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Genome Sequence of the Streptomycin-Producing Microorganism *Streptomyces griseus* IFO 13350▽†

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We determined the complete genome sequence of *Streptomyces griseus* IFO 13350, a soil bacterium producing an antituberculosis agent, streptomycin, which is the first aminoglycoside antibiotic, discovered more than 60 years ago. The linear chromosome consists of 8,545,929 base pairs (bp), with an average G+C content of 72.2%, predicting 7,138 open reading frames, six rRNA operons (16S-23S-5S), and 66 tRNA genes. It contains extremely long terminal inverted repeats (TIRs) of 132,910 bp each. The telomere's nucleotide sequence and secondary structure, consisting of several palindromes with a loop sequence of 5′-GGA-3′, are different from those of typical telomeres conserved among other *Streptomyces* species. In accordance with the difference, the chromosome has pseudogenes for a conserved terminal protein (Tpg) and a telomere-associated protein (Tap), and a novel pair of Tpg and Tap proteins is instead encoded by the TIRs. Comparisons with the genomes of two related species, *Streptomyces coelicolor* A3(2) and *Streptomyces avermitilis*, clarified not only the characteristics of the *S. griseus* genome but also the existence of 24 *Streptomyces*-specific proteins. The *S. griseus* genome contains 34 gene clusters or genes for the biosynthesis of known or unknown secondary metabolites. Transcriptome analysis using a DNA microarray showed that at least four of these clusters, in addition to the streptomycin biosynthesis gene cluster, were activated directly or indirectly by AdpA, which is a central transcriptional activator for secondary metabolism and morphogenesis in the A-factor (a γ-butyrolactone signaling molecule) regulatory cascade in *S. griseus*.

The gram-positive, soil-inhabiting, filamentous bacterial genus *Streptomyces* is characterized by its ability to produce a wide variety of secondary metabolites, such as antibiotics, parasiticides, herbicides, and pharmacologically active substances, including antitumor agents and immunosuppressants. Another characteristic feature of the genus is its complex multicellular development. Spores germinate to form a branched, multinucleoid substrate mycelium, which then produces an aerial mycelium. After septa have been formed at regular intervals along the aerial hyphae, long chains of uninucleoid spores are formed. Because of its complex morphogenesis and industrial and medical importance, *Streptomyces* has become a model prokaryote for the study of multicellular differentiation and secondary metabolism. The complete genomic sequences of two *Streptomyces* species, a model strain, *Streptomyces coelicolor* A3(2) (3), and an industrial strain, *Streptomyces avermitilis* (20, 36), have been published.

Unlike most other eubacterial chromosomes, the chromosome of *Streptomyces* is linear and contains a centrally located origin of replication (*oriC*) and unique terminal inverted repeats (TIRs) with terminal proteins (Tpgs) covalently bound to the 5′ ends. Replication proceeds bidirectionally from *oriC*, and a terminal single-stranded gap on the discontinuous lagging strand is filled in by DNA synthesis primed by Tpg (1). A telomere-associated protein (Tap) which binds specifically to the terminal single-stranded DNA is also involved in the replication of telomeres (2). Tpg and Tap are conserved among several *Streptomyces* species; accordingly, the terminal sequences of linear chromosomes and some linear plasmids of *Streptomyces* show extensive homology (19).

Streptomycin, the first aminoglycoside antibiotic, was discovered in S. A. Waksman's laboratory more than 60 years ago (41). This antibiotic, which has saved many people from tuberculosis, is produced industrially by *Streptomyces griseus*. *S. griseus* also provided arguably the first well-studied bacterial example of extracellular signaling by a diffusible low-molecular-weight compound. In *Streptomyces*, chemical-signaling molecules that have a γ-butyrolactone are commonly used as the switch for secondary metabolite production and/or morphological development. A-factor (2-isocapryloyl-3*R*-hydroxymethyl-γ-butyrolactone) of *S. griseus*, originally discovered by Khokhlov et al. (25) 40 years ago, is one such γ-butyrolactone (16, 17). When A-factor produced in a growth-dependent manner reaches a critical concentration as low as 10^{-9} M, it binds an A-factor-specific receptor (ArpA) that has bound to the promoter of *adpA* and dissociates ArpA from the promoter (34). The transcriptional activator AdpA then activates

