JOURNAL OF BACTERIOLOGY, Dec. 2004, p. 8490–8498 Vol. 186, No. 24
0021-9193/04/$08.00+0 DOI: 10.1128/JB.186.24.8490–8498.2004

Regulation of σ^B by an Anti- and an Anti-Anti-Sigma Factor in *Streptomyces coelicolor* in Response to Osmotic Stress

Eun-Jin Lee,[1] You-Hee Cho,[2] Hyo-Sub Kim,[1] Bo-Eun Ahn,[1] and Jung-Hye Roe[1]*

School of Biological Sciences and Institute of Microbiology, Seoul National University,[1] and Department of Life Science, Sogang University,[2] Seoul, Korea

Received 26 April 2004/Accepted 25 August 2004

σ^B, a homolog of stress-responsive σ^B of *Bacillus subtilis*, controls both osmoprotection and differentiation in *Streptomyces coelicolor* A3 (2). Its gene is preceded by *rsbA* and *rsbB* genes encoding homologs of an anti-sigma factor, RsbW, and its antagonist, RsbV, of *B. subtilis*, respectively. Purified RsbA bound to σ^B and prevented σ^B-directed transcription from the *sigB*p1 promoter in vitro. An *rsbA*-null mutant exhibited contrasting behavior to the *sigB* mutant, with elevated *sigB*p1 transcription, no actinorhodin production, and precocious aerial mycelial formation, reflecting enhanced activity of σ^B in vivo. Despite sequence similarity to RsbV, RsbB lacks the conserved phosphorylatable serine residue and its gene disruption produced no distinct phenotype. RsbV (SCO7325) from a putative six-gene operon (*rsbV-rsbR-rsbS-rsbT-rsbU1-rsbU*) was strongly induced by osmotic stress in a σ^B-dependent manner. It antagonized the inhibitory action of RsbA on σ^B-directed transcription and was phosphorylated by RsbA in vitro. These results support the hypothesis that the rapid induction of σ^B target genes by osmotic stress results from modulation of σ^B activity by the kinase-anti-sigma factor RsbA and its phosphorylatable antagonist RsbV, which function by a partner-switching mechanism. Amplified induction could result from a rapid increase in the synthesis of both σ^B and its inhibitor antagonist.

Transcriptional regulators, especially sigma factors, play pivotal roles in the bacterial survival strategies such as stress responses, differentiation, social behavior, and pathogenesis (25, 29). While the amounts of sigma factors are regulated through controlled synthesis and degradation, their activities are also regulated by other proteins, such as anti-sigma factors (28, 34). The activity of anti-sigma factors can be regulated by a network of other proteins, as best exemplified by the regulation of stress response and sporulation in *Bacillus subtilis*. There, the binding of anti-sigma factors RsbW and SpoIIAB to σ^B and σ^F, respectively, is regulated by their antagonist proteins, by a so called "partner-switching" mechanism, and involves interplay of various kinases and phosphatases (2, 43, 51).

Streptomyces coelicolor is a gram-positive spore-forming soil bacterium that undergoes a complex cycle of morphological and physiological differentiation resembling that of filamentous fungi. The differentiation of *S. coelicolor* is dependent upon its ability to respond to changes in the environment, especially nutrient limitation, and is recognized as one of the processes to escape from the mitotic (vegetative) growth. Interplay of regulatory cascades with metabolic, morphological, homeostatic, and stress-related checkpoints has been proposed (15). Evidence for coupling differentiation with stress-related signals has accumulated in recent years. These include coregulation of stress stimulons with growth (developmental) transitions (45, 58) and participation of several stress-related sigma factors and/or their anti-sigma factors in initiating and completing differentiation process (20, 24, 37, 48, 53).

S. coelicolor devotes more than 12% of its genes (>900 gene products) to encoding transcriptional regulators (7). The presence of over 60 sigma factors reflects the complexity of its gene regulation. Phylogenetic relatedness (30, 41) reveals 1 major sigma (group 1; σ^{HrdB}), three group 2 sigmas (σ^{HrdA}, σ^{HrdC}, and σ^{HrdD}), 10 group 3 sigmas (σ^{WhiG}, σ^B, σ^F, σ^G, σ^H, σ^I [SCO3068], σ^K [6520], σ^L [7278], σ^M [7314], and σ^N [4034]), and 50 group 4 (ECF) sigmas that include σ^E, σ^R, σ^{BldN}, σ^U, and σ^T (25, 27, 37, 49). Among these, only a handful of sigma factors are known to control differentiation and/or stress response. These are σ^{BldN}, σ^{WhiG}, and σ^F for differentiation (8, 14, 50); σ^B and σ^H for both osmotic control and differentiation (20, 37, 53); σ^E and σ^R for stress response (46, 47); and σ^R and σ^U for indirect control of differentiation (24, 48). Involvement of anti-sigma factors to regulate the activity (availability) of sigmas has been demonstrated for σ^R (36), σ^H (54, 57), and σ^U (24), even though it is very likely that the majority of sigma factors are regulated by protein-protein interactions.

σ^B, a group 3 sigma homologous to σ^B from *B. subtilis*, is induced by osmotic stress and starvation and is responsible for osmoprotection and proper differentiation in *S. coelicolor* (20). It regulates the expression of catalase B that is required for osmoprotection and differentiation of *S. coelicolor* (19). Whether the signal transduction path that responds to osmotic stress and starvation employs the partner switching of anti-sigma factor through serine phosphorylation and dephosphorylation, as is used in *B. subtilis*, has not been demonstrated. In *B. subtilis*, σ^B is released free of its anti-sigma factor, RsbW, under both energy and environmental stress conditions (6). RsbW, being a serine kinase, binds and phosphorylates RsbV (22), whose phosphate group can be removed by two phosphatases (RsbP and RsbU) in response to energy and environmental stresses (55, 60). The unphosphorylated form of RsbV

* Corresponding author. Mailing address: School of Biological Sciences and Institute of Microbiology, Seoul National University, Seoul 151-742, Korea. Phone: 82 2 880 6706. E-mail: jhroe@plaza.snu.ac.kr.

serves as an antagonist of the anti-sigma factor RsbW. Even though the *sigB* gene in *S. coelicolor* has as its neighbors an *rsbW* homolog (*rsbA*) and an *rsbV* homolog (*rsbB*) (20), experimental evidence for the presence of anti- and anti-anti-sigma factors for σ^B has been lacking. In addition, a similarity search revealed 48 *rsbW* homologs and 17 *rsbV* homologs in the genome of *S. coelicolor* (44), revealing the complexity and hence the difficulty of elucidating the system.

This work describes an initial effort to find a regulatory path for σ^B in *S. coelicolor*. We present evidences for its anti- and anti-anti-sigma factors, which could be regulated by phosphorylation in a partner-switching mechanism. Regulation of the synthesis of σ^B and its anti-anti-sigma factor is also presented. Our finding will provide a clue to unravel a signal transduction path from nutritional and osmotic stresses to σ^B-directed gene expression in *S. coelicolor* and related bacteria, necessary for proper differentiation and/or stress survival.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Growth and maintenance of *S. coelicolor* A3(2) strains (J1501, M145, and their derivatives) were done as described by Hopwood et al. (33) and Cho et al. (19). For liquid culture, YEME medium (33) containing either 34 or 10.3% sucrose was inoculated with pregerminated spores (about 10^8 to $\sim 10^9$ spores/100 ml of broth). For plate culture, 10^7 pregerminated spores or patches of mycelia were streaked on R2YE, NA, or minimal agar media (33). To facilitate harvesting of aerial and sporulated mycelia, inocula were spread on cellophane membrane on solid media. The growth rates and phases were determined as described by Cho and Roe (17). To apply osmotic stress in liquid culture, 0.2 M KCl was added to cultures of exponentially growing cells in YEME for various lengths of time before harvest.

Preparation of cell extracts and determination of antibiotic pigments. Harvested mycelia were suspended in TGED (50 mM NaCl, 50 mM Tris-HCl [pH 7.8], 5% glycerol, 0.1 mM EDTA, 0.1 mM dithiothreitol [DTT]) buffer containing 1 mM phenylmethylsulfonyl fluoride. They were disrupted by sonication (Sonics and Materials Inc.), and the suspension was clarified by centrifugation at 4°C. The concentration of total protein in cell extract was determined with a Bio-Rad protein assay kit. Extraction of antibiotic pigments and their spectrophotometric quantification were carried out as described by Hobbs et al. (32) and Adamidis et al. (1).

DNA manipulations. Restriction and modifying enzymes were used according to the manufacturer's recommendations (POSCOCHEM, Roche, New England Biolabs). Standard recombinant DNA methods were used. DNA fragments were purified from agarose gels with a GeneClean kit II (BIO101) or the freeze-squeeze method. *Escherichia coli* DH5α, methylation-negative *E. coli* ET12567 (42), and *S. coelicolor* A3(2) J1501 cells were used as hosts for various recombinant DNAs.

