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CebR as a Master Regulator for Cellulose/Cellooligosaccharide Catabolism Affects Morphological Development in *Streptomyces griseus*▽†

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Streptomyces griseus **mutants exhibiting deficient glucose repression of β-galactosidase activity on lactose-containing minimal medium supplemented with a high concentration of glucose were isolated. One of these mutants had a 12-bp deletion in *cebR*, which encodes a LacI/GalR family regulator. Disruption of *cebR* in the wild-type strain caused the same phenotype as the mutant, indicating that CebR is required for glucose repression of β-galactosidase activity. Recombinant CebR protein bound to a 14-bp inverted-repeat sequence (designated the CebR box) present in the promoter regions of *cebR* and the putative cellobiose utilization operon, *cebEFG-bglC*. The DNA-binding activity of CebR was impaired by cellooligosaccharides, including cellobiose, cellotriose, cellotetraose, cellopentaose, and cellohexaose. In agreement with this observation, transcription from the *cebE* and *cebR* promoters was greatly enhanced by the addition of cellobiose to the medium. Seven other genes containing one or two CebR boxes in their upstream regions were found in the *S. griseus* genome. Five of these genes encode putative secreted proteins: two cellulases, a cellulose-binding protein, a pectate lyase, and a protein of unknown function. These five genes and *cebEFG-bglC* were transcribed at levels 4 to 130 times higher in the Δ*cebR* mutant than in the wild-type strain, as determined by quantitative reverse transcription-PCR. These findings indicate that CebR is a master regulator of cellulose/cellooligosaccharide catabolism. Unexpectedly, the Δ*cebR* mutant formed very few aerial hyphae on lactose-containing medium, demonstrating a link between carbon source utilization and morphological development.**

The gram-positive filamentous bacteria of the genus *Streptomyces* are representative soil microorganisms. Although recent metagenomic studies revealed the surprising genetic diversity of soil microorganisms, including bacteria, archaea, and fungi (10, 18), *Streptomyces* spp. have been considered to comprise the major fraction of soil decomposers (26). Accounting for its niche, studies of complete *Streptomyces* genome sequences have revealed a huge number of genes encoding enzymes for the utilization of divergent nutrient sources, such as cellulose, chitin, xylan, and their hydrolysis products. For example, the model organism *Streptomyces coelicolor* A3(2) encodes 172 sets of secreted proteins that probably function in the metabolism of complex soil substrates and 81 ATP-binding cassette (ABC) transporters that might be used in the uptake of saccharides, oligopeptides, and nucleosides (5, 6).

Another characteristic of *Streptomyces* is that these prokaryotic cells undergo complex morphological development resembling that of eukaryotic filamentous fungi. The typical stages of the streptomycete life cycle on solid medium include germination from spores, substrate growth, formation of multinucleoid aerial hyphae, and sporulation by septation of aerial hyphae. Studies of the *bld* and *whi* series of morphological differentiation mutants, which are arrested at the stages of substrate growth and formation of aerial hyphae, respectively, have made extraordinary contributions to our knowledge of *Streptomyces* morphological development (8). The checkpoint mechanisms for these complicated developmental events have been investigated and elucidated in *S. coelicolor* A3(2) and *Streptomyces griseus* (9). Interestingly, most of the *bld* mutants also lack the capability for catabolite control or "carbon catabolite repression" (31). However, the molecular mechanism that links morphogenesis to the regulation of carbon source utilization, including carbon catabolite repression, is largely unknown.

In our studies of carbon catabolite repression in *S. griseus*, we have observed that *S. griseus* colonies turn blue when grown on lactose-containing minimal medium with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). The blue color indicates the production of β-galactosidase, which hydrolyzes the β-galactoside bond of X-Gal to yield a blue pigment. However, the colonies remain white when glucose is present at a high concentration, indicating that glucose represses β-galactosidase production, while the mechanism of induction of β-galactosidase activity by lactose is unknown in *Streptomyces*.

Here, we isolated several *S. griseus* mutants that formed blue colonies even in the presence of high concentrations of glucose, indicating an apparent escape from glucose catabolite repression of β-galactoside activity. Analysis of one of the mutants, GRD1, revealed that a mutation in the *cebR* gene encoding a LacI/GalR family transcriptional regulator was re-

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sponsible for the phenotype. The purpose of this study was to characterize the gene product, CebR, and investigate the involvement of CebR in catabolite repression. CebR was found to be a master regulator of cellulose/cellooligosaccharide catabolism in *S. griseus*, indicating that CebR is indirectly involved in glucose catabolite repression. Although molecular mechanisms for the apparent escape from catabolite repression in a strain with *cebR* deleted (Δ*cebR*) remained to be elucidated, we found that the Δ*cebR* strain formed very few aerial hyphae on lactose-containing medium. Thus, the Δ*cebR* mutant is the first conditional bald mutant that almost fails to form aerial mycelia in the presence of lactose rather than glucose. This result reemphasizes a possible link between morphogenesis and the regulation of carbon source utilization, including carbon catabolite repression.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The wild-type strain *S. griseus* IFO13350 (equivalent to NBRC102592) was obtained from the Institution of Fermentation (Osaka, Japan) (29). The *S. griseus* mutant GRD1 was derived from the wild-type strain IFO13350 by UV-induced mutagenesis. *Escherichia coli* strains JM109 and JM110 (Takara Biochemicals) and BL21(DE3)Gold (Stratagene) and plasmids pUC19 and pET-26b(+) (Invitrogen) were used for DNA manipulation in *E. coli*.

S. griseus was routinely cultured at 30°C in YMP medium or standard minimal medium (SMM) supplemented with 5 μg/ml thiostrepton or neomycin when necessary. YMP is a nutrient-rich medium (pH 7.2) consisting of 0.2% yeast extract, 0.14% Ehrlich's fish extract (Kyokuto), 0.75% meat extract powder (Kyokuto), 0.4% Bacto peptone, 0.5% NaCl, and 0.2% $MgSO_4 \cdot 7H_2O$. SMM (pH 7.2) consisted of 0.9% glucose, 0.9% L-asparagine, 0.2% $(NH_4)_2SO_4$, 0.24% Tris, 0.1% NaCl, 0.05% K_2SO_4, 0.02% $MgSO_4 \cdot 7H_2O$, 0.01% $CaCl_2$, 1% trace element solution (14), and 2.5 mM KH_2PO_4. To examine the morphological development of *S. griseus* strains, glucose or another carbon source was added to the media at various concentrations. R5 medium (14) was used for the regeneration of protoplasts. Inorganic salt (IS) medium (0.07% K_2HPO_4, 0.03% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, 0.03% NH_4NO_3, and appropriate sugar [27]) was used for screening for *S. griseus* mutants defective in glucose catabolite repression. The media and growth conditions for *E. coli* were described by Maniatis et al. (24). Ampicillin (50 μg/ml) or kanamycin (20 μg/ml) was used when necessary. The *Streptomyces* plasmid pIJ922 (22), which contained the thiostrepton resistance gene and had an estimated copy number of one per genome, was used for construction of a chromosomal DNA library of *S. griseus*.

General recombinant DNA studies. Restriction enzymes, T4 DNA ligase, PrimeStar HS DNA polymerase, ExTaq DNA polymerase, and other DNA-modifying enzymes were purchased from Takara Biochemicals. [γ-^{32}P]ATP (220 TBq/mmol) for 5′-end labeling with T4 polynucleotide kinase was purchased from Perkin-Elmer Inc. DNA was manipulated in *Streptomyces* (14, 20) and in *E. coli* (3, 24) as described previously. When DNA fragments were amplified by PCR, the absence of PCR errors was confirmed by nucleotide sequencing on a Beckman CEQ 8000 DNA analysis system. All primers used in this study are shown in Table S1 in the supplemental material.