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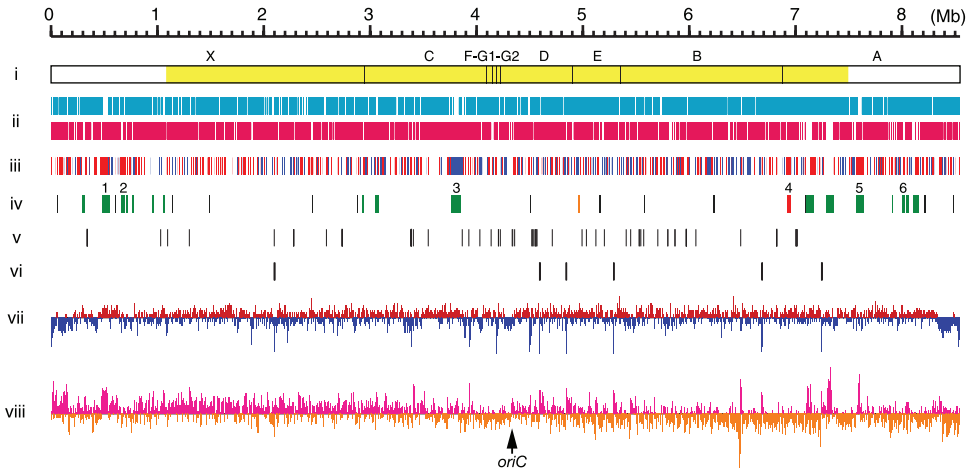


FIG. 1. Schematic representation of the *S. griseus* chromosome. (i) DraI physical map. Fragments observed after pulsed-field gel electrophoresis are indicated above the bar. The largest fragment, X, was not detected by Southern blotting using linkage clones as probes. The yellow color indicates the "core region." (ii) Distribution of ORFs according to direction of transcription (positive strand, upper line; negative strand, lower line). (iii) Distribution of genes that were affected by *adpA* disruption shown by DNA microarray analysis ($P < 0.05$, $n = 4$). Red, >2-fold upregulated in the wt strain in comparison with the Δ*adpA* mutant; blue, >2-fold downregulated in comparison with the Δ*adpA* mutant. (iv) Distribution of secondary metabolite gene clusters. Red, streptomycin (*str*, *sts*); orange, grixazone (*gri*); green, PKS and/or NRPS; black, other. Six secondary metabolite gene clusters, the transcription of which is affected by *adpA* disruption, are indicated as numerals above the line. 1, SGR443 to SGR455 (NRPS); 2, SGR574 to SGR593 (NRPS); 3, SGR3239 to SGR3288 (type II PKS and NRPS); 4, SGR5914 to SGR5940 (streptomycin); 5, SGR6360 to SGR6387 (type I PKS); 6, SGR6709 to SGR6717 (NRPS). (v) Distribution of tRNA genes. (vi) Distribution of rRNA operons. (vii) G+C content percentage variation for nonoverlapping 5-kb window. Red and blue are above and below the mean, respectively. (viii) GC-skew for 3-kb window and 1-kb step. Deep pink and dark orange are above and below zero, respectively. The putative *oriC* gene is indicated by an arrow.

the transcription of a number of genes that are required for secondary metabolism and morphological differentiation, forming an AdpA regulon (33). *strR*, which encodes the pathway-specific transcriptional activator for streptomycin biosynthesis, is one of the targets of AdpA, which explains how A-factor induces streptomycin production (34).

Here, we present the complete nucleotide sequence of the *S. griseus* IFO 13350 genome and compare it with the two other sequenced *Streptomyces* genomes. The *S. griseus* genome sequence has revealed a novel pair of telomeric proteins, which makes us speculate that *S. griseus* acquired the unique telomere from a linear plasmid during evolution. In addition, comparison of the genome sequences of three *Streptomyces* species has revealed a core genome sequence of *Streptomyces*, as well as genes specific to *S. griseus*, including gene clusters for secondary metabolite biosynthesis. We also describe the results of the *S. griseus* transcriptome analysis using a DNA microarray to show genome-wide transcriptional regulation by A-factor.