Expression and purification of proteins. His-tagged σ^B protein was obtained as described previously (20). Either full-length (FL) or the C-terminal half (C-terminal domain [CTD]) of RsbA protein was prepared through PCR and expression through the pET-3a system (Novagen) in *E. coli*. Mutagenic forward primers containing an NdeI site (5′-GGTGGCGCCCATATGAGCACG-3′ for full length and 5′-CCAGCACGAGCATATGATCAACGC-3′ for CTD; NdeI site underlined) and a backward primer hybridizing immediately downstream of the termination codon (5′-CTCCCGGTGGATCGGTCGTCGTTG-3′) were used. The PCR products of 703 and 1,062 bp were cloned into pUC18, recovered by NdeI and BamHI digestion, and then cloned into pET-3a (Novagen) to yield pET3307 and pET3312, respectively. Soluble fractions of freshly grown *E. coli* cells harboring pET3307 or pET3312 were dialyzed twice against TGED binding buffer (10 mM Tris-HCl [pH 7.8], 20% glycerol, 1 mM EDTA, 1 mM DTT) with 50 mM NaCl. The dialysate was subjected to chromatography through a Resource Q column (Pharmacia) and eluted with a 20-ml gradient of NaCl from 50 to 500 mM in TGED. The eluates were concentrated to about 0.3 mg/ml and dialyzed against storage buffer (50 mM Tris-HCl [pH 7.8], 10 mM $MgCl_2$, 0.1 M KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol). To prepare glutathione *S*-transferase (GST)-tagged RsbA protein, forward primer containing a BamHI site (5′-GCCACGATGGGATCCGCGACCGCC-3′; BamHI site underlined) and the same reverse primer given above were used to amplify a 1,053-bp fragment containing the entire *rsbA* gene. The PCR product was cloned into pUC18, recovered by BamHI and EcoRI digestion, and then cloned into pGEX-4T1 (Amersham Pharmacia) to yield pGEX3312. To prepare GST-tagged RsbV protein, mutagenic forward (5′-GAGGTACACGGATCCATAGCC-3′; BamHI site underlined) and reverse (5′-GGCGGCTCAGAATTCGGGAAC-3′; EcoRI site underlined) primers were used to amplify a 408-bp fragment containing the entire *rsbV* gene with replacement of the first two and the last (130th) codons. The PCR product was digested with BamHI and EcoRI and cloned in pGEX-4T1 (Amersham Pharmacia) to yield pGEX5104. GST-tagged proteins were purified according to the recommendations of the manufacturer (Amersham Pharmacia). Purified proteins were divided into aliquots and stored at −70°C.

σ^B-RsbA interaction on an Ni-NTA column. Partially purified His-tagged σ^B, GST-tagged RsbA, and bovine serum albumin (Sigma) at 20 μg each were incubated at 25°C for 30 min in the binding buffer (10 mM Tris-HCl, pH 7.6, 0.1 M NaCl) and loaded onto 0.5 ml of an Ni-nitrilotriacetic acid (NTA) column (Novagen). The column was washed with 10 volumes of 5 mM imidazole in the binding buffer (W1) and again with 10 volumes of 25 mM imidazole in the binding buffer (W2). Finally the bound proteins were eluted with 3 volumes of 500 mM imidazole in the binding buffer (E). The two wash fractions and eluates were concentrated to 0.5 ml each with a Centricon-10 (Amicon), and 20-μl aliquots were subjected to 10% polyacrylamide gel electrophoresis (PAGE) with sodium dodecyl sulfate (SDS).

In vitro phosphorylation assay. The phosphorylation assay was performed as described by Min et al. (43). The reaction mixture (20-μl total volume) contained 1 μg each of purified RsbA (either full-length or C-terminal domain) and GST-tagged RsbV in 50 mM Tris-HCl (pH 7.6), 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, and 0.1 mM EDTA. The reaction was started by adding 5 μCi of [γ-^{32}P]ATP (5,000 Ci/mmol) and unlabeled ATP to 20 μM, which was then incubated at 22°C for 30 min, and the reaction was terminated by adding 5 μl of SDS-PAGE sample buffer (250 mM Tris-HCl [pH 6.8], 10% glycerol, 1% SDS, 150 mM 2-mercaptoethanol, 0.02% bromophenol blue). The mixtures were heated at 85°C for 3 min and separated on a 13% polyacrylamide gel containing SDS. The labeled proteins were visualized by autoradiography, whereas the positions of protein bands were confirmed by Coomassie blue staining.

RNA isolation and S1 nuclease mapping. RNA isolation and S1 nuclease mapping analysis were performed as described previously (20). Probes used for mapping *sigB*p1, *sigB*p2, and *catB*p transcripts were prepared as described previously (19, 20). The DNA probe for *rsbV*p transcript was prepared by PCR and contained 12 bp of unrelated sequence linked to 382 bp of *rsbV* gene sequence. The 5′ end position labeled with ^{32}P corresponds to 72 nucleotides downstream from the ATG start codon.

In vitro transcription assay. Transcription assays were carried out as described by Kang et al. (35) with slight modifications. To examine the effect of anti-sigma factor, RsbA protein with or without GST tag (10 to 20 pmol) was preincubated with His-tagged σ^B protein (10 pmol) at 37°C for 30 min, prior to incubation with *S. coelicolor* core RNA polymerase (1.5 pmol) for another 30 min in 16 μl of transcription buffer. Template DNAs containing *sigB*p1 or *catB*p (0.2 pmol) were added and incubated for an additional 30 min to allow sufficient formation of the promoter open complexes. For comparison, transcription from *sigR*p2 promoter by His-tagged σ^R was followed in parallel. Transcription was restricted to a single round by adding heparin (50 μg/ml) 2 min after initiation by nucleoside triphosphate (NTP) mix containing [α-^{32}P]CTP. Transcripts were resolved in 5% PAGE containing 7 M urea, followed by autoradiography. To examine the effect of RsbV protein on antagonizing RsbA, GST-tagged RsbV protein (40 to 160 pmol) was preincubated with GST-RsbA (10 to 20 pmol) and His-tagged σ^B (10 pmol) for 30 min prior to the addition of core RNA polymerase.

Gene disruption and complementation. To prepare an internally deleted *rsbA* gene, a 425-bp BclI-BamHI fragment encompassing the majority of the C-terminal half of the *rsbA* gene was deleted from the 1.8-kb SalI fragment and cloned into pDH5, resulting in pDH314. Nonmethylated pDH314 DNA prepared from *E. coli* ET12567 (42) was alkaline denatured and introduced into the J1501 protoplast by transformation (31). Single-crossover recombinants were identified as thiostrepton-resistant colonies and verified by Southern hybridization. YI3105, a cointegrate in which the recombination took place in the desired region, was selected and allowed to sporulate on a nonselective minimal medium to ensure second crossover. Two types of thiostrepton-sensitive colonies were obtained by plating: one with wild-type morphology and the other with the white phenotype. The white colonies all retained the deleted *rsbA* gene as judged by Southern analysis. One representative mutant (YD352) was selected and used for further analysis. The Southern result excluded the possibility of remaining the wild-type *rsbA* gene in the mutant, arising from duplication of a long terminal region where *rsbA* and *sigB* genes reside in the J1501 strain (59). For complementation, the 4,611-bp SmaI-BamHI fragment containing the entire *sigB* operon was isolated from pUC246 (18) and cloned into pSET152 (9), resulting