UV mutagenesis. Spores of *S. griseus* IFO13350 were irradiated with long-wavelength UV light to yield a survival ratio of approximately 0.5% and spread on IS agar medium (IS-GLX) containing 1% glucose, 0.5% lactose, and 0.006% X-Gal. After incubation for 7 days at 28°C, blue colonies were picked for further analyses.

Shotgun cloning. *S. griseus* IFO13350 chromosomal DNA was partially digested with Sau3AI. The resultant 6- to 10-kb DNA fragments were ligated into BamHI-digested pIJ922, and the ligation mixture was introduced by protoplast transformation into the *S. griseus* mutant strain GRD1. Thiostrepton (5 μg/ml)-resistant transformants were selected on R5 regeneration medium and then inoculated onto IS-GLX agar medium. Transformants that formed white colonies on the medium were isolated and further analyzed.

Gene disruption and complementation. A 7.3-kb EcoRI-BglII fragment of pGRL2 (Fig. 1A), which contained the full-length shotgun-cloned *S. griseus* sequence and part of the pIJ922 sequence, was subcloned into pUC19 between the EcoRI and BamHI sites to form pUC-GRL2. A 2.3-kb NruI fragment containing a 3′ portion of *cebG*, all of *bglC*, and a 5′ portion of *cebR* was excised from pUC-GRL2 and introduced into the HincII site of pUC19 to yield

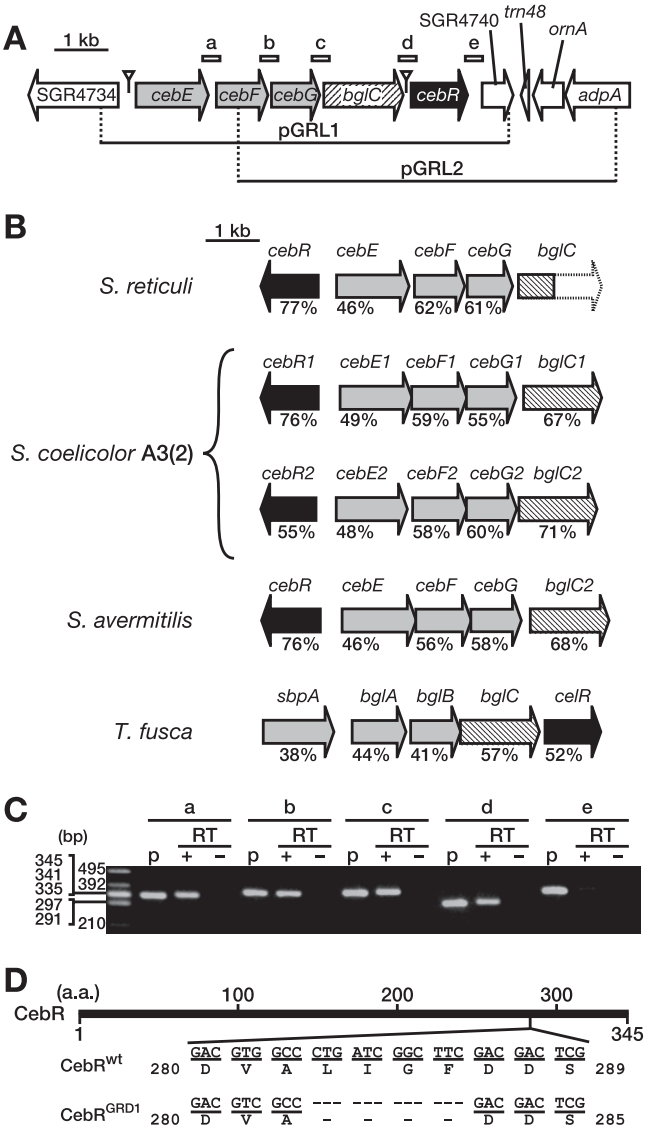

FIG. 1. Cloning and sequence analysis of the *ceb* operon in *S. griseus*. (A) Gene organization of the insert DNA fragments (indicated as horizontal lines) of pGRL1 and pGRL2. The ORFs and their directions on the cloned fragments are indicated by arrows. Light-gray, hatched, and black arrows indicate the genes encoding ABC transporter subunits, a β-glucosidase, and a LacI/GalR family transcriptional regulator, respectively. The open triangles indicate complete CebR boxes (Table 1). The regions amplified by RT-PCR in panel C (described below) are indicated by short bars labeled a, b, c, d, and e. (B) Gene organization of the *ceb* homologs in actinomycetes. The values below the arrows indicate amino acid identity with the corresponding genes in *S. griseus*. The genome of *S. coelicolor* A3(2) includes two *ceb* gene clusters. The entire sequence of *bglC* of *S. reticuli* has not yet been determined. (C) RT-PCR analysis of the transcriptional units in the *ceb* gene cluster. Amplification of five intergenic regions, a (between *cebE* and *cebF*), b (*cebF* and *cebG*), c (*cebG* and *bglC*), d (*bglC* and *cebR*), and e (*cebR* and SGR4740) (Fig. 1A), was attempted. To confirm amplification of the DNA fragments, pGRL1 was used as a PCR template (lanes p). No amplification occurred when reverse transcriptase was omitted from the reaction mixture (lanes RT−), indicating the absence of DNA in the mRNA samples. (D) Site of the *cebR* mutation in the GRD1 mutant. The bold horizontal line represents the CebR protein. The nucleotide and deduced amino acid (a.a.) sequences of *cebR* of the wild-type strain (CebR^{wt}) and the GRD1 mutant (CebR^{GRD1}) are shown.

pDCEBR-1. A 0.6-kb fragment containing a 3′ portion of *cebR* and a 5′ portion of SGR4740 was amplified by PCR with the primers DR-for (containing EcoRI and NcoI sites) and DR-rev (containing a HindIII site), digested with EcoRI and HindIII, and subcloned between the EcoRI-HindIII sites of pUC18 to yield pDCEBR-2. The 0.5-kb NcoI-XbaI fragment of pDCEBR-2 and the 2.9-kb XbaI-EcoRI fragment of pUC-GRL2, which contains the 3′ portion of SGR4740, *ornA*, the 5′ portion of *adpA*, and a 0.6-kb fragment derived from pIJ922, were subcloned together into pDCEBR-1 between the NcoI and EcoRI sites by three-fragment ligation to yield pDCEBR. On pDCEBR, a 2.3-kb fragment upstream from *cebR* and a 2.3-kb fragment downstream from *cebR* are directly connected. The 3.4-kb kanamycin resistance (Km^r) determinant obtained by digestion of Tn*5* (4) with HindIII was cloned into the HindIII site of pUC19. A 1.3-kb fragment containing the Km^r gene (*aphII*) was excised from the plasmid by digestion with BamHI (on the multiple-cloning site of pUC19) and SmaI (downstream from *aphII*) and subcloned between the BamHI-SmaI sites of pUC19 to yield pUC-APH. The 1.3-kb HindIII fragment containing the Km^r gene (*aphII*) was excised from pUC-APH and introduced into the HindIII site of pDCEBR, resulting in pDCEBR-aph. On pDCEBR-aph, *aphII* is located next to a 4.6-kb recombinant fragment containing both upstream and downstream sequences from *cebR*. This plasmid was amplified in *E. coli* JM110, isolated, alkali denatured, and introduced by protoplast transformation into *S. griseus* IFO13350, and neomycin (5 μg/ml)-resistant colonies by a single crossover were isolated. After one of the neomycin-resistant transformants had been cultured in the absence of neomycin several times, neomycin-sensitive colonies by a second crossover were isolated as candidates for true *cebR* disruptants. The disruption was confirmed by PCR and sequencing of the amplified DNA fragment.