MATERIALS AND METHODS

Strain. The *S. griseus* strain IFO 13350 was originally transferred from the IMRU (Institute of Microbiology, Rutgers, The State University of New Jersey) as *Streptomyces bikiniensis* IMRU 3514 to the IFO (Institute for Fermentation, Culture Collection of Microorganisms, Osaka, Japan), via the National Institute of Health (Tokyo, Japan); however, it was later shown not to be *S. bikiniensis* IMRU 3514 but rather *S. griseus* (Krainsky) Waksman and Henrici 1948 (38). To avoid confusion, the genome-sequenced strain has been deposited as *S. griseus* strain NBRC 102592 in the Biological Resource Center of the National Institute of Technology and Evaluation (NITE; Chiba, Japan).

Genome sequencing and assembly. The nucleotide sequence of the *S. griseus* IFO 13350 genome was determined using a whole-genome shotgun strategy. We constructed small-insert (2-kb) and large-insert (10-kb) genomic libraries and generated approximately 110,000 sequences, giving 9.5-fold coverage, from both ends of each of the genomic clones. The sequence data were assembled using the Phred/Phrap/Consed package and in-house scripts. Sequence gaps were closed by transcriptional sequencing (Nippon GeneTech) or by primer walking. The left and right TIR sequences were determined to be a single sequence.

The telomere sequence was determined as follows. Adenine-homopolymer [poly(A)] tails were attached to the naked 3′ ends of the linear chromosome by incubating the purified chromosomal DNA (1 μg) with 15 units of terminal deoxynucleotidyl transferase (Invitrogen) at 37°C for 15 min, according to the manufacturer's instructions. After being heated at 60°C for 10 min, with the reaction mixture as a template, the terminal sequence was amplified by PCR using primers 5′-ACGAGCACAACGCTGATC-3′ (430R; positions 430 to 413) and 5′-GTAAAACGACGGCCAGTATAGGGTTTTTTTTTTT-3′, followed by nested PCR using primers 5′-GTAAAACGACGGCCAG-3′ (M13) and 5′-AAATGATGGATTCTCATATCCG-3′ (182R; positions 182 to 161). The amplified fragment was cloned into pCR4Blunt-TOPO (Invitrogen) and sequenced. Similarly, thymine-homopolymer [poly(T)] was attached to the 3′ ends of the chromosomal DNA using dTTP instead of dATP. The terminal sequence was amplified by PCR using primers 430R and 5′-GTAAAACGACGGCCAGTAT AGGGAAAAAAAAAAAA-3′, followed by nested PCR using primers M13 and 182R, and sequenced.

According to the method described by Ômura et al. (36), we also cloned every DraI linking clone and constructed a DraI physical map of the chromosome to evaluate the validity of the final sequence assembly (Fig. 1, line i). Finally, the entire sequence was estimated to have an error rate of <1 per 10,000 bases (Phrap score, ≥40).

Genome annotation and analysis. Putative protein-coding sequences were predicted using the GLIMMER program trained with all annotated open reading frames (ORFs) of *S. coelicolor* A3(2) and *S. avermitilis* and then manually examined using the BLASTP program and the FRAME PLOT program optimized to handle genomic-size sequences. The tRNA and transfer-messenger RNA genes were predicted using the tRNAscan-SE and ARAGORN programs, respectively. Proteins were clustered using the BLASTCLUST program.

Microarray preparation. An array of 13,042 specific oligonucleotides (45-mer or 35-mer; melting temperature, 72 ± 5°C) was designed based on the *S. griseus* genome sequence. Sequences from the 7,138 genes that encoded putative ORFs were used in the design. The oligonucleotides were designed and synthesized by Sigma-Genosys (Sigma-Aldrich, Japan) and printed onto a glass slide produced by KakenGeneqs (Chiba, Japan) according to the manufacturer's protocol. As a positive control, all of the oligonucleotides were mixed and printed at the corners of each subgrid.