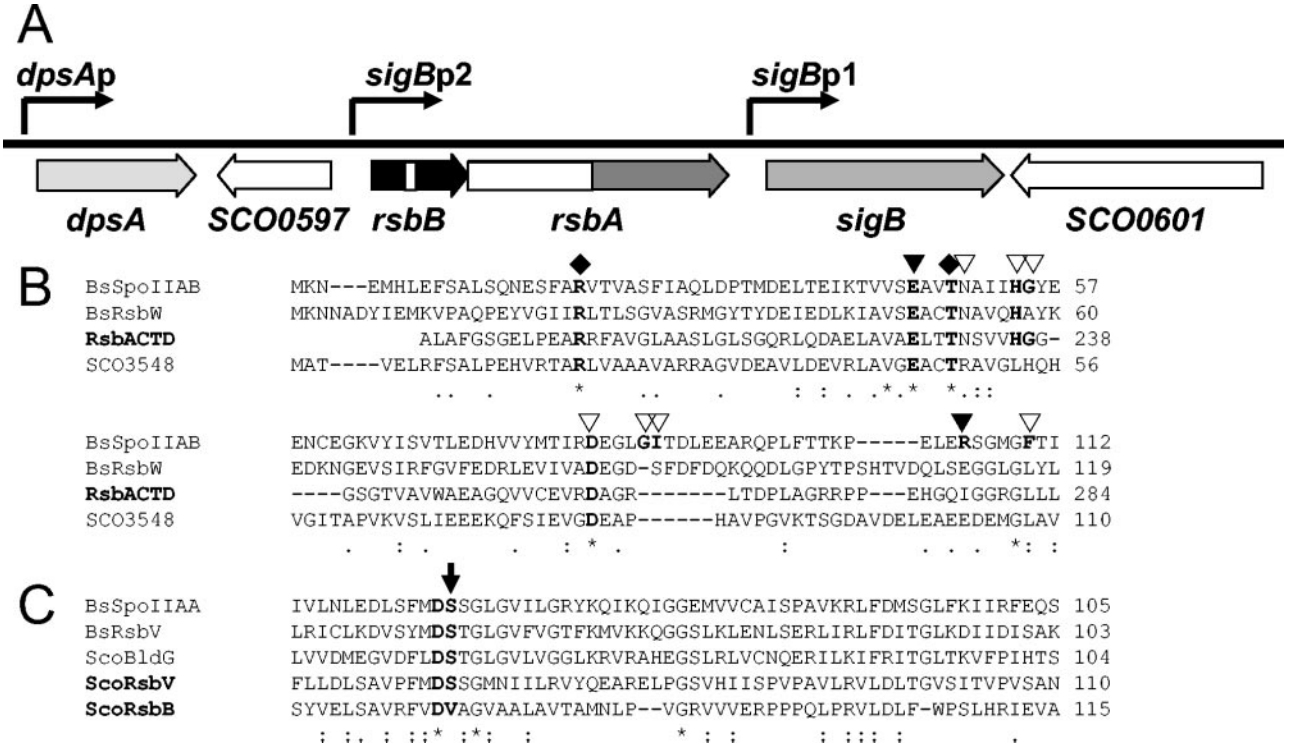

FIG. 1. Sequence analysis of *S. coelicolor* RsbA and RsbB. (A) Gene structure around *sigB* operon. The *sigB* gene is cotranscribed with *rsbB* and *rsbA* genes from the upstream promoter *sigB*p2 in a constitutive manner and from the downstream promoter *sigB*p1 induced by salt, late growth phase, or during differentiation (20). The *rsbB* gene encodes a sequence homolog of anti-anti-sigma factors SpoIIAA and RsbV of *B. subtilis*, whereas *rsbA* encodes a protein whose C-terminal half resembles anti-sigma factors SpoIIAB and RsbW. (B) Comparison of the C-terminal half of RsbA (RsbA-CTD) with those of related anti-sigma factors. C-terminal aa 190 to 284 of RsbA were compared with those of SpoIIAB and RsbW from *B. subtilis* (BsRsbW), and SCO3548, a putative anti-sigma factor encoded downstream of *bldG* in *S. coelicolor* (10, 11) around the regions of the conserved motif. Conserved residues are denoted with asterisks and dots. Residues known to serve for ATP binding (▽) or possibly for kinase function (▼) are indicated along with proposed residues for SpoIIAA-related antagonist interaction (♦) (13, 23). (C) Comparison of *S. coelicolor* RsbB with related antagonist family proteins. An internal portion of aa 59 to 115 from *S. coelicolor* RsbB (ScoRsbB) was compared with corresponding regions of *B. subtilis* SpoIIAA (BsSpoIIAA), *B. subtilis* RsbV (BsRsbV), *S. coelicolor* BldG (ScoBldG), and *S. coelicolor* RsbV (ScoRsbV; SCO7325). The position of a serine residue, which is phosphorylated in *B. subtilis* by serine kinase anti-sigma factors, is marked by an arrow on top. In ScoRsbB, the corresponding residue is valine.

in pSET246. Introduction of pSET246 into YD352 by conjugation and isolation of exconjugants were done as described by Cho et al. (19). To construct the *rsbB* mutant, the REDIRECT technology was employed (26) to replace the *rsbB* gene with a disruption cassette. The cassette containing *oriT* and the *aac(3)V* gene conferring apramycin resistance was generated by PCR using a gel-purified, 1.4-kb EcoRI-HindIII fragment from pIJ773 as a template and the oligonucleotide primers *rsbB*PTN (5′-GGCGTATGCCCGGCGAAGTTCCGAAGGGGATCGAGTGTGATTCCGGGGATCCGTCGACC) and *rsbB*PTC (5′-CCGGATGGATGAATGCCTCGTCGGCGGTCGCCGTGCTCATGTAGGCTGGAGCTGCTTC), which contain 39-nucleotide gene-specific extensions (underlined) at 5′ positions. Cosmid F55 containing the *rsbB* gene was introduced into *E. coli* BW25113 (21) by electroporation along with *rsbB*-specific disruption cassettes, and the whole *rsbB* gene from the first to the last (117th) codons was replaced with the *oriT*/*aac(3)V* cassette in *E. coli*. The resulting cosmid was either directly introduced into nonmethylating *E. coli* ET12567 and then to *S. coelicolor* J1501 via conjugation, to obtain *rsbB* mutant with cassette integration (YD403) or further processed to remove the disruption cassette in frame by the FLP recombination system (21, 26). The resulting cosmid containing an in-frame *rsbB* deletion without the cassette was further modified to replace the kanamycin resistance marker with the *oriT*/*aadA* cassette from pIJ778, in order to circumvent poor selection by kanamycin on our culture plates. The final in-frame *rsbB* deletion mutant (YI403) was selected first by spectinomycin resistance and then by its subsequent loss resulting from double crossover. The expected mutations in YD403 and YI403 were confirmed by PCR and Southern hybridization.

RESULTS

RsbA is an anti-sigma factor for σ^B. The *sigB* gene of *S. coelicolor* is preceded by *rsbA* and *rsbB* genes that are cotranscribed (20) (Fig. 1A). The *rsbA* gene encodes a 309-amino-acid (aa) protein whose C-terminal half shares critical residues with anti-sigma factors RsbW and SpoIIAB of *B. subtilis* containing conserved domains of serine kinases (13) (Fig. 1B). The N-terminal domain of RsbA does not show significant similarity to any proteins in the current database except a homolog in *S. avermitilis* (http://avermitilis.ls.kitasato-u.ac.jp). The *rsbB* gene encodes a protein of 117 aa that reveals prominent sequence similarity with anti-anti-sigma factors SpoIIAA and RsbV of *B. subtilis*, which are phosphorylated by anti-sigma factors with kinase activity (Fig. 1C). However, RsbB lacks the conserved serine residue that is phosphorylated in *B. subtilis*.

Based on the similarity in amino acid sequence and gene organization, we postulated that RsbA may function as an anti-sigma factor for σ^B in *S. coelicolor* in a similar manner to RsbW for σ^B in *B. subtilis*. To test this idea, we performed affinity chromatography. GST-tagged RsbA and His-tagged σ^B

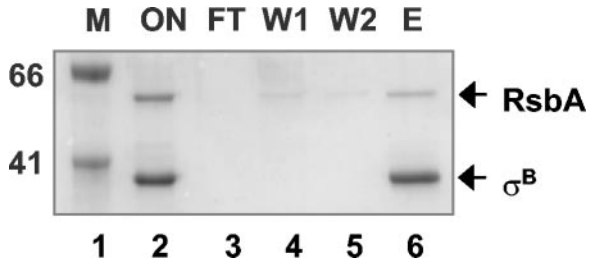

FIG. 2. Interaction between RsbA and σ^B monitored by affinity chromatography. His-tagged σ^B and GST-RsbA in binding buffer were applied to an Ni-NTA column as described in Materials and Methods (ON; lane 2). Fractions from flow-through (FT; lane 3), wash with 5 mM imidazole (W1; lane 4), wash with 25 mM imidazole (W2; lane 5), W2), and elution with 500 mM imidazole (E; lane 6) were pooled, concentrated, and subjected to SDS-PAGE. Proteins were visualized by Coomassie staining and marked along with size markers (M; lane 1) of 66 and 41 kDa.

were purified from *E. coli* and applied to Ni-NTA column as described above. Figure 2 demonstrates that RsbA coelutes with σ^B from the column by 0.5 M imidazole, suggesting tight interaction with σ^B. When the C-terminal half of RsbA (from residues 121 to 309; RsbA-CTD) in a GST-tagged form was applied, it also was coeluted with σ^B, suggesting that the interaction domain resides in the conserved CTD (data not shown). His-tagged σ^R was not able to hold RsbA in the column in a parallel experiment, confirming the specificity of the σ^B-RsbA interaction (data not shown).