Peptidoglycan degradation activity assay. To examine the peptidoglycan degradation activities of *S. griseus* strains, we supplemented YMP solid medium with dead cells of *Micrococcus lysodeikticus* ATCC 4698 (Sigma) at a concentration of 0.25%. *S. griseus* cells were grown on the medium at 28°C for 36 h, and peptidoglycan degradation activity was detected by the observation of clear halos caused by degradation of the *M. lysodeikticus* cell walls.

Production and purification of CebR. The *cebR* sequence was amplified by PCR with the primers PR-for (containing BamHI and NdeI sites) and PR-rev (containing EcoRI and XhoI sites). The *cebR* coding sequence was excised as a BamHI-EcoRI fragment and subcloned into BamHI/EcoRI-digested pUC19. The NdeI-XhoI fragment was excised from this plasmid and subcloned into NdeI/XhoI-digested pET-26b(+) to create pET-CEBR. *E. coli* BL21(DE3)Gold cells harboring pET-CEBR were cultured at 16°C for 24 h in the presence of 0.3 mM isopropyl-β-D-thiogalactopyranoside. CebR with a C-terminal His tag (CebR-H) was purified from the soluble fraction with an Ni-nitrilotriacetic acid spin column (Qiagen) according to the manufacturer's instructions. Protein concentrations were measured using a Bio-Rad protein assay kit with bovine serum albumin as a standard.

The molecular mass of the recombinant protein CebR-H was determined by Superdex 200 gel filtration chromatography on an AKTA FPLC Purifier (GE Healthcare) in a mobile phase consisting of 50 mM Tris-HCl (pH 8.0), 200 mM NaCl, and 10% glycerol at a flow rate of 0.2 ml/min.

EMSA. DNA fragments were amplified by PCR using appropriate primers, 5′ ^{32}P labeled, and used as probes in electrophoretic mobility shift assays (EMSAs). For the binding assay, 0.5 nM of ^{32}P-labeled probe DNA was incubated with CebR-H at room temperature for 15 min in a buffer containing 20 mM HEPES (pH 7.6), 1 mM EDTA, 10 mM $(NH_4)_2SO_4$, 1 mM dithiothreitol, 30 mM KCl, 0.2% (vol/vol) Tween-20, and 10% (vol/vol) glycerol in a total volume of 20 μl. The resulting DNA/CebR-H complexes and free DNA were resolved on nondenaturing 6% polyacrylamide gels (mono/bis ratio, 79:1) in a running buffer containing 45 mM Tris-borate and 1 mM EDTA. The dissociation constant was calculated as the concentration of CebR-H that caused 50% of the amount of the probe DNA to form a complex with the protein.

S1 nuclease mapping. Spores (2×10^6 CFU) were inoculated into 200 ml of liquid SMM and incubated at 30°C for 50 h with reciprocal shaking (120 rpm). Cells were collected by centrifugation, and total RNA was prepared from the cells using Isogen (Nippon Gene). S1 nuclease mapping was performed as described by Bibb et al. (7) and Kelemen et al. (19). Hybridization probes were prepared by PCR with a pair of ^{32}P-labeled and nonlabeled primers. The PCR primers used for high-resolution S1 nuclease mapping were HR-for and HR-rev* for *cebR* and HLE-for and HE-rev* for *cebE*. The asterisks indicate 5′ labeling performed using T4 polynucleotide kinase with [γ-^{32}P]ATP before PCR. The primers for low-resolution S1 mapping were LR-for and LR-rev* for *cebR* and HLE-for and LE-rev* for *cebE*. The *hrdB* (SGR1701) gene encoding a principal sigma factor of RNA polymerase was used as a control for the purity and amount of RNA, as described previously (51). Protected fragments were analyzed on 6%-polyacrylamide DNA-sequencing gels by the method of Maxam and Gilbert (25).

RT-PCR. Total RNA was used for a standard reverse transcription (RT) reaction using the ThermoScript RT-PCR system (Invitrogen) with random hexamers according to the manufacturer's instructions. The resulting cDNA fragments, a to e, were amplified by PCR with the primer pairs rA-for/A-rev, rB-for/rB-rev, rC-for/rC-rev, rD-for/rD-rev, and rE-for/rE-rev, respectively (Fig. 1A). The amplified PCR fragments were analyzed by agarose gel electrophoresis.

Quantitative RT-PCR. A pair of primers for each gene was designed using the SYBR green method. The housekeeping gene *hrdB* was used as an internal standard because it is presumably transcribed consistently throughout growth. The cDNA was synthesized using the ThermoScript RT-PCR system (Invitrogen) with random hexamers according to the manufacturer's instructions. All reactions were performed in the Takara SYBR Premix ExTaq II reaction mixture using a SmartCycler II real-time PCR system (Cepheid) with an initial denaturation at 95°C for 10 s and 50 cycles of 5 s at 95°C (denaturing) and 30 s at 60°C (annealing and extension). All reactions were performed in triplicate, and the data were normalized to the average of the internal standard.

RESULTS

Isolation of *S. griseus* GRD (for *g*lucose catabolite *r*epression-*d*eficient) mutants by UV mutagenesis. During our study of carbon catabolite repression in *S. griseus*, we noticed that β-galactosidase production was repressed by glucose. To find novel genes involved in carbon catabolite repression, we attempted to isolate mutants with deficiencies of glucose catabolite repression such that β-galactosidase activity was not repressed in the presence of a high concentration of glucose. More than 10,000 colonies formed from UV-mutated spores were screened on IS-GLX medium (1% glucose, 0.5% lactose, and 0.006% X-Gal), and 14 blue colonies that appeared to produce β-galactosidase even in the presence of a high concentration of glucose, were obtained. The wild-type *S. griseus* strain formed no blue colonies on this IS-GLX medium.

The morphological development of these 14 strains was tested on solid YMP medium containing a high concentration of glucose. Of the 14 mutant strains, 4 were distinct from the wild-type strain; while the wild-type strain exhibited vegetative arrest on 2% glucose-containing medium, the 4 mutant strains formed aerial mycelia even on 3% glucose-containing medium (data not shown). These mutants were designated GRD1, -2, -3, and -4.

Previous studies showed that mutations in the glucose kinase gene *glkA* cause a defect in glucose catabolite repression in *Streptomyces* (1, 2, 13, 16, 21, 35, 47). Therefore, we next determined the nucleotide sequence of the *glkA* locus of each mutant. The GRD2 mutant was found to harbor a point mutation at position +238 of *glkA* (taking the first nucleotide of the start codon as position +1), resulting in an Asp80→His mutation. However, no mutations were found in the *glkA* loci of the other three mutants. Because the GRD1 mutant exhibited the most extreme glucose catabolite repression-deficient phenotype (data not shown), we chose it for further analysis.

Cloning and nucleotide sequencing of DNA fragments complementing the glucose repression deficiency in the *S. griseus* mutant GRD1. A chromosomal DNA library of wild-type *S. griseus* was constructed using a low-copy-number plasmid, pIJ922, as the cloning vector. This library was introduced into mutant GRD1 by protoplast transformation. The β-galactosidase activities of approximately 5,000 transformants were examined on IS-GLX medium, and two white colonies with apparently repressed β-galactosidase activities were isolated.

Like the wild-type strain, the two transformants exhibited vegetative arrest on medium containing 2% glucose (data not shown). Two plasmids (designated pGRL1 and pGRL2) were individually extracted from these transformants, and their insert DNA fragments were identified by end sequencing and found to overlap (Fig. 1A).