DNA microarray analysis. *S. griseus* wild-type (wt) and *adpA* deletion (Δ*adpA*) (34) strains were precultured at 30°C for 2 days in 100 ml of YMPD medium containing 0.5% glycine (in a 500-ml shaking [Sakaguchi] flask) with reciprocal shaking (120 rpm). YMPD is a nutrient-rich medium (pH 7.2) consisting of 0.2% yeast extract, 0.22% meat extract, 0.4% Bacto peptone, 0.5% NaCl, 0.2% $MgSO_4 \cdot 7H_2O$, and 1% glucose. Two milliliters of each culture was inoculated into 100 ml of YMPD medium (in a 500-ml shaking [Sakaguchi] flask). The culture was incubated at 30°C with reciprocal shaking (120 rpm) until the cells entered the early stationary phase (wt, 30 h; Δ*adpA* mutant, 19 h). Note that the difference in the cultivation times resulted from the following two reasons. (i) The growth lag time of the wt strain was approximately 3 h longer than that of the Δ*adpA* mutant, probably due to the difference in the physiological conditions of the inoculated cells; some of the wt cells produced submerged spores in the preculture, but the Δ*adpA* cells did not. (ii) The maximum cell mass of the Δ*adpA* mutant in the culture was reproducibly smaller than that of the wt strain, resulting in an apparently earlier entry into the stationary phase. The difference in maximum cell mass between the wt and an A-factor-deficient mutant (strain HH1) has long been observed (our unpublished observation), although the reason is not yet known. The cells were harvested by centrifugation. To preserve RNA, RNA*later* (Ambion) was added to the cells, and the mixture was incubated at 4°C for 12 h according to the manufacturer's instructions. The cells were collected by centrifugation and stored at −80°C. Total RNA was isolated from the cells using the RNAqueous mini kit (Ambion) according to the manufacturer's instructions. The RNA was quantified by measuring the absorbance at 260 nm. The cDNA probes were indirectly labeled using reverse transcription in the presence of amino allyl dUTP (GE Healthcare). Six micrograms of total RNA was mixed with 0.75 μg of random hexamers (Invitrogen) and 0.75 μg of high-GC-content (70%) random hexamers and made up to 13.3 μl with diethyl pyrocarbonate-treated water. The RNA was denatured at 70°C for 10 min and cooled on ice for 5 min. The following were then added to the RNA sample: 3 mM (final concentration) (each) dATP, dCTP, and dGTP; 1.2 mM dTTP; 1.8 mM amino allyl dUTP (Ambion); 0.01 mM dithiothreitol; 40 U cloned RNase inhibitor (Takara); 6 μl of 5× reverse transcriptase (RT) buffer; and 380 U Superscript II RT (Invitrogen). After the mixture had been incubated at 42°C for 2 h for cDNA synthesis, the RNA template was hydrolyzed by adding 10 μl of 1.0 M NaOH and 10 μl of 0.5 M EDTA and incubated at 65°C for 30 min to degrade the RNA. The sample was then neutralized with 25 μl of 1 M HEPES (pH 7.5), purified using a Microcon YM30 column (Amicon), and dried in a vacuum. Either Cy3 or Cy5 dye was coupled to the amino allyl dUTP in the cDNA in the presence of 4.5 μl of 0.1 M sodium bicarbonate (pH 9.0) for 1 h. The labeled probe was purified using the QIAquick PCR purification system (Qiagen) and concentrated using a Microcon YM30 column.