We then tested whether RsbA inhibits σ^B-directed transcription in vitro. Full-length RsbA protein with a GST tag was added to the transcription mixture containing σ^B and template DNAs containing its cognate promoter *sigB*p1 or *catB*p. Addition of a twofold molar excess of RsbA inhibited the σ^B-dependent transcription from *sigB*p1 and *catB*p promoters to near completion (Fig. 3A and B). Either the full-length or CTD fragment of RsbA without GST tag produced a similar result (data not shown). In contrast, RsbA only marginally inhibited transcription from *sigR*p2 promoter directed by an ECF sigma factor, σ^R (Fig. 3C), suggesting that its inhibitory action is specific toward σ^B.

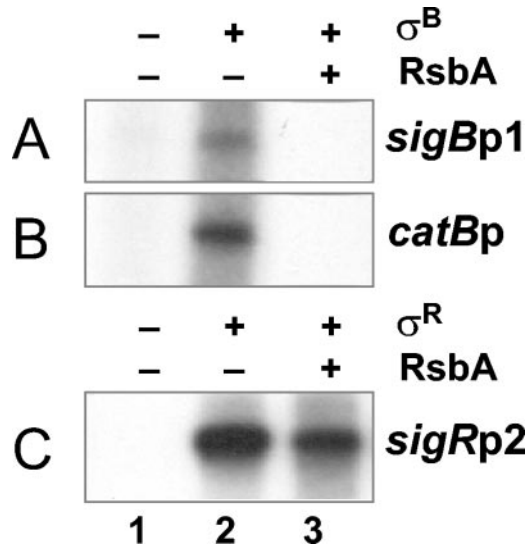

FIG. 3. Inhibition of σ^B-directed transcription by RsbA in vitro. Single-round transcription assays were done with core RNA polymerase (lanes 1), with added σ^B or σ^R (lanes 2), using σ^B-specific promoters *sigB*p1 (A) or *catB*p (B) or σ^R-specific promoter *sigR*p2 (C). Partially purified RsbA was preincubated with each sigma factor at a 2:1 molar ratio to observe its effect (lanes 3). Transcripts were electrophoresed on a 5% PAGE gel with 7 M urea and visualized by autoradiography.

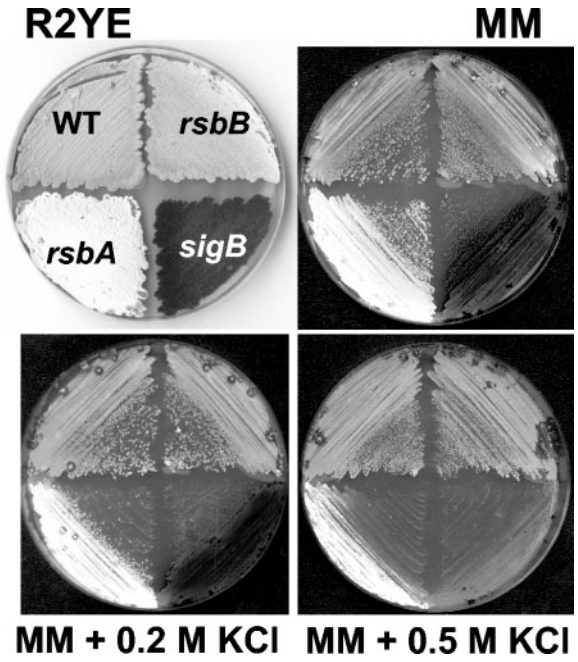

FIG. 4. Phenotypes of *rsb* mutants. Colony morphologies of the wild type (J1501) and *rsbB* (YD403), *rsbA* (YD352), and *sigB* (YD2108) mutants on R2YE (upper left) and MM (upper right) plates. The wild type and *rsbB* mutant sporulated normally, whereas the *rsbA* mutant demonstrated a white phenotype with no sporulation. The *sigB* mutant exhibited a bald phenotype with no aerial mycelium formation. Salt sensitivity was examined by streaking cells on MM plates containing 0.2 M KCl (lower left) or 0.5 M KCl (lower right). Retarded growth at 0.2 M or more KCl was observed for *rsbA* and *sigB* mutants.

The growth and morphological phenotype of an *rsbA*-null mutant. To find out the role of RsbB and RsbA proteins in vivo, we made *rsbA*- and *rsbB*-null mutants from an *S. coelicolor* A3(2) J1501 strain and compared them with a *sigB* mutant. The *rsbB*-null mutant showed no obvious phenotype for any of the properties tested. In contrast, the *rsbA*-null mutant had multiple defects, consistent with a regulatory role. The *rsbA* mutant (YD352) showed a white phenotype when plated on R2YE, SFM, and MM plates (Fig. 4, upper left). On SFM and MM-mannitol plates, some spores were formed after prolonged incubation of more than 2 weeks, whereas no sporulation occurred on R2YE during this period. The delayed spores from SFM or MM-mannitol plates lost plating efficiency significantly by a freeze-thaw cycle, suggesting the compromised integrity of the spore. The mutants were then examined for salt sensitivity by plating on MM with or without additional KCl (Fig. 4). The *rsbB* mutant grew as well as the wild type in the presence of high salt, whereas the *rsbA* mutant was sensitive to KCl as *sigB* mutant. The antibiotic production was disturbed in the *rsbA* mutant, with almost complete absence of blue-pig-

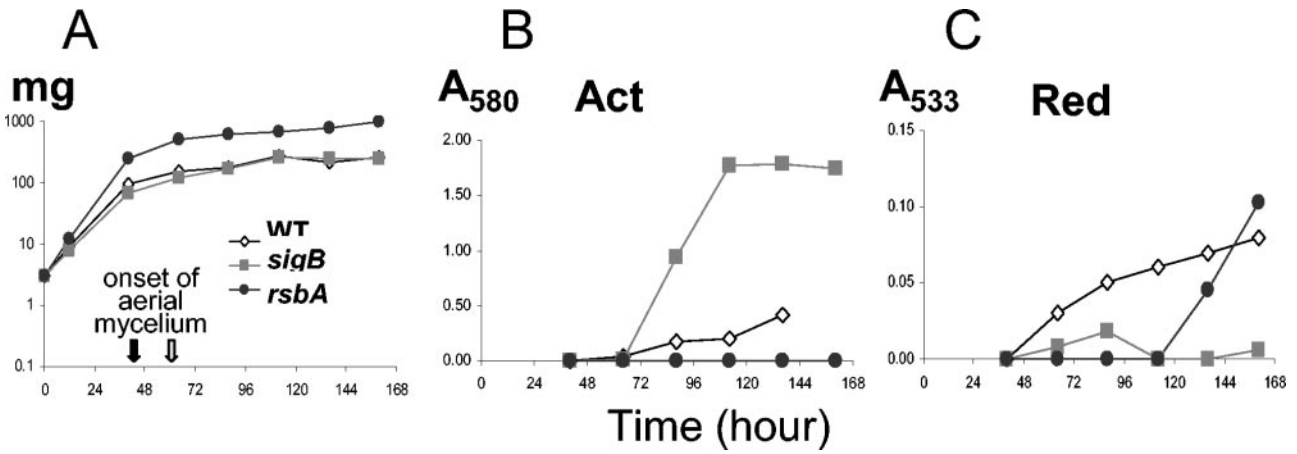

FIG. 5. Antibiotic production in *rsbA* mutant. Shown is production of biomass (A), actinorhodin (B; Act), and undecylprodigiosin (C; Red) by each mutant grown on R2YE plates. Increase in biomass is shown as wet weight (milligrams), and the timing of aerial mycelium formation for the wild type (WT) and *rsbA* mutant is indicated by filled and open arrows, respectively. The amount of actinorhodin was measured by determining the A_{580} after alkaline extraction of pigments from 10 mg (wet weight) of cells grown for different lengths of time. The amount of undecylprodigiosin was measured by determining the A_{533}, after extracting red pigments with methanol-HCl from 33 mg of cells. Measurements were made in duplicate from two separate cultures. The average values are presented.

mented actinorhodin, in sharp contrast to its precocious production in the *sigB* mutant (Fig. 4, upper left).

The growth and antibiotic production of *rsbA* mutant were compared with those of the *sigB* mutant and wild type more quantitatively on R2YE plates. As observed previously (20), the *sigB* mutant grew as well as the wild type, with an exponential doubling time of about 8 h without forming aerial mycelium. In contrast, the *rsbA* mutant grew faster (with doubling at every 6 h) to a higher biomass and formed aerial mycelium earlier than the wild type by about 15 h (Fig. 5A). The production of blue antibiotic (actinorhodin) in *rsbA* was not detected at all, in sharp contrast to the wild type and the *sigB* mutant (Fig. 5B). On the other hand, delayed but abundant production of red antibiotic (undecylprodigiosin) was evident in the *rsbA* mutant, in contrast to scanty production in the *sigB* mutant (Fig. 5C). To verify the effect of the *rsbA* mutation, we introduced a wild-type *rsbA* gene on the pSET246 plasmid that integrates into the chromosome of the *rsbA* mutant and observed that the wild-type phenotypes were all restored (data not shown).