Plasmid pGRL1 was found to contain five complete open reading frames (ORFs), SGR4735 to SGR4739. On the basis of sequence homology and synteny, these five ORFs were predicted to compose a gene cluster for cellobiose/cellotriose utilization; similar gene clusters in *Streptomyces reticuli* and *Thermobifida fusca* (Fig. 1B) have been characterized and identified as cellobiose/cellotriose (37) and cellobiose (41) utilization systems, respectively. Therefore, these genes were designated *cebE* (SGR4735), *cebF* (SGR4736), *cebG* (SGR4737), *bglC* (SGR4738), and *cebR* (SGR4739). The *cebE*, *cebF*, and *cebG* genes appeared to encode subunits of a sugar ABC transporter, since CebE was a homolog of sugar-binding proteins and CebF and CebG were homologs of membrane transport proteins. BglC, an apparent β-glucosidase, probably hydrolyzed cellobiose (or cellotriose) imported through the ABC transporter consisting of CebE, CebF, CebG, and an ATP-binding protein encoded by a gene not linked to the *ceb* operon. CebR, homologous to LacI/GalR regulators, was probably a transcriptional repressor for the gene cluster. *S. coelicolor* A3(2) has two gene clusters homologous to the *ceb* gene cluster (5), and *Streptomyces avermitilis* has one such cluster (17), as shown in Fig. 1B. Plasmid pGRL2 was found to contain two complete ORFs, SGR4740, encoding a chitin-binding protein, and *ornA*, encoding an oligoribonuclease (30), in addition to *cebG*, *bglC*, and *cebR*.

In *S. reticuli*, *cebE*, *cebF*, *cebG*, and *bglC* are cotranscribed (37). Therefore, the possible cotranscription of ORFs SGR4735 (*cebE*) through SGR4740 was analyzed by RT-PCR. The intervening regions between *cebE* and *cebF* (Fig. 1A, a, and C, a), *cebF* and *cebG* (b), *cebG* and *bglC* (c), and *bglC* and *cebR* (d) were amplified by RT-PCR (Fig. 1C), indicating that these five genes (*cebE* through *cebR*) were cotranscribed as an operon. In contrast, no amplification of the intervening region between *cebR* and SGR4740 was detected (Fig. 1C, e), indicating that SGR4740 was not cotranscribed with its upstream gene *cebR*. As described below, *cebR* appeared to harbor its own promoter in the intergenic region between *bglC* and *cebR*. Thus, *cebR* seems to be transcribed from not only the *cebE* promoter, but also the *cebR* promoter.

Identification of the GRD1 mutation. As described above, three complete ORFs, *cebG*, *bglC*, and *cebR*, were common to pGRL1 and pGRL2. Therefore, we thought that the GRD1 mutant chromosome might harbor mutations in the coding or regulatory regions of *cebG*, *bglC*, and *cebR*. Nucleotide sequencing of the region covering these genes revealed a 12-bp deletion in the *cebR* coding region (positions +847 to +858, taking the first nucleotide of the start codon as +1), causing a deletion of amino acid residues Leu283 through Phe286 of CebR (Fig. 1D).

The glucose repression-defective phenotype of a *cebR*-disrupted strain. To confirm that the chromosome DNA mutation found in GRD1 was responsible for its glucose repression-defective phenotype, we disrupted the chromosomal *cebR* gene of *S. griseus* IFO13350 by deleting approximately 70% of the *cebR* coding sequence. This Δ*cebR* mutant showed the same phenotype as GRD1, i.e., defective glucose repression of β-galactosidase activity (Fig. 2A) and morphological development on 2% glucose-containing medium (Fig. 2C and D). Moreover, we found that deletion of *cebR* caused a defect in glucose repression of peptidoglycan degradation activity (Fig. 2B). When carried on the low-copy-number plasmid pIJ922, *cebR* was sufficient to rescue the glucose repression-deficient phenotype of the Δ*cebR* mutant (Fig. 2A to D). On the other hand, a plasmid containing *cebR* with the GRD1-type 12-bp deletion (pIJ922-*cebR*^{GRD1}) failed to complement glucose re-

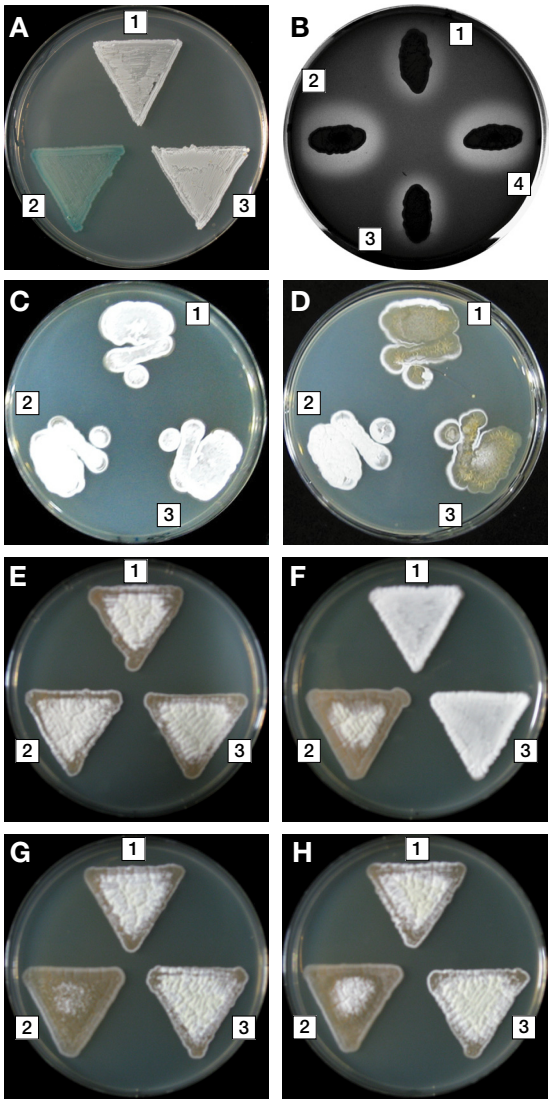

FIG. 2. Glucose catabolite repression-defective phenotypes of the *S. griseus* Δ*cebR* mutant. (A) β-Galactosidase activity on IS-GLX agar medium. (B) Halo formation by peptidoglycan degradation activity on YMP medium containing 1% glucose. (C to H) Morphology on YMP agar medium supplemented with no carbohydrates (C), 2.0% glucose (D), 1.5% glucose (E), 1.5% lactose (F), 1.0% glucose plus 0.5% lactose (G), and 1.0% glucose plus 0.5% lactose and 100 mM TES (pH 7.5) (H). 1, the wild-type strain harboring pIJ922; 2, the Δ*cebR* mutant harboring pIJ922; 3, the Δ*cebR* mutant harboring pIJ922-*cebR*^{wt}; and 4, the Δ*cebR* mutant harboring pIJ922-*cebR*^{GRD1}.

TABLE 1. CebR boxes found in the genome of *S. griseus*

Gene ID	Deduced function	Sequence of CebR box[a]	Position[b]	Location	Transcription level (Δ*cebR*/wt)[c]
SGR199	Secreted cellulose-binding protein	TGGGAGCGCTCCCA, CGGGAGCGCTCCCC	−324.5, −519.5	TL	39
SGR217	Secreted pectate lyase	TGGGAGCGCTCCCG	−103.5	TL	4.2
SGR1971	Secreted protein of unknown function	TGGGAGCGCTCCCC	−298.5	TL	5.5
SGR2445	Secreted cellulase	TGGGAGCGCTCCCA	−23.5	TL	15
SGR3391	Acetyltransferase	CGGGAGCGCTCCCG	−25.5	TL	1.6
SGR4735	*cebE*; cellobiose ABC transporter solute-binding protein	TGGGAGCGCTCCCA	+13.5	TC	10
SGR4738	*bglC*; β-glucosidase	AGGGAGCGCTCCCA	−8.5	TL	5.8
SGR4739	*cebR*; LacI/GalR family transcriptional regulator	TGGGAGCGCTCCCA	−21.5[d]	TC	–[e]
SGR6927[f]	Hypothetical protein	TGGGAGCGCTCCCA, TTGGAGCGCTCCCA	−296.5, −209.5	TL	2.6
SGR6928[f]	Secreted cellulase	TGGGAGCGCTCCAA, TGGGAGCGCTCCCA	−199.5, −112.5	TL	130

[a] Nucleotides that match the consensus CebR box sequence are underlined.
[b] Nucleotide position of the center of the CebR box relative to the transcriptional start site (TC) or the translational start site (TL).
[c] Average value from three quantitative-RT-PCR experiments. wt, wild type.
[d] From the transcriptional start site of $cebRp_1$ (Fig. 5E).
[e] Not detected because the *cebR* coding region was deleted in the Δ*cebR* mutant.
[f] SGR6927 and SGR6928 are divergent. Two CebR boxes exist in the intergenic region between SGR6927 and SGR6928.

pression of peptidoglycan degradation activity in the Δ*cebR* mutant, confirming that the 12-bp deletion was sufficient to abolish the function of CebR (Fig. 2B).