Microarray hybridization was carried out at 42°C for 18 h by mixing using 120 μl per slide of SlideHyb no. 1 hybridization solution (Ambion) in an automated hybridization machine, GeneTac HybStation (Genomic Solutions). The posthybridization washing consisted of three cycles of 20-s incubations with each of the following solutions: 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) plus 0.1% sodium dodecyl sulfate (medium stringency) at 42°C, 0.1× SSC plus 0.05% sodium dodecyl sulfate (high stringency) at 25°C, and 0.1× SSC (low stringency) at 25°C. The slides were washed, dried by centrifugation at 350 × *g* for 5 min at room temperature, and scanned using a model 428 array scanner (Affymetrix). The spot intensities were quantified using Imagene 6.1 (BioDiscovery). RNA samples isolated from four independent cultures of each strain were used for cDNA synthesis. Two cDNA samples of each strain were labeled with Cy5, and the other two were labeled with Cy3. We performed duplicate competitive hybridization experiments using Cy3-labeled wt cDNA plus Cy5-labeled Δ*adpA* mutant cDNA or Cy5-labeled wt cDNA plus Cy3-labeled Δ*adpA* mutant cDNA. Equal amounts of Cy3- and Cy5-labeled cDNA were used in all hybridizations. The expression ratios (wt strain to Δ*adpA* mutant) were normalized by the LIMMA (linear model for microarray analysis) loess (subgrid) method using ArrayPipe 2.0. Normalized expression ratios were calculated for each gene and tested for significance (expression ratio of either >2.0 or <0.5 and a P of <0.05). We designed two or more specific oligonucleotides for each of the 5,643 ORFs and one oligonucleotide for each of the 1,495 ORFs of small size. When an ORF had two or more oligonucleotides, distinctive change values were obtained for the ORF due to the difference in signal intensity caused by the distinct hybridization efficiencies of the oligonucleotides. Among them, the change value with the lowest P value in a statistical analysis (t test) was employed as the most reliable one.

Quantitative RT-PCR. For validation of the microarray data, transcripts from 17 genes that encoded known or putative regulatory proteins were quantified using RT-PCR. Primers for each gene (Table 1) were designed for use with the Sybr green method. As an internal standard, we used *hrdB*(SGR1701), which encodes a principal sigma factor of RNA polymerase, because this gene is presumably transcribed similarly throughout growth in the wt and Δ*adpA* strains. The cDNA was synthesized using the ThermoScript RT-PCR system with random hexamers according to the manufacturer's instructions (Invitrogen). All reactions were performed in the Takara Sybr Premix Ex Taq II (Takara) reaction mixture using a SmartCycler II real-time PCR system (Cepheid) under the following conditions: 10 s at 95°C, followed by 50 cycles of 5 s at 95°C for denaturing and 30 s at 60°C for annealing and extension. All reactions were performed in triplicate, and the data were normalized using the average for the internal standard.

TABLE 1. Primers used for quantitative RT-PCR

Primer	Positions[a]	Sequence (5′ to 3′)
SGR1701 *hrdB* F	1,182 to 1,203	GAAGGTCATCGAGGTCCAGAAG
SGR1701 *hrdB* R	1,506 to 1,488	GTGGCGGAGCTTCGACATC
SGR2064 F	195 to 213	CGGCATGATCCACCAACTG
SGR2064 R	669 to 649	TTCGGAGTACGAGGTCTTCTG
SGR2271 *sigH* F	1 to 17	GTGAGCGACGGGAACGG
SGR2271 *sigH* R	303 to 285	CATCCGCACCAGTCTGTTG
SGR2274 F	83 to 103	TGCAGATCGAGGAAGCCAAGG
SGR2274 R	257 to 241	CAGGCGCTTGCGTTGCG
SGR2378 F	256 to 276	CGCATCACGACCAATCTCTTC
SGR2378 R	575 to 558	CGGTGCTTGAGCGCCTTG
SGR2760 F	4 to 23	GCAGATTTCTCCCGCCTTCC
SGR2760 R	275 to 259	CTGGCCCGGCCCATCAG
SGR3271 F	50 to 69	GTATGGATCAGGCGGCGAAG
SGR3271 R	203 to 186	GCGGACGGCAGGATCATG
SGR3340 F	21 to 39	GAGCCTCGATTCGGGAGAG
SGR3340 R	194 to 178	GCATCGGCCAGGCACTC
SGR3555 F	106 to 122	TCCGCCGCCGTACTGAC
SGR3555 R	470 to 454	CTGCACTCGCCGCTGTG
SGR3905 *atrA* F	259 to 275	CTGGTGCGCCGGATAGC
SGR3905 *atrA* R	625 to 607	CCACGACCTCCAACAGCTC
SGR4240 *griR* F	215 to 233	GCCTCCGAAGACTCCTCTC
SGR4240 *griR* R	599 to 583	AGGCACAGCGACTCCTG
SGR4276 *hrdD* F	675 to 693	CCCGGTCAGCCTGAACATG
SGR4276 *hrdD* R	982 to 964	CCGTGTCGTGAGCCATTCG
SGR5049 F	685 to 702	CAGCCGTCCTTCTTCTGC
SGR5049 R	993 to 976	GGTCAGTGTGTGCAGGAC
SGR5931 *strR* F	86 to 106	AATTATCCGCCGTGACAATGG
SGR5931 *strR* R	422 to 404	GGATGGGTCTCCAGGACAC
SGR6369 F	473 to 489	GCGTGGAGGCGGAGTTC
SGR6369 R	942 to 924	CACCACCCAGCGGATCTTG
SGR6380 F	170 to 188	TCGCCTGCCTCGACAAGTC
SGR6380 R	469 to 453	GTGCCGAGCGCCTCCTG
SGR6385 F	345 to 365	CCAATGGGAGCTGAACGAAGG
SGR6385 R	680 to 659	ATGCCGTAGAGGTTCTGGTAGG