The sharply contrasting behavior between the *rsbA* and *sigB* mutants in producing aerial mycelium and antibiotics coincides well with the proposal that RsbA is an antagonist of σ^B. The similar salt sensitivity of *rsbA* and *sigB* mutants, together with their defective developmental phenotypes, suggests that a proper activity level of σ^B (and RsbA) is necessary for osmotic balance and completion of the differentiation process.

Inhibition of σ^B-dependent transcription by RsbA in vivo. Previous studies demonstrated that σ^B is responsible for osmotic induction of its own structural gene (from *sigB*p1) and that of catalase B (from *catB*p) (20). In order to find the effect of *rsb* mutations in vivo, we examined the salt induction of these promoters by S1 mapping. As expected, the level of *sigB*p1 transcripts was enhanced in the *rsbA* mutant even in the absence of any salt stress. The *rsbB* mutant (YD403) showed similar induction behavior to the wild type (Fig. 6A). The in-frame-deleted *rsbB* mutant (YI403) also exhibited similar behavior, excluding the possibility of a polar mutation effect in YD403 (data not shown). The wild-type induction pattern was restored in the *rsbA* mutant by introducing pSET246 plasmid containing the wild-type *rsbA* gene (Fig. 6B). We then monitored the time course of induction of these promoters by S1 mapping. Figure 6C demonstrates that in the wild-type cell *sigB*p1 transcripts were induced by 0.2 M KCl rapidly with a peak at 20 to 30 min, whereas *catB*p induction was delayed with a peak at around 1 h. In the *sigB* mutant, induction of both promoters was markedly reduced. In the *rsbA* mutant, the level

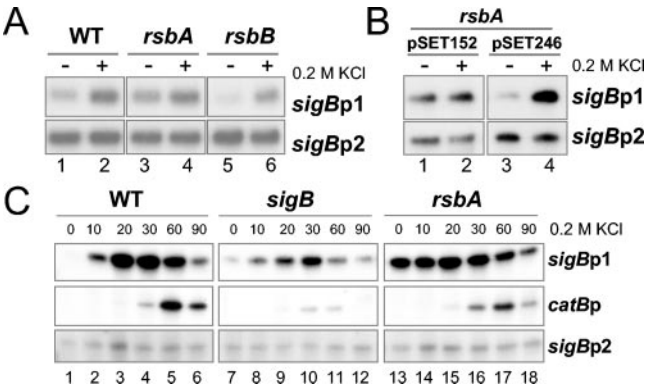

FIG. 6. Effect of *rsbA* mutation on σ^B-dependent transcription. (A) The amount of *sigB*p1 transcripts was monitored by S1 mapping in the wild type (WT) and *rsbA* (YD352) and *rsbB* (YD403) mutants. As a constitutive control, *sigB*p2 transcripts were analyzed in parallel. Cells were grown in YEME containing 10.3% sucrose for 15 h and treated with (lanes 2, 4, and 6) or without (lanes 1, 3, and 5) 0.2 M KCl for an hour. (B) Complementation of the *rsbA* mutant. The *sigB* transcripts from the *rsbA* mutants harboring the parental pSET152 vector or the recombinant plasmid with the wild-type *rsbA* gene (pSET246) were analyzed by S1 mapping. Cells were prepared as described above. (C) Induction kinetics of the *sigB* and *catB* transcripts. The time course of σ^B-dependent induction of *sigB*p1 and *catB*p transcripts upon 0.2 M KCl treatment was monitored in the wild type and *sigB* and *rsbA* mutants grown in YEME liquid medium to the early exponential phase. Samples were taken at 10-min intervals over 90 min, and S1 mapping analysis was done.

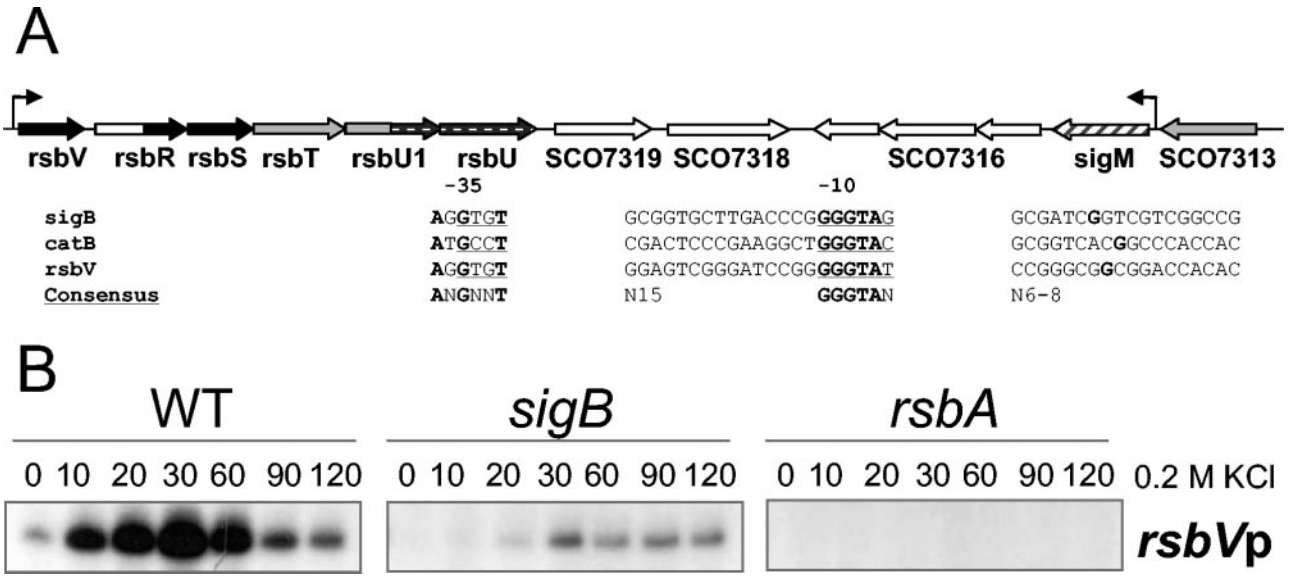

FIG. 7. σ^B-dependent expression of the *rsbV* gene. (A) Gene organization near the *rsbV* operon in *S. coelicolor*. The *rsbV* gene is followed by homologs of *rsbR*, *rsbS*, *rsbT*, and *rsbU*, similar to the gene order near the *B. subtilis rsbV* gene. The *rsbU1* gene contains an *rsbU*-like PP2C phosphatase domain plus an *rsbT*-like sequence with low similarity. A σ^B-like sigma factor, SigM (SCO7314), is encoded nearby in the opposite orientation. A putative σ^B-dependent *rsbV* promoter sequence was compared with those of *sigB*p1 and *catB* promoters. Conserved nucleotides within −35 and −10 regions are marked in bold. (B) σ^B-dependent induction of the *rsbV* transcripts upon KCl treatment. The time course of induction of *rsbV* transcripts was monitored in the wild type (WT) and *sigB* and *rsbA* mutants grown in YEME liquid medium to the early exponential phase and treated with 0.2 M KCl. Samples were taken at 10- to 30-min intervals over 2 h and analyzed by S1 mapping.

of *sigB*p1 transcripts was elevated to near its full induction level even without salt treatment, consistent with the proposal that RsbA acts as an anti-sigma factor for σ^B. The delayed induction of *catB* persisted in the *rsbA* mutant, suggesting that *catB* and *sigB* genes are not regulated in the same way. The residual induction of *sigB*p1 and *catB* transcripts in *sigB* mutant may reflect the action of another σ^B-like sigma factor or factors with overlapping promoter specificity. The *rsbB* mutant showed a similar induction pattern to that of the wild type (data not shown). We therefore propose that the primary sigma factor that induces *sigB*p1 transcription in response to osmotic stress is σ^B itself, and it is antagonized by RsbA in vivo, consistent with the in silico prediction and in vitro observations.

RsbV is an antagonist for RsbA. Since RsbB lacks the conserved serine residue that is usually phosphorylated by a cognate anti-sigma factor/kinase and since the *rsbB*-null mutant does not display any noticeable phenotype, we searched for other antagonist candidates. Inspection of the *S. coelicolor* genome revealed 17 antagonist (anti-anti-sigma factor) candidates, 13 of which contain the conserved phosphorylatable serine residue. We noted a genetic locus of clusters of genes with predicted amino acid sequences highly homologous to RsbV, -R, -S, -T, and -U of *B. subtilis* (51) and hence named it *rsbV-rsbR-rsbS-rsbT-rsbU1-rsbU* (Fig. 7A). Inspection of the *rsbV* upstream region revealed a σ^B consensus sequence closely resembling *sigB*p1 and *catB* promoters (Fig. 7A).