Binding of CebR to a CebR box located upstream of *cebE*. Two CebR homologs in *S. reticuli* and *T. fusca* have been characterized; CebR of *S. reticuli* is a regulator for cellobiose/cellotriose utilization genes and the avicelase gene *cel1*, and CelR of *T. fusca* is a regulator for six cellulase genes. Both proteins have been shown to bind to the same 14-bp inverted-repeat sequence, TGGGAGCGCTCCCA, located in the promoter regions of their target genes (37, 40). We found the same inverted-repeat sequence (hereafter designated the "CebR box") in the *ceb* gene cluster of *S. griseus*; the CebR box was present in the promoter regions of *cebE* and *cebR* (Fig. 1A and Table 1).

To examine binding of the *S. griseus* CebR protein to the CebR box, we first expressed *cebR* in *E. coli* and purified recombinant CebR (Fig. 3A). The recombinant CebR having the sequence CebR-Leu-Glu-His_6 was designated CebR-H. The subunit structure of CebR-H was determined using gel filtration chromatography under nondenaturing conditions. A single peak representing CebR-H eluted at 81 kDa, as determined on the basis of its elution volume, V_e. Because CebR-H had a calculated molecular mass of 38 kDa, we concluded that it formed a homodimer in solution. We confirmed that the V_e did not vary in the presence of the CebR effector molecule cellobiose (10 mM) (see below), indicating that the binding of the effector molecule does not affect the subunit structure of CebR-H.

Finally, we confirmed binding of CebR-H to the CebR box using EMSAs. The recombinant protein bound to a 120-bp DNA fragment of the *cebE* promoter region containing a CebR box (Fig. 3B). The apparent K_d (dissociation constant) value was 8.6 nM (Fig. 3C), indicating that the DNA/CebR-H complex was quite stable.

Effects of cellooligosaccharides on DNA binding by CebR. Regulators of the LacI/GalR family generally bind low-molecular-weight effectors that abolish their DNA-binding activities

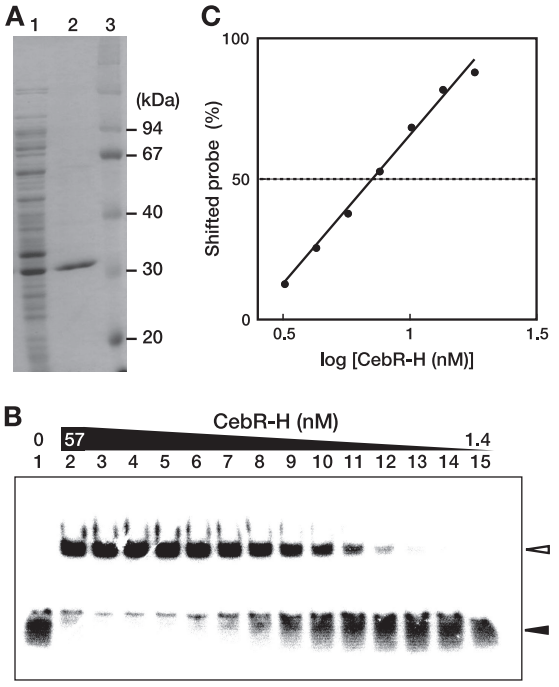

FIG. 3. Binding of CebR-H to the *cebE* promoter region. (A) CebR-H used in this study was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Lane 1, crude extract of *E. coli* BL21(DE3)Gold cells harboring pET-CebR; lane 2, purified CebR-H; lane 3, molecular mass standards (phosphorylase *b* [94 kDa], bovine serum albumin [67 kDa], ovalbumin [43 kDa], carbonic anhydrase [30 kDa], and soybean trypsin inhibitor [20 kDa]). (B) EMSA using CebR-H and a DNA fragment containing the *cebE* promoter region. The CebR-H homodimer was diluted stepwise by 75% from 57 nM (lane 2) to 1.4 nM (lane 15). Lane 1 shows a negative control without CebR-H. The positions of free probe (solid arrowhead) and DNA-CebR-H complexes (open arrowhead) are shown. (C) Plot of percentages of CebR-H-bound probe versus log[CebR-H (nM)] from 18 nM (lane 6) to 3.2 nM (lane 12). The apparent K_d value for CebR-H is indicated by a dashed line.

(50). The major effector of CebR of *S. reticuli* is cellopentaose, a cellooligosaccharide comprising five glucose units joined with β(1→4) linkages (38). On the other hand, the effector of *T. fusca* CelR is cellobiose rather than cellopentaose (40).

To investigate the possible effectors of *S. griseus* CebR, we used EMSAs to examine the binding of cellooligosaccharides consisting of two to six glucose units, i.e., cellobiose, cellotriose, cellotetraose, cellopentaose, and cellohexaose. The DNA-binding activity of CebR-H, unlike that of its *S. reticuli* and *T. fusca* CebR homologs, was impaired by all cellooligosaccharides tested (Fig. 4A). For each cellooligosaccharide, the apparent K_d was calculated as the concentration that caused 50% dissociation of the protein-DNA complex. These values were 0.22 mM for cellobiose, 0.99 mM for cellotriose, 4.1 mM for cellohexaose, 7.9 mM for cellopentaose, and approximately 12 mM for cellotetraose (Fig. 4B). Monosaccharides and other oligosaccharides, such as glucose (Fig. 4), glucose-6-phosphate, fructose-6-phosphate, maltose, lactose, or gentiobiose (data not shown), had no effect on DNA binding by CebR at concentrations of at least 20 mM. Although cellobiose was bound about 50 times more effectively than cellotetraose, the apparent K_d value of cellotetraose for CebR-H (12 mM) was nonetheless remarkable compared to those of CebR homologs from other species. For example, the DNA-binding activity of *T. fusca* CelR was not affected by 50 mM cellotriose (40), and the DNA-binding activity of *S. reticuli* CebR was not affected by 10 mM cellotriose or cellopentaose (38).

Transcriptional analysis of *cebE* and *cebR* by S1 nuclease mapping. As described above, cellobiose was the most effective inhibitor of the in vitro DNA-binding activity of CebR. Therefore, we used low-resolution S1 nuclease mapping to determine whether cellobiose in the medium affected transcription from the CebR box-containing *cebE* and *cebR* promoters. RNAs were prepared from the wild-type strain grown in liquid SMM containing (i) 1% glucose, (ii) 1% cellobiose, (iii) 1% glucose plus 1% cellobiose, and (iv) 1% glycerol. The *hrdB* gene, which is transcribed throughout growth, was used as an internal control for the integrity and amount of mRNA. As shown in Fig. 5A, the *cebE* transcript was detected at a high level in the wild-type strain grown in cellobiose-containing medium (lane 3), whereas it was barely detectable in the wild-type strain grown in glucose- or glycerol-containing medium (lanes 2 and 5). Furthermore, we also analyzed transcription of *cebE* in the Δ*cebR* mutant grown in liquid SMM containing 1% glucose. As we expected, the *cebE* transcript was clearly detected in the Δ*cebR* mutant even in the absence of cellobiose (lane 1).