[a] Nucleotide positions are relative to the first nucleotide of the start codon, which is 1.

Accession numbers. The nucleotide sequence has been deposited in the DDBJ DNA database under accession no. AP009493. The fully annotated genomic sequence is also available at http://park.itc.u-tokyo.ac.jp/hakko/genome. Details of the microarray design, transcriptome experimental design, and transcriptome data have been deposited in the NCBI Gene Expression Omnibus under accession no. GSE9882.

RESULTS AND DISCUSSION

General features of the *S. griseus* genome. The complete genome sequence indicated a single linear chromosome composed of 8,545,929 bp with no plasmids. The main features of the *S. griseus* IFO 13350 chromosome are summarized in Table 2 and Fig. 1. Table 2 also includes those of the chromosomes of *S. coelicolor* A3(2) and *S. avermitilis* for comparison. The *S. griseus* chromosome contains at least 7,138 ORFs (Fig. 1, line ii). A total of 4,464 ORFs (62.6%) were assigned to known or putative functions, and 2,674 ORFs (37.4%) were annotated as genes for hypothetical proteins. The chromosome also contains six rRNA operons (16S-23S-5S) (Fig. 1, line vi) and 66 tRNA genes (42 species) (Fig. 1, line v). The average G+C content of

TABLE 2. General features of the chromosomes of three *Streptomyces* species[c]

Species	Length (bp)	TIR (bp)	Avg G+C content (%)	No. of protein-coding genes	Avg CDS length (bp)	Coding density (%)	No. of rRNA (16S-23S-5S) operons	No. of tRNA genes	No. of tmRNAs	No. of 4.5S RNAs
S. griseus	8,545,929	132,910	72.2	7,138	1,055	88.1	6	66	1	1
S. coelicolor A3(2)[a]	8,667,507	21,653	72.1	7,825	991	88.9	6	63	1	1
S. avermitilis[b]	9,025,608	49	70.7	7,583	1,027	86.3	6	68	1	1

[a] Data reported by Bentley et al. (3).
[b] Up-to-date annotation data are shown.
[c] tmRNAs, transfer-messenger RNAs; CDS, protein coding sequence. The topology of the chromosome each species is linear.

the chromosome is 72.2%, but the approximately 300-kb regions (including the 133-kb TIR sequence) at both ends show a remarkably lower G+C content (Fig. 1, line vii). The replication origin, *oriC* (positions 4,324,631 to 4,325,203), which contains 19 DnaA box-like sequences (22), was predicted to be located in the middle of the chromosome (52 kb away from the center toward the right end). A GC-skewed inversion observed between the left and right arms of the chromosome supports this prediction (Fig. 1, line viii).

Telomere structure of the *S. griseus* chromosome. Since we could not clone the terminal fragments carrying covalently bound Tpgs by the glass bead binding method (19), we determined the telomere sequence of the *S. griseus* chromosome by a newly devised method. Briefly, either poly(A) or poly(T) was attached to the naked 3′ end of the chromosome using terminal deoxynucleotidyl transferase, and the terminal DNA fragment was amplified by PCR and then sequenced. Only one guanine was found between the poly(A) [or poly(T)] sequence and the tentative *S. griseus* chromosomal end that had been determined using whole-genome shotgun sequencing. Thus, we concluded that the additional guanine was the true 3′ end. We also confirmed that specific amplification of the terminal sequence of the *S. coelicolor* A3(2) chromosome was achieved by this method (data not shown). Our new method for the determination of the telomere sequence of a given linear chromosome is very convenient once the sequence near the chromosomal end is available.