When monitored by S1 mapping, *rsbV* transcript was induced dramatically by 0.2 M KCl treatment within 10 min with a peak at around 30 min (Fig. 7B). In the *sigB* mutant, on the other hand, no such induction was observed, except a low but delayed induction. Therefore, *rsbV* transcription and possibly the entire operon of at least six genes are under the control of σ^B in vivo. In the *rsbA* mutant, however, the *rsbV* gene was not expressed at all. Since the activity of σ^B is enhanced greatly in the *rsbA* mutant, as judged from the highly elevated transcription from the *sigB*p1 promoter, the absence of σ^B-dependent *rsbV* transcription in the *rsbA* mutant most likely reflects the involvement of another additional regulator or regulators.

An anti-RsbA activity of RsbV was examined by an in vitro transcription assay using the *sigB*p1 promoter. Transcription by σ^B-containing RNA polymerase was inhibited by RsbA as described above (Fig. 8, lanes 3 and 4). Addition of RsbV restored *sigB*p1 transcription (Fig. 8, lanes 5 to 7). In contrast, addition of RsbV did not antagonize the inhibitory action of RsrA on σ^R-directed transcription (data not shown), reflecting its specificity toward the RsbA-σ^B system. The stoichiometry of

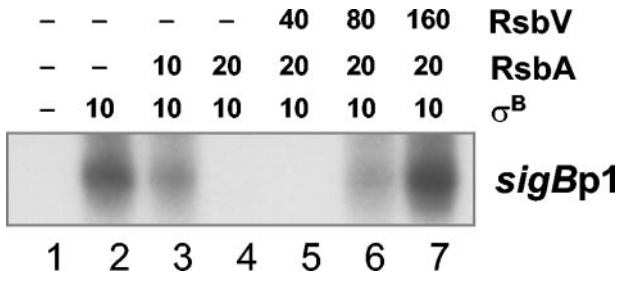

FIG. 8. Anti-RsbA activity of RsbV. Antagonistic activity of RsbV against RsbA was assessed with an in vitro transcription assay as described in Materials and Methods. All of the transcription mixture contained *sigB*p1 promoter DNA and core RNA polymerase. The amount of additional components is indicated in picomoles above each lane.

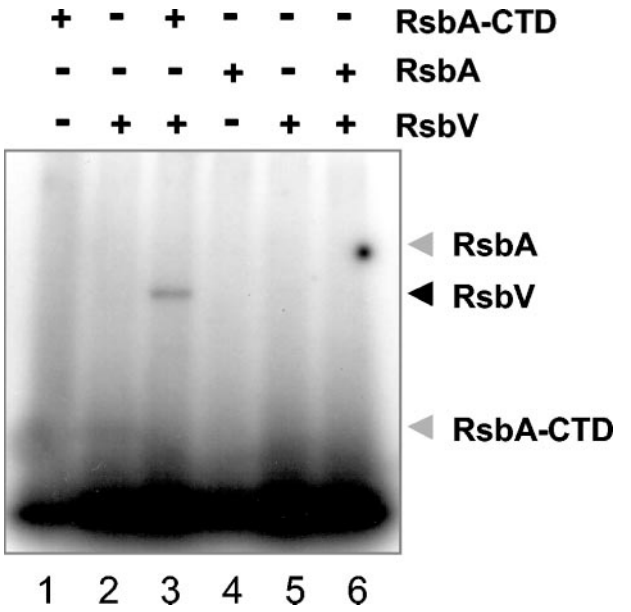

FIG. 9. Phosphorylation of RsbV by RsbA. Purified RsbA (full-length or CTD) and GST-tagged RsbV were incubated with [γ-^{32}P]ATP and separated by SDS-PAGE. The radiolabeled protein band was detected by autoradiography. The positions of RsbA and RsbV proteins, determined by Coomassie blue staining, are indicated by arrowheads.

RsbA-RsbV at maximal action was well above 1:4. This may reflect a low fraction of functionally active GST-RsbV protein.

We then examined whether RsbV can be phosphorylated by RsbA in vitro. Either full-length or C-terminal half (CTD) of RsbA proteins was tested for its ability to phosphorylate GST-tagged RsbV with [γ-^{32}P]ATP. A radiolabeled protein band was detected by autoradiography following SDS-PAGE. We found that RsbV was phosphorylated by the CTD of RsbA in vitro (Fig. 9, lane 3). Full-length RsbA failed to phosphorylate RsbV to a detectable level. Since RsbA-CTD bound σ^B and inhibited σ^B-dependent transcription in vitro, we interpret our result to suggest that (i) RsbA indeed possesses kinase activity; (ii) RsbV is a phosphorylation target of RsbA; and (iii) the unconserved N-terminal domain of RsbA blocks its kinase activity in vitro, whereas this blockage is lifted in vivo, possibly through interaction with some other factor(s). When purified RsbB was examined in a parallel experiment, we observed no phosphorylation by either the CTD or full-length RsbA protein (data not shown).

DISCUSSION

We propose from this study that the osmotic induction of σ^B-dependent promoters is regulated by an anti-sigma factor and its phosphorylatable antagonist. In an uninduced state, σ^B is bound by an anti-sigma factor RsbA. In response to osmotic stress such as 0.2 M KCl, σ^B is released free of its anti-sigma factor, RsbA, due to the binding of an antagonist RsbV to RsbA. The free σ^B then combines with core RNA polymerase to form a functional holoenzyme, which then recognizes and transcribes its target promoters such as *sigB*p1 and *rsbV*p within 10 min of stress. Increase in σ^B synthesis as well as RsbV could further amplify the response. Among putative members of σ^B regulon, there are genes for at least four σ^B-related sigma factors (40). An avalanche of expression of regulons controlled by σ^B and its down-stream target regulators would constitute a critical part of the osmotic stress response. Inspection of the *S. coelicolor* genome for putative σ^B-dependent promoters revealed 118 putative promoters with an ANGNNT-N_{14-16}-GGGTA(C/T) sequence motif within 500 bp upstream of known and predicted genes (40). The catalase B gene that is dependent on σ^B is most likely an example of genes controlled by one or more of these secondary σ^B-related sigma factors that share some promoter specificity, as judged from its slow induction kinetics.

Among 48 *rsbW*-like genes, those neighboring a sigma factor gene are only a few. The gene order partly syntenic to *B. subtilis* (*rsbV-rsbW-sigB*) is found in *rsbB-rsbA-sigB*, *SCO7313-sigM*, *prsI-arsI-sigI*, *sigL-SCO7277*, and *prsH-sigH* operons. Among these, *arsI-prsI* is cotranscribed divergently from *sigI*, and *SCO7277* is transcribed convergently to *sigL*. PrsH serves as an anti-sigma factor for σ^H, but it lacks a kinase domain and hence has no enzyme activity (54, 57). Therefore, in both gene structure and regulation partner, σ^B parallels most closely its homolog in *B. subtilis*. Even though σ^H is reported to share a similar function with σ^B in *S. coelicolor*, in being induced by osmotic stress and influencing differentiation, its regulation mechanisms are quite different from those of σ^B. First, the amount of σ^B increases during osmotic induction and growth transition in liquid as well as on solid media (data not shown), whereas the σ^H level does not change significantly throughout growth and osmotic stress. Disruption of *sigB* produces a bald phenotype with altered antibiotic production and osmosensitivity, whereas the *sigH* mutant does not produce any morphologically distinct phenotype in our hands, in agreement with Viollier et al. (56). In addition, σ^B is regulated by an anti-sigma factor that can bind and phosphorylate its antagonist, whereas PrsH regulation appears not to involve such a mechanism.

The positive regulation of RsbV by σ^B is intriguing, since it can amplify the response by titrating out anti-sigma factors. However, in the absence of an anti-sigma factor for σ^B as in the *rsbA* mutant, the *rsbV* operon is not expressed nor induced, demonstrating an efficient control that avoids unnecessary expression of regulatory modules. Either depletion of activator or overproduction of a repressor for the *rsbV* operon in the *rsbA* mutant can be postulated to ensure coordinated expression of these two genes.

The link between osmotic stress response and differentiation in streptomycetes has been reported in several cases, even though its underlying mechanism is unclear. The change in glycogen synthesis and breakdown that may affect osmolarity accompanies the onset of aerial mycelial growth and sporulation (12, 52), suggesting that controlled osmolarity might be necessary for differentiation. Catalase B is required for both hyperosmotic survival and proper differentiation (19) and is thought to play this dual function through N-terminal peptide degradation and secretion. Deletion of σ^B and σ^H impairs both osmotic response and differentiation (20, 53). Sensitivity to high osmolarity was observed in the presence of a high concentration of A-factor, a differentiation signal molecule in *Streptomyces griseus* (3). The white and osmosensitive phenotype of *rsbA* mutant suggests that even though aerial mycelium forms precociously in the presence of abundant free σ^B, proper

balance of the intracellular osmotic state may be necessary to complete differentiation.