Using high-resolution S1 nuclease mapping, we determined the transcriptional start sites of *cebE* to be the C and G located 128 and 127 nucleotides, respectively, upstream from the translational start point of *cebE* (Fig. 5B). The −35 and −10 sequences for the *cebE* promoter (*cebEp*) appeared to be TT GACA and CAACCT, respectively (Fig. 5D), although the putative −10 sequence differed somewhat from the consensus −10 sequence (TAGRRT, where R = A or G) for promoters of housekeeping genes (43). Thus, the CebR box was located immediately downstream of the two adjacent transcriptional start sites of *cebE*. Together, these results indicated that CebR acted as a repressor for *cebE* and that cellobiose released the transcriptional repression of *cebE* by CebR. A considerable amount of *cebE* transcript was detected in medium containing cellobiose and glucose (lane 4), suggesting that transcription of *cebE* was not subject to glucose catabolite repression.

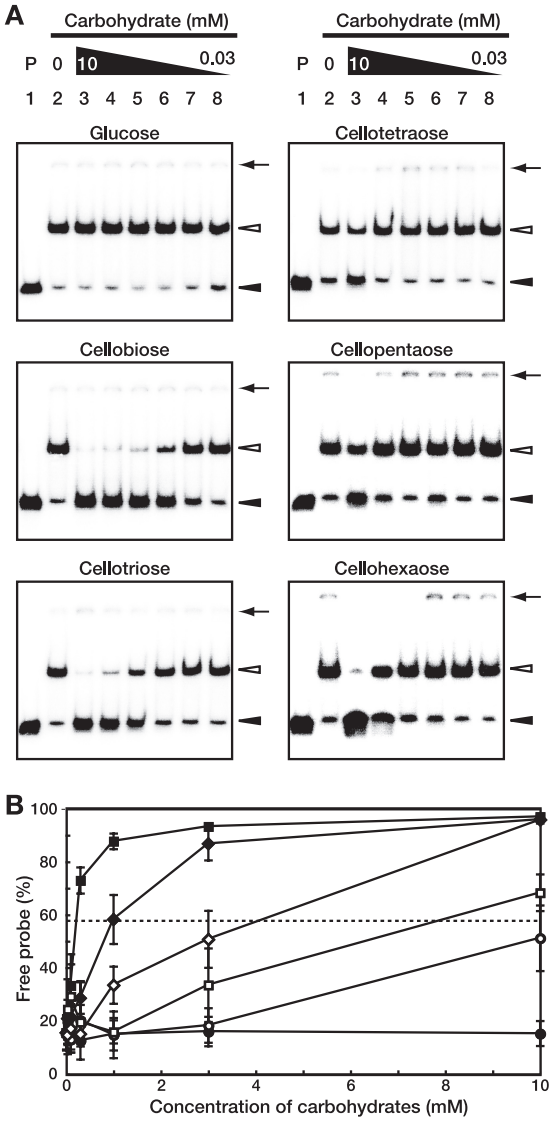

FIG. 4. Inhibition of the DNA-binding activity of CebR by cellooligosaccharides. (A) EMSAs using CebR-H and a DNA fragment containing the *cebE* promoter region in the presence of the indicated carbohydrates. Lane 1, a negative control without CebR-H; lanes 2 to 8, CebR-H homodimer (at 15 nM, so that approximately 85% of the probe is shifted in the absence of carbohydrates) with carbohydrate at 0 mM (lane 2; control), 10 mM (lane 3), 3 mM (lane 4), 1 mM (lane 5), 0.3 mM (lane 6), 0.1 mM (lane 7), and 0.03 mM (lane 8). The positions of free probes (solid arrowheads), DNA-CebR-H complexes (open arrowheads), and the wells (solid arrows) are shown. (B) Plot of percentages of free probe versus concentrations of each carbohydrate: glucose (●), cellobiose (■), cellotriose (◆), cellotetraose (○), cellopentaose (□), and cellohexaose (◇). The values shown are averages and standard deviations from triplicate assays. A dashed line indicates 50% dissociation of the protein-DNA complex for calculation of the apparent K_d value for each cellooligosaccharide.

In contrast to *cebE*, *cebR* was transcribed from two different promoters (Fig. 5A). Transcription from the upstream promoter ($cebRp_1$) was detected only in the presence of cellobiose,

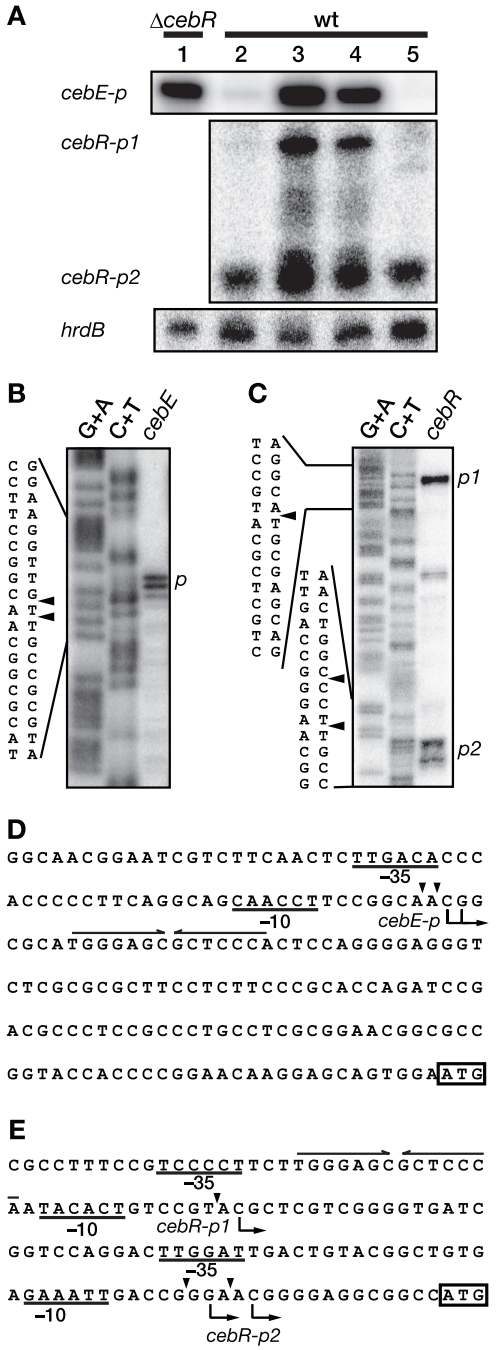

FIG. 5. Transcriptional analysis of *cebE* and *cebR*. (A) Transcription of *cebE* and *cebR* in *S. griseus* was examined by low-resolution S1 nuclease mapping. Total RNA was prepared from the Δ*cebR* mutant (lane 1) or the wild-type strain (lanes 2 to 5) grown in liquid SMM containing 1% glucose (lanes 1 and 2), 1% cellobiose (lane 3), 1% glucose plus 1% cellobiose (lane 4), and 1% glycerol (lane 5) at 30°C for 50 h. (B and C) High-resolution S1 nuclease mapping of *cebE* (B) and *cebR* (C). S1-protected fragments were analyzed in parallel with the sequence ladders (lanes G+A and C+T) (24). The arrowheads indicate the positions of S1 nuclease-protected fragments. The 5′ terminus of the mRNA was assigned to the positions shown in panels D and E because fragments generated by the chemical sequencing reactions migrated 1.5 nucleotides further than the corresponding fragments generated by S1 nuclease digestion of the DNA/RNA hybrids (one-half residue from the presence of the 3′-terminal phosphate group and one residue from the elimination of the 3′-terminal nucleotide). (D and E) Nucleotide sequences of the upstream regions of *cebE* (D) and *cebR* (E). Transcriptional start points are shown by arrows below the sequences. The probable −35 and −10 sequences are underlined. A CebR box is indicated with a pair of convergent arrows above the sequence. Probable translational start codons are boxed.

like transcription from *cebEp* (Fig. 5A). However, transcription from the downstream promoter (*cebRp*$_2$) was detected regardless of the carbon source in the medium (Fig. 5A). Although cellobiose induced a low level of transcription from *cebRp*$_2$, this result suggested that *cebRp*$_2$ was constitutively active at a basal level.

