes, such as *gabP*, *comK*, *rapA* (31), the *hag* regulon (23), and, to a lesser extent, the *dpp* operon. However, there is no obvious *scoC* effect on *srA*, *rapC*, and *citB*, which were reported as being regulated by *codY* (31). In addition, the expression of *codY* itself has an unchanging profile between the wild-type and *scoC* strains.

A strong (fivefold) expression increase was exerted in the *scoC4* mutant on the glutamine transporter operon (*glnQHMP*). This may indicate a higher level and/or demand of glutamine, a key intermediary in nitrogen utilization in *B. subtilis*. While the *scoC* mutation represses nitrogen utilization by lowering the expression of the *hut*, *kbd*, and *ure* operons, it elevates the expression of the genes involved in the transport of the central player for nitrogen metabolism, glutamine. This may help explain the observation (33) that a strain carrying a mutation in *scoC* can affect catabolite repression exerted by glucose but not that exerted by glucose and glutamine together.

The slight but observable increase in the expression level of *sinI* is bound to antagonize SinR activity. It has been reported that *sinR* is a positive regulator of *sigD* (30), involved in motility functions. Therefore, a higher level of *sinI*, by inactivating *sinR*, would depress the motility of a *scoC* mutant by repressing *sigD* transcription. This may explain the observation that *scoC* mutants are somewhat less motile than wild-type cells (16). We have shown that a great many of the genes involved in motility and chemotaxis are indeed transcribed at lower levels in the *scoC4* strain (Fig. 4).

Given the large number of genes affected by ScoC, as determined by comparing the *scoC4* mutant and wild-type transcriptomes, it is perhaps not surprising that a large number of affected genes themselves encode regulatory proteins, thus transmitting the direct effects of ScoC to other genes. ScoC is known to be a pleiotropic regulatory protein, and we suggest that some of these effects are likely due to an indirect action mediated through other regulators (Tables 5 and 6). Potential ScoC DNA binding sites were found upstream of several regulatory genes, including *ykoM*, *ykvE*, *cheY*, *cheB*, *rhsR*, *ywrC*, and *cggR*, in which transcription appears to be decreased in the *scoC* loss-of-function mutant in at least one of the four time points assayed (Tables 5 and 6). The bidirectionality of these regulatory effects and of the effects seen on genes involved in nitrogen metabolism points to the interesting possibility that ScoC acts both in a negative and, perhaps directly or indirectly, in a positive manner.

The effect of the *scoC* mutation on *comK* expression (discussed above), together with the effect on the transcription of the *opp* operon, may explain the lower level of competence reached by a *scoC* mutant (Causey and Ferrari, unpublished). It is interesting that *comK* appears to have a putative *scoC* binding site at upstream position −147 (Table 5).

The *yctF* gene is unique in our set of significantly changed expressions in *scoC*. It is the only gene that showed an increased expression level at all four time points tested. In the
amino acid sequence shows high similarity (E value 492-amino-acid in which this repression would be relaxed. The function of the elements are double underlined.

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repressing TATY (Fig. 6). These sequences are potential binding sites matches to the ScoC-binding consensus sequence, RATAN-quences. Overlying the sequences of this region are two close binding site. The

yclF promoter region of Lactococcus lactis dtpT there are clear and canonical

tinct from the multisubunit ABC peptide transporters, such as proton symporters, which are structurally and functionally dis-

novel di- and tripeptide transporter (11, 18). Both DtpT and yclF in B. subtilis gene product, a

35 boxes of the

yclF protein in B. subtilis transcriptome. This

FIG. 6. The 280-bp promoter region of the yclF gene (and the yclG gene). Start codons are underlined and in bold capitals. R.B.S., ribosomal binding site. The –10 and –35 boxes of the yclF promoter are indicated. The ScoC-binding consensus sequences overlying these promoter elements are double underlined.

promoter region of yclF, upstream of its ribosomal binding site, there are clear and canonical –10 and –35 ρ4 promoter se-
quences. Overlying the sequences of this region are two close matches to the ScoC-binding consensus sequence, RATAN-
TATY (Fig. 6). These sequences are potential binding sites repressing yclF in wild-type cells relative to the scoC4 mutant, in which this repression would be relaxed. The function of the 492-amino-acid yclF protein in B. subtilis is unknown, but its amino acid sequence shows high similarity (E value = 10^{-106}) to the 463-amino-acid Lactococcus lactis dtpT gene product, a novel di- and tripeptide transporter (11, 18). Both DtpT and YdF appear to be members of the PTR superfamily, peptide/ proton symporters, which are structurally and functionally dist-
tinct from the multisubunit ABC peptide transporters, such as Opp and Dpp (35). A point mutation in dtpT leads to increases in protease gene transcription in L. lactis (21). Research aimed at investigating the role of yclF in B. subtilis is in progress.

In this paper, we present a global analysis of the effects of the scoC4 null mutation on the B. subtilis transcriptome. This study has correlated known phenotypes of the scoC null muta-
tion (lower motility, sporulation phenotype, development of competence, and degradative enzyme production) with changes detected in the expression pattern of a large number of genes with both known and unknown function. The data suggest (Table 7) that there are many additional genes in-
volved in the complex network of sporulation genes, an obser-

vation echoed by the recent report by Fawcett et al. (7). In addi-
tion, our work suggests a close and unforeseen link of ScoC to the regulation of nitrogen metabolism. We propose that there may exist a strong interaction of scoC with the global nitrogen metabolism regulators, such as CodY. Finally, we have demonstrated that ScoC clearly plays a major role in various cellular functions of the life cycle of this bacterium.

These observations allow us to paint a picture in which Spo0A is still the master switch of the transition phase, but the real differentiation occurring within the transition state population is mediated by a number of transition state regulators, one of which is scoC. By way of the global regulatory network imple-
mented by these transition state regulators, the bacterial culture can swiftly achieve a redistribution of functions within its populations to carry out the most needed tasks. Elucidating the mechanistic details of how ScoC exercises control among the various processes it participates in will usher in an exciting era for the investigation of Bacillus growth and development.

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