The presence of a partner-switching mechanism of regulation involving phosphorylation or dephosphorylation has been suggested in a wide variety of eubacteria through comparative genomics (38, 44). From this study, we demonstrated that *S. coelicolor* employs such a mechanism to control σ^B that is required for osmotic stress response and differentiation process. Existence of an anti-sigma factor (UshX) and its phosphorylatable antagonist has been proposed to regulate σ^F in *Mycobacterium tuberculosis*, which is required for expression of virulence and adaptation genes (4, 16). This kind of regulatory mechanism may indeed be utilized in a multitude of gram-positive pathogenic bacteria, such as *Staphylococcus aureus* and *Listeria monocytogenes*, where a σ^B-like stress sigma factor influences pathogenicity (5, 39).

ACKNOWLEDGMENTS

We are grateful to J.-B. Bae and M.-Y. Hahn for their technical assistance in protein purification and in vitro transcription.

This work was supported by Basic Science Research grants (2000-DP0372 and KRF-2003-041-C00332) from the Korean Research Foundation to J.-H. Roe. E.-J. Lee, H.-S. Kim, and B.-E. Ahn were recipients of a BK21 fellowship for graduate students in Life Sciences at SNU.

REFERENCES

1. **Adamidis, T., P. Riggle, and W. Champness.** 1990. Mutations in a new *Streptomyces coelicolor* locus which globally block antibiotic biosynthesis but not sporulation. J. Bacteriol. **172:**2962–2969.
2. **Alper, S., L. Duncan, and R. Losick.** 1994. An adenosine nucleotide switch controlling the activity of a cell type-specific transcription factor in *B. subtilis*. Cell **77:**195–205.
3. **Ando, N., K. Ueda, and S. Horinouchi.** 1997. A *Streptomyces griseus* gene (*sgaA*) suppresses the growth disturbance caused by high osmolality and a high concentration of A-factor during early growth. Microbiology **143:**2715–2723.
4. **Beaucher, J., S. Rodrigue, P. E. Jacques, I. Smith, R. Brzezinski, and L. Gaudreau.** 2002. Novel *Mycobacterium tuberculosis* anti-sigma factor antagonists control sigmaF activity by distinct mechanisms. Mol. Microbiol. **45:**1527–1540.
5. **Becker, L. A., M. S. Çetin, R. W. Hutkins, and A. K. Benson.** 1998. Identification of the gene encoding the alternative sigma factor σ^B from *Listeria monocytogenes* and its role in osmotolerance. J. Bacteriol. **180:**4547–4554.
6. **Benson, A. K., and W. G. Haldenwang.** 1993. Regulation of σ^B levels and activity in *Bacillus subtilis*. J. Bacteriol. **175:**2347–2356.
7. **Bentley, S. D., K. F. Chater, A. M. Cerdeno-Tarraga, G. L. Challis, N. R. Thomson, K. D. James, D. E. Harris, M. A. Quail, H. Kieser, D.Harper, A. Bateman, S. Brown, G. Chandra, C. W. Chen, M. Collins, A. Cronin, A. Fraser, A. Goble, J. Hidalgo, T. Hornsby, S. Howarth, C. H. Huang, T. Kieser, L. Larke, L. Murphy, K. Oliver, S. O'Neil, E. Rabbinowitsch, M. A. Rajandream, K. Rutherford, S. Rutter, K. Seeger, D. Saunders, S. Sharp, R. Squares, S. Squares, K. Taylor, T. Warren, A. Wietzorrek, J. Woodward, B. G. Barrell, J. Parkhill, and D. A. Hopwood.** 2002. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature **417:**141–147.
8. **Bibb, M. J., V. Molle, and M. J. Buttner.** 2000. σ^{BldN}, an extracytoplasmic function RNA polymerase sigma factor required for aerial mycelium formation in *Streptomyces coelicolor* A3(2). J. Bacteriol. **182:**4606–4616.
9. **Bierman, M., R. Logan, K. O'Brien, E. T. Seno, R. N. Rao, and B. E. Schoner.** 1992. Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces* spp. Gene **116:**43–49.
10. **Bignell, D. R., J. L. Warawa, J. L. Strap, K. F. Chater, and B. K. Leskiw.** 2000. Study of the *bldG* locus suggests that an anti-anti-sigma factor and an anti-sigma factor may be involved in *Streptomyces coelicolor* antibiotic production and sporulation. Microbiology **146:**2161–2173.
11. **Bignell, D. R., L. H. Lau, K. R. Colvin, and B. K. Leskiw.** 2003. The putative anti-anti-sigma factor BldG is post-translationally modified by phosphorylation in *Streptomyces coelicolor*. FEMS Microbiol. Lett. **225:**93–99.
12. **Bruton, C. J., K. A. Plaskitt, and K. F. Chater.** 1995. Tissue-specific glycogen branching isoenzymes in a multicellular prokaryote, *Streptomyces coelicolor* A3(2). Mol. Microbiol. **18:**89–99.
13. **Campbell, E. A., S. Masuda, J. L. Sun, O. Muzzin, C. A. Olson, S. Wang, and S. A. Darst.** 2002. Crystal structure of *Bacillus stearothermophilus* anti-sigma factor SpoIIAB with the sporulation σ factor σ^F. Cell **108:**795–807.
14. **Chater, K. F., C. J. Bruton, K. A. Plaskitt, M. J. Buttner, C. Mendez, and J. D. Helmann.** 1989. The developmental fate of *S. coelicolor* hyphae depends upon a gene product homologous with the motility sigma factor of *B. subtilis*. Cell **59:**133–143.
15. **Chater, K. F.** 2001. Regulation of sporulation in *Streptomyces coelicolor* A3(2): a checkpoint multiplex? Curr. Opin. Microbiol. **4:**667–673.
16. **Chen, P., R. E. Ruiz, Q. Li, R. F. Silver, and W. R. Bishai.** 2000. Construction and characterization of a *Mycobacterium tuberculosis* mutant lacking the alternate sigma factor gene, *sigF*. Infect. Immun. **68:**5575–5580.
17. **Cho, Y. H., and J. H. Roe.** 1997. Isolation and expression of the *catA* gene encoding the major catalase in *Streptomyces coelicolor* Müller. J. Bacteriol. **179:**4049–4052.
18. **Cho, Y.-H.** 1999. Gene expression and the role of catalases in *Streptomyces coelicolor* A3(2). Ph.D. thesis. Seoul National University, Seoul, Korea.
19. **Cho, Y. H., E. J. Lee, and J. H. Roe.** 2000. A developmentally regulated catalase required for proper differentiation and osmoprotection of *Streptomyces coelicolor*. Mol. Microbiol. **35:**150–160.
20. **Cho, Y. H., E. J. Lee, B. E. Ahn, and J. H. Roe.** 2001. SigB, an RNA polymerase sigma factor required for osmoprotection and proper differentiation of *Streptomyces coelicolor*. Mol. Microbiol. **42:**205–214.
21. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA **97:**6640–6645.
22. **Dufour, A., and W. G. Haldenwang.** 1994. Interaction between a *Bacillus subtilis* anti-σ factor (RsbW) and its antagonist (RsbV). J. Bacteriol. **176:**1813–1820.
23. **Garsin, D. A., L. Duncan, D. M. Paskowitz, and R. Losick.** 1998. The kinase activity of the antisigma factor SpoIIAB is required for activation as well as inhibition of transcription factor sigmaF during sporulation in *Bacillus subtilis*. J. Mol. Biol. **284:**569–578.
24. **Gehring, A. M., N. J. Yoo, and R. Losick.** 2001. RNA polymerase sigma factor that blocks morphological differentiation by *Streptomyces coelicolor*. J. Bacteriol. **183:**5991–5996.
25. **Gruber, T. M., and C. A. Gross.** 2003. Multiple sigma subunits and the partitioning of bacterial transcription space. Annu. Rev. Microbiol. **57:**441–466.
26. **Gust, B., G. L. Challis, K. Fowler, T. Kieser, and K. F. Chater.** 2003. PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. Proc. Natl. Acad. Sci. USA **100:**1541–1546.
27. **Hahn, M. Y., J. B. Bae, J. H. Park, and J. H. Roe.** 2003. Isolation and characterization of *Streptomyces coelicolor* RNA polymerase, its sigma, and antisigma factors. Methods Enzymol. **370:**73–82.
28. **Helmann, J. D.** 1999. Anti-sigma factors. Curr. Opin. Microbiol. **2:**135–141.
29. **Helmann, J. D., and C. P. Moran, Jr.** 2002. RNA polymerase and sigma factor, p. 289–312. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and its closest relatives: from genes to cells. ASM Press, Washington, D.C.
30. **Helmann, J. D.** 2002. The extracytoplasmic function (ECF) sigma factors. Adv. Microb. Physiol. **46:**47–110.
31. **Hillemann, D., A. Pühler, and W. Wohlleben.** 1991. Gene disruption and gene replacement in *Streptomyces* via single stranded DNA transformation of integration vectors. Nucleic Acids Res. **19:**727–731.
32. **Hobbs, G., C. M. Frazer, D. C. J. Gardner, F. Flett, and S. G. Oliver.** 1990. Pigmented antibiotic production by *Streptomyces coelicolor* A3(2): kinetics and the influence of nutrients. J. Gen. Microbiol. **136:**2291–2296.
33. **Hopwood, D. A., M. J. Bibb, K. F. Chater, T. Kieser, C. J. Bruton, H. M. Kieser, D. J. Lydiate, C. P. Smith, J. M. Ward, and H. Schrempf.** 1985. Genetic manipulation of *Streptomyces*, a laboratory manual. The John Innes Foundation, Norwich, United Kingdom.
34. **Hughes, K. T., and K. Mathee.** 1998. The anti-sigma factors. Annu. Rev. Microbiol. **52:**231–286.
35. **Kang, J. G., M. Y. Hahn, A. Ishihama, and J. H. Roe.** 1997. Identification of sigma factors for growth phase-related promoter selectivity of RNA polymerases from *Streptomyces coelicolor* A3(2). Nucleic Acids Res. **25:**2566–2573.
36. **Kang, J. G., M. S. Paget,Y. J. Seok, M. Y. Hahn, J. B. Bae, J. S. Hahn, C. Kleanthous, M. J. Buttner, and J. H. Roe.** 1999. RsrA, an anti-sigma factor regulated by redox change. EMBO J. **18:**4292–4298.
37. **Kelemen, G. H., P. H. Viollier, J. Tenor, L. Marri, M. J. Buttner, and C. J. Thompson.** 2001. A connection between stress and development in the multicellular prokaryote *Streptomyces coelicolor* A3(2). Mol. Microbiol. **40:**804–814.
38. **Koonin, E. V., L. Aravind, and M. Y. Galperin.** 2000. A comparative-genomic view of the microbial stress response, p. 417–444. *In* G. Storz and R. Hengge-Aronis (ed.), Bacterial stress responses. ASM Press, Washington, D.C.
39. **Kullik, I., P. Giachino, and T. Fuchs.** 1998. Deletion of the alternative sigma factor σ^B in *Staphylococcus aureus* reveals its function as a global regulator of virulence genes J. Bacteriol. **180:**4814–4820.
40. **Lee, E. J., Y. H. Cho, H. S. Kim, and J. H. Roe.** 2004. Identification of