The transcriptional start sites of *cebR* were determined by high-resolution S1 nuclease mapping (Fig. 5C). Transcription from *cebRp*$_1$ was found to begin at the C located 78 nucleotides upstream from the translational start point of *cebR*. The −35 and −10 sequences for *cebRp*$_1$ appeared to be TCCCCT and TACACT (Fig. 5E), respectively, although the −35 sequence differed somewhat from the consensus −35 sequence (TTGACR) for promoters of housekeeping genes (43). Thus, the CebR box was located only in *cebRp*$_1$, consistent with the observation that *cebRp*$_1$ was regulated by CebR.

Transcription from *cebRp*$_2$ was found to begin at the G and C located 16 and 13 nucleotides, respectively, upstream from the translational start point of *cebR* (Fig. 5C and E). Its −35 and −10 sequences for *cebRp*$_2$ appeared to be TTGGAT and GAAATT (Fig. 5E). Thus, the CebR box was located far from *cebRp*$_2$, in agreement with the observation that *cebRp*$_2$ was essentially independent of CebR.

Regulation of the other CebR box-containing genes by CebR. When we searched the *S. griseus* genome (29) for the 14-bp CebR box sequence (TGGGAGCGCTCCCA), allowing up to two mismatches, we identified 11 sequences (Table 1) in addition to those located in the *cebE* and *cebR* upstream regions. These CebR boxes were generally located in the upstream regions of genes encoding secreted proteins for cellulose/cellooligosaccharide catabolism (Table 1). Two CebR boxes were found in the upstream region of SGR199, and two additional CebR boxes were found in the intergenic region between the divergent genes SGR6927 and SGR6928. Using EMSAs, we confirmed that CebR-H bound to the nine DNA fragments containing one or two CebR boxes (Fig. 6). CebR-H appeared to have similar binding affinities for these DNA fragments, although two shifted bands were detected for the probes for SGR199 and SGR6927/SGR6928, both of which had two CebR boxes.

We next compared the transcription of these CebR box-containing genes in the wild-type and Δ*cebR* strains using quantitative RT-PCR. The strains were grown on IS agar medium supplemented with 1% glucose as the sole carbon source. Although the expression ratio (Δ*cebR*/wild-type) varied from 1.6 to 130, all of these genes were upregulated in the Δ*cebR* mutant compared with the wild-type strain (Table 1). We concluded that at least five genes (SGR199, SGR217, SGR1971, SGR2445, and SGR6928), all of which were clearly upregulated in the Δ*cebR* mutant (by at least fourfold), were CebR target genes, as well as *cebEFG-bglC* and *cebR*.

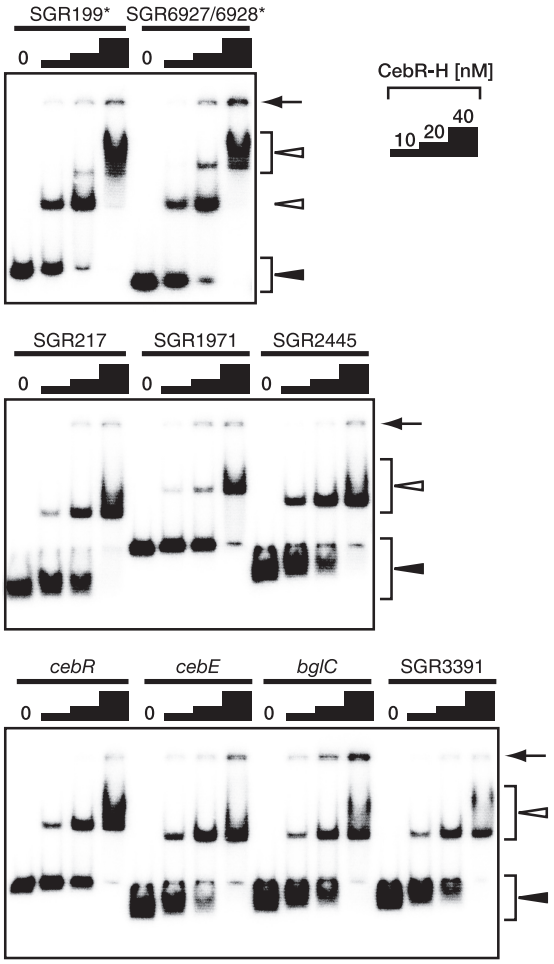

FIG. 6. Binding of CebR-H to DNA fragments containing CebR boxes, as determined by EMSAs. DNA fragments of the indicated upstream gene regions were used as probes. Fragments containing two CebR boxes are marked with asterisks. The CebR-H dimer was used at 0, 10, 20, and 40 nM. The positions of free probes (solid arrowheads), DNA-CebR-H complexes (open arrowheads), and the wells (solid arrows) are shown.

Carbon source-dependent morphological defects of the Δ*cebR* mutant. We observed consistently incomplete morphological differentiation for the Δ*cebR* mutant on IS-GLX medium. To confirm this observation, the Δ*cebR* mutant was inoculated onto YMP solid medium, which has been routinely used for culture of *S. griseus* in our laboratory, supplemented with various carbon sources. The mutant was unable to complete morphological differentiation on YMP solid medium supplemented with 1.5% lactose as a carbon source (Fig. 2F). The morphological defect was enhanced for YMP medium supplemented with 1.0% glucose and 0.5% lactose (YMP-GL) (Fig. 2G), whereas the Δ*cebR* mutant was indistinguishable from the wild-type strain when the carbon source was 1.5% glucose (Fig. 2E) or 1.5% maltose, 1.5% galactose, 1.5% glycerol, or 1.0% glucose plus 0.5% galactose (data not shown).

In previous studies, the morphological differentiation of some *S. coelicolor* A3(2) mutants was inhibited by acidification of the medium, and the addition of appropriate buffers to the medium allowed these mutants to develop normally (44, 48, 49). Therefore, we added 50 mM HEPES or TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] to the YMP-GL solid medium to maintain the pH at ~7.5. The morphological differentiation of the Δ*cebR* mutant did not improve, even on solid YMP-GL media containing HEPES (data not shown) and TES (Fig. 2H), indicating that its morphological defect on lactose-containing medium was not caused by acidification of the medium.

DISCUSSION

In this study, we identified *cebR* as a gene required for glucose catabolite repression of β-galactosidase activity in *S. griseus*. A Δ*cebR* mutant was deficient in glucose catabolite repression; it exhibited β-galactosidase and peptidoglycan degradation activities in the presence of a high concentration of glucose, and it exhibited morphological development on medium containing 3% glucose. However, *cebR* is not directly involved in glucose catabolite repression; rather, we found that CebR acts as a master regulator of cellulose/cellooligosaccharide catabolism. Identification of genes encoding a β-galactosidase(s) and an enzyme(s) for peptidoglycan degradation is essential for elucidation of the molecular mechanism for catabolite repression in *S. griseus*. Disruption of some candidate genes on the *S. griseus* chromosome is in progress in our laboratory.