The telomere sequence of the *S. griseus* IFO 13350 chromosome shows no similarity to the typical telomere sequences conserved among many linear chromosomes and some linear plasmids of *Streptomyces* (Fig. 2A). The telomere has several palindromes whose loop sequences are 5′-GGA-3′ (Fig. 2B), whereas typical *Streptomyces* telomeres carry 5′-GCA-3′ loops in their palindromes (19). The positions of the palindromes also do not correspond to those of palindromes in other *Streptomyces* telomeres. *S. griseus* seems not to produce any conserved Tpg or Tap because the chromosome has pseudogenes for them (SGR6986 and SGR6987, respectively). These pseudogenes have apparently undergone rearrangements and mutations. SGR6986, which once encoded a Tpg protein conserved in typical *Streptomyces* telomeres, is disrupted by a 73-bp deletion. SGR6987, with its upstream region, was assumed to be a ruin of the conserved *tac* gene; several mutations in both the 5′ and the 3′ portions of SGR6987, including deletions and frameshift mutations, are found. Recently, a novel pair of Tpg and Tap proteins for the *S. coelicolor* A3(2) linear plasmid SCP1, the telomere of which is also unique, was identified to be encoded by the plasmid (18). The diversity of telomere palindromic sequences among *Streptomyces* linear plasmids was also shown (46). Therefore, we assumed that *S. griseus* acquired the unique telomere together with a novel pair of Tpg and Tap proteins from a certain linear plasmid during its evolution and that the original *tpg* and *tap* genes became unnecessary and decayed.

We expected that a novel pair of Tpg and Tap proteins must be encoded as an operon near the chromosomal ends. In fact, we found two copies of candidates for such an operon in both TIRs (SGR98t to SGR97t and SGR7041t to SGR7042t). We found that SGR97t (SGR7042t), a 185-amino-acid (aa) protein showing very low similarity (18% identity) to conserved Tpgs, was attached to the chromosome ends and that SGR98t (SGR7041t), an 837-aa protein with a C-terminal DnaB-like-helicase domain, was able to bind specifically to the single-stranded DNA of the chromosomal end (H. Suzuki, K. Marushima, Y. Ohnishi, and S. Horinouchi, submitted for publication). We therefore propose that SGR97t (SGR7042t) and SGR98t (SGR7041t) code for functional Tpg and Tap, respectively, in *S. griseus*.

The telomere of *S. griseus* IFO 13350 was also shown to be different from the unique telomere of *S. griseus* 2247 (14). The length of the TIRs of *S. griseus* 2247 (24 kb) is much shorter than that of the TIRs of *S. griseus* IFO 13350 (133 kb). We also found that the DraI physical map of *S. griseus* IFO 13350 (Fig. 1, line i) was somewhat different from that of *S. griseus* 2247 (27), implying the heterogeneity of species *S. griseus*.

Comparative analysis of the *S. griseus* genome against the genomes of *S. coelicolor* A3(2) and *S. avermitilis*. We compared all annotated proteins on the chromosomes of three *Streptomyces* species, namely, *S. griseus*, *S. coelicolor* A3(2), and *S. avermitilis* (SGR, SCO, and SAV, respectively, are used in their locus tags). Ortholog (reciprocal best-hit pair) plots using pairwise BLASTP searches clearly demonstrated the existence of a highly conserved internal core region of each chromosome, and several large inversions centered at *oriC* were found (Fig. 3). Comparison of the results from the three chromosomes predicted the core regions to be SGR923 to SGR6311 (6.39 Mb) (Fig. 1, line i), SCO6804 to SCO1209 (6.28 Mb), and SAV1625 to SAV7128 (6.50 Mb). The subtelomeric regions were less conserved among these three chromosomes with regard to sequence and ortholog distribution. Variability in the subtelomeric regions was also found in the *Streptomyces ambofaciens* chromosome (7). Thus, a conserved internal core region of approximately 6.4 Mb, with a couple of variable subtelomeric regions of 1 to 2 Mb, seems to be a common feature of *Streptomyces* linear chromosomes.

We clustered *S. griseus*, *S. coelicolor* A3(2), and *S. avermitilis*

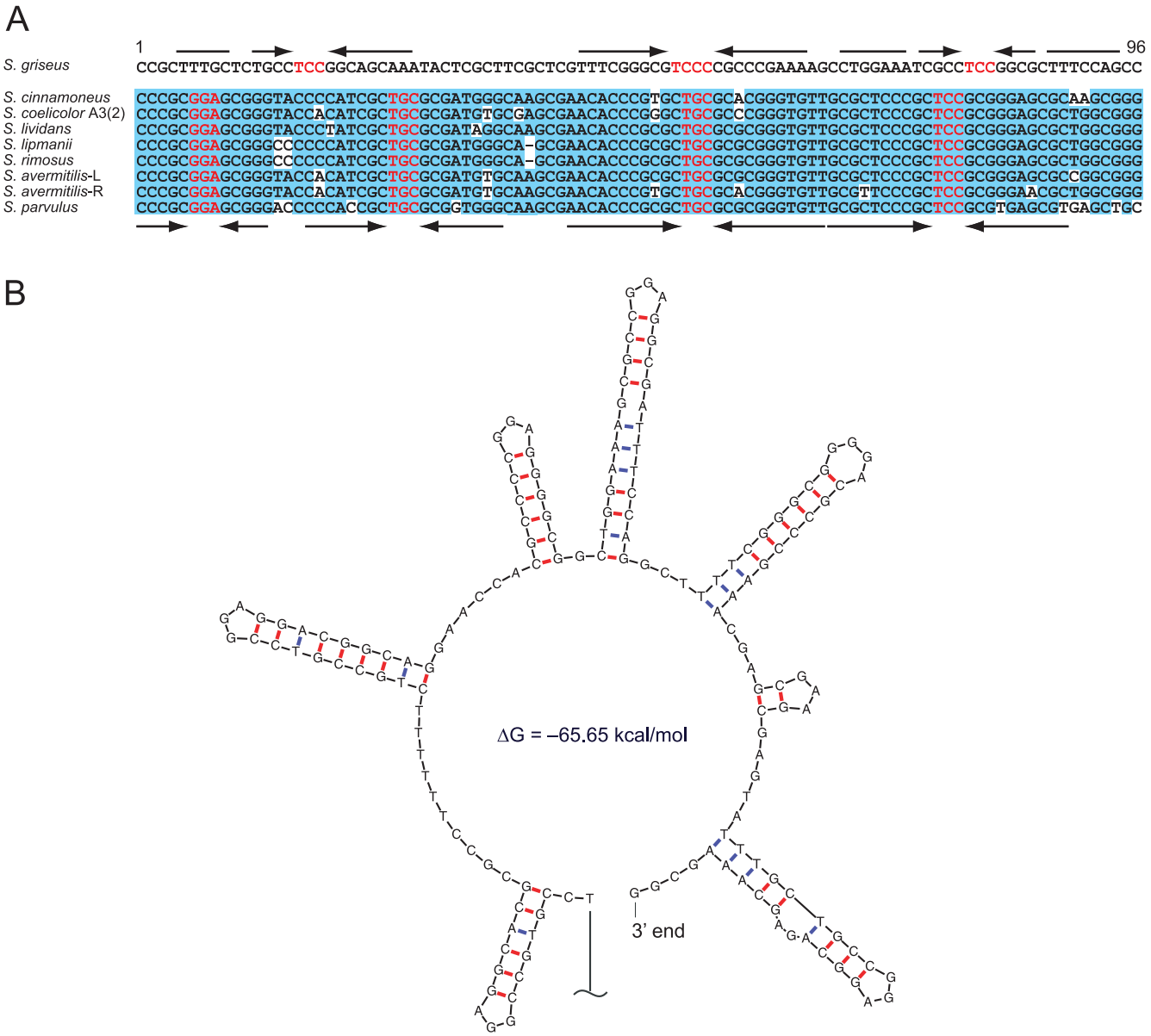