σ^B-dependent promoters using consensus-directed search of *Streptomyces coelicolor* genome. J. Microbiol. **42:**147–151.

41. **Lonetto, M. A., K. L. Brown, K. E. Rudd, and M. J. Buttner.** 1994. Analysis of the *Streptomyces coelicolor sigE* gene reveals the existence of a subfamily of eubacterial RNA polymerase sigma factors involved in the regulation of extracytoplasmic functions. Proc. Natl. Acad. Sci. USA **91:**7573–7577.
42. **MacNeil, D. J.** 1988. Characterization of a unique methyl-specific restriction system in *Streptomyces avermitilis*. J. Bacteriol. **170:**5607–5612.
43. **Min, K.-T., C. M. Hilditch, B. Diederich, J. Errington, and M. D. Yudkin.** 1993. σ^F, the first compartment-specific transcription factor of *B. subtilis*, is regulated by an anti-σ factor that is also a protein kinase. Cell **74:**735–742.
44. **Mittenhuber, G.** 2002. A phylogenomic study of the general stress response sigma factor σ^B of *Bacillus subtilis* and its regulatory proteins. J. Mol. Microbiol. Biotechnol. **4:**427–452.
45. **Novotna, J., J. Vohradsky, P. Berndt, H. Gramajo, H. Langen, X. M. Li, W. Minas, L. Orsaria, D. Roeder, and C. J. Thompson.** 2003. Proteomic studies of diauxic lag in the differentiating prokaryote *Streptomyces coelicolor* reveal a regulatory network of stress-induced proteins and central metabolic enzymes. Mol. Microbiol. **48:**1289–1303.
46. **Paget, M. S., J. G. Kang, J. H. Roe, and M. J. Buttner.** 1998. σ^R, an RNA polymerase sigma factor that modulates expression of the thioredoxin system in response to oxidative stress in *Streptomyces coelicolor* A3(2). EMBO J. **17:** 5776–5782.
47. **Paget, M. S., L. Chamberlin, A. Atrih, S. J. Foster, and M. J. Buttner.** 1999. Evidence that the extracytoplasmic function sigma factor σ^E is required for normal cell wall structure in *Streptomyces coelicolor* A3(2). J. Bacteriol. **181:** 204–211.
48. **Paget, M. S., J. B. Bae, M. Y. Hahn, W. Li, C. Kleanthous, J. H. Roe, and M. J. Buttner.** 2001. Mutational analysis of RsrA, a zinc-binding anti-sigma factor with a thiol-disulphide redox switch. Mol. Microbiol. **39:**1036–1047.
49. **Paget, M. S., H. J. Hong, M. J. Bibb, and M. J. Buttner.** 2002. The ECF sigma factors of *Streptomyces coelicolor* A3(2), p. 105–125. *In* D. A. Hodgson and C. M. Thomas (ed.), SGM Symposium 61: signals, switches, regulons and cascades: control of bacterial gene expression. Cambridge University Press, Cambridge, United Kingdom.
50. **Potuckova, L., G. H. Kelemen, K. C. Findlay, M. A. Lonetto, M. J. Buttner, and J. Kormanec.** 1995. A new RNA polymerase sigma factor, sigma F, is required for the late stages of morphological differentiation in *Streptomyces* spp. Mol. Microbiol. **17:**37–48.
51. **Price, C. W.** 2002. General stress response, p. 369–384. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and its closest relatives: from genes to cells. ASM Press, Washington, D.C.
52. **Schneider, D., C. J. Bruton, and K. F. Chater.** 2000. Duplicated gene clusters suggest an interplay of glycogen and trehalose metabolism during sequential stages of aerial mycelium development in *Streptomyces coelicolor* A3(2). Mol. Gen. Genet. **263:**543–553.
53. **Sevcikova, B., O. Benada, O. Kofronova, and J. Kormanec.** 2001. Stress-response sigma factor sigma(H) is essential for morphological differentiation of *Streptomyces coelicolor* A3(2). Arch. Microbiol. **177:**98–106.
54. **Sevcikova, B., and J. Kormanec.** 2002. Activity of the *Streptomyces coelicolor* stress-response sigma factor σ^H is regulated by an anti-sigma factor. FEMS Microbiol. Lett. **209:**229–235.
55. **Vijay, K., M. S. Brody, E. Fredlund, and C. W. Price.** 2000. A PP2C phosphatase containing a PAS domain is required to convey signals of energy stress to the sigmaB transcription factor of *Bacillus subtilis*. Mol. Microbiol. **35:**180–188.
56. **Viollier, P. H., G. H. Kelemen, G. E. Dale, K. T. Nguyen, M. J. Buttner, and C. J. Thompson.** 2003. Specialized osmotic stress response systems involve multiple SigB-like sigma factors in *Streptomyces coelicolor*. Mol. Microbiol. **47:**699–714.
57. **Viollier, P. H., A. Weihofen, M. Folcher, and C. J. Thompson.** 2003. Post-transcriptional regulation of the *Streptomyces coelicolor* stress responsive sigma factor, SigH, involves translational control, proteolytic processing, and an anti-sigma factor homolog. J. Mol. Biol. **325:**637–649.
58. **Vohradsky, J., X.-M. Li, G. Dale, M. Folcher, L. Nguyen, P. H. Viollier, and C. J. Thompson.** 2000. Developmental control of stress stimulons in *Streptomyces coelicolor* revealed by statistical analyses of global gene expression patterns. J. Bacteriol. **182:**4979–4986.
59. **Weaver, D., N. Karoonuthaisiri, H. H. Tsai, C. H. Huang, M. L. Ho, S. Gai, K. G. Patel, J. Huang, S. N. Cohen, D. A. Hopwood, C. W. Chen, and C. M. Kao.** 2004. Genome plasticity in *Streptomyces*: identification of 1 Mb TIRs in the *S. coelicolor* A3(2) chromosome. Mol. Microbiol. **51:**1535–1550.
60. **Yang, X., C. M. Kang, M. S. Brody, and C. W. Price.** 1996. Opposing pairs of serine protein kinases and phosphatases transmit signals of environmental stress to activate a bacterial transcription factor. Genes Dev. **10:**2265–2275.