Concerning the β-galactosidase activity observed in the Δ*cebR* mutant in the presence of glucose at a high concentration, a β-glucosidase, BglC, which is ectopically overproduced in the mutant, may have some activity toward β-galactoside in addition to β-glucoside, because BglC of *T. fusca*, which shows 57% identity to *S. griseus* BglC in their amino acid sequences, can hydrolyze a β-galactosidic bond of *o*-nitrophenyl-β-D-galactopyranoside (41). If this is the case, the release from the catabolite repression of β-galactosidase activity in the Δ*cebR* mutant must be ascribed to a kind of "artifact." However, because catabolite repression of not only β-galactosidase activity, but also peptidoglycan degradation activity and morphological development, was abolished in the Δ*cebR* mutant, disruption of *cebR* undoubtedly caused a defect in catabolite repression through some unknown mechanisms. Again, identification of genes that encode a β-galactosidase(s) and an enzyme(s) for peptidoglycan degradation is important.

Unexpectedly, the Δ*cebR* mutant produced very few aerial hyphae on medium containing 1.0% glucose and 0.5% lactose, although the IS-GLX medium that was used for the isolation of glucose catabolite repression-deficient mutants also contained 1.0% glucose and 0.5% lactose. Why is aerial mycelium production in the Δ*cebR* mutant so poor in medium containing lactose? We assume that the lack of CebR in the presence of lactose triggers a drastic disturbance in the sugar-sensing and utilization systems, which might result in vegetative arrest. Deficient morphological development caused by a disturbance of the chitin/chitooligosaccharide utilization system has been reported in *S. coelicolor* A3(2). In *S. coelicolor* A3(2), the transcriptional repressor DasR, a member of the GntR family (HutC subfamily), acts as a global regulator of chitin/chitooligosaccharide catabolism and regulates the phosphotransferase system components specific to the uptake of *N*-acetylglucosamine (a monomer of chitin), the ABC transporter system

for the import of chitobiose, and several enzymes for chitin/chitooligosaccharide utilization (32, 33, 36). Glucosamine 6-phosphate, a central molecule in *N*-acetylglucosamine metabolism, is an effector of DasR that abolishes its DNA-binding activity (32). Disruption of *dasR* results in a bald phenotype on glucose-containing media, not only in *S. coelicolor* A3(2), but also in *S. griseus* (32, 39). Rigali et al. (32) have suggested that the significant deregulation of gene expression in the *dasR* mutant is a major reason for its vegetative arrest. Additionally, they have proposed that, since the polysaccharides cellulose and chitin are the most abundant on Earth, both compounds are therefore potentially crucial sources of carbon (and chitin also of nitrogen) and can constitute an important marker for nutrient availability in soil-dwelling microorganisms, implying their possible involvement in developmental control. DasR is apparently involved in the sensing of chitin and chitooligosaccharides. Here, we propose that CebR is involved in the sensing of cellulose and cellooligosaccharides in *S. griseus*. It is very interesting that disruption of either *dasR* or *cebR* results in vegetative arrest in medium containing a sugar that is not apparently related to the metabolites controlled by the regulator; the *dasR* and *cebR* mutants showed a bald phenotype on media containing glucose and lactose, respectively. We speculate that ectopic production of a sugar-utilizing system, in the absence of its substrate and in the presence of some other sugar, should dramatically disturb the balance in glucose catabolite repression and/or morphological development. Further studies are required to examine this speculation.

We showed that CebR binds to CebR boxes in *S. griseus* to repress the transcription of its target genes. In addition to the cellobiose/cellotriose uptake ABC-transporter genes *cebEFG-bglC* and *cebR* itself, at least five genes encoding secreted proteins (two cellulases, a cellulose-binding protein, a pectate lyase, and a protein with unknown function) were identified as target genes of CebR. The binding of CebR to the short intergenic region between *cebG* and *bglC* (Table 1) may inhibit elongation of transcription from *cebEFG* to *bglC*, which would enable strict regulation of the *cebEFG-bglC* operon by CebR. In contrast, we cannot exclude the possibility that *bglC* additionally has its own promoter and the promoter is repressed by the binding of CebR to the intergenic region between *cebG* and *bglC*. Because only two cellulases, both of which have been shown to be regulated by CebR in this study, are annotated in the *S. griseus* genome and because CebEFG, which seems to be the only transporter for cellobiose (cellooligosaccharide), is also regulated by CebR, we conclude that CebR is a master regulator of cellulose/cellooligosaccharide catabolism in *S. griseus*.

Despite the high frequency of CebR boxes in the *T. fusca* genome (e.g., in the upstream regions of six cellulase-encoding genes), Lykidis et al. (23) reported that CelR appears not to be a global regulator in *T. fusca*; *celR* transcription was diminished when *T. fusca* was grown on glucose- or xylan-containing medium. In comparing the transcriptional control of *cebR* in *S. griseus* with that of *celR* in *T. fusca*, it is noteworthy that *cebR* is transcribed constitutively from $cebRp_2$ and in a cellobiose-dependent manner from $cebRp_1$ in *S. griseus*. The constitutive transcription of *cebR* from $cebRp_2$ is important for the repression of transcription of the target genes in the absence of cellulose/cellooligosaccharide. On the other hand, the cellobiose-dependent transcription from $cebRp_1$ may contribute to strict control of CebR at the intracellular level, allowing a quick and adequate response to extracellular cellulose/cellooligosaccharide. A similar regulation (self-repression) of sugar utilization by a LacI/GalR family repressor has been reported for GlyR3, a regulator of cellulose/hemicellulose catabolism in *Clostridium thermocellum* (28).

We found that the DNA-binding activity of CebR was impaired by all cellooligosaccharides tested (cellobiose, cellotriose, cellotetraose, cellopentaose, and cellohexaose). This result was unexpected, because relatively strict ligand specificities have been reported for two CebR homologs. The DNA-binding activity of CelR of *T. fusca* is impaired by cellobiose (apparent K_d, 0.5 mM), but only minimally by cellotriose, sophorose, or xylobiose at 50 mM and not at all by other tested mono- or disaccharides at up to 100 mM (40). The DNA-binding activity of CebR of *S. reticuli* is abolished by cellopentaose (apparent K_d, 0.5 mM), only slightly impaired by cellobiose at 10 mM, and not at all affected by glucose, cellotriose, or cellotetraose at up to 10 mM (38). As an effector, *S. griseus* CebR accepts neither lactose, which differs from cellobiose only in the stereochemistry at the C-4 position of the nonreducing end of the disaccharide, nor maltose, an α-1,4-linked glucose dimer. These results suggest that *S. griseus* CebR recognizes only the terminal cellobiose structure of cellooligosaccharides, rather than their entire structures.

The morphological development of *Streptomyces* spp. involves several DNA-binding proteins belonging to large families of bacterial regulatory proteins. A representative example is WhiH, a regulatory protein of the GntR family in *S. coelicolor* A3(2). WhiH is specifically needed for the orderly multiple-sporulation septation of aerial hyphae (34). Many DNA-binding proteins have recently been identified as regulators of morphological development in *S. coelicolor* A3(2): a TetR family regulator (XdhR) (12), an AsnC family regulator (BkdR) (42), two IclR family regulators (SamR [45] and SsgR [46]), and three GntR family regulators (DevA [15], DasR [32], and Agl3R [11]). In particular, DasR and Agl3R are regulators of carbon source utilization. In this study, we have identified the first LacI/GalR family regulator involved in morphological development in *Streptomyces*. As described by Weickert and Adhya (50), proteins belonging to the LacI/GalR family appear to be regulators of primary metabolism, including the utilization of sugars and nucleotides. Further analyses of the integral relationships between morphological development, nutrient utilization regulation, and primary metabolism may reveal the nutrient signals required for morphological development in *Streptomyces*.

ACKNOWLEDGMENTS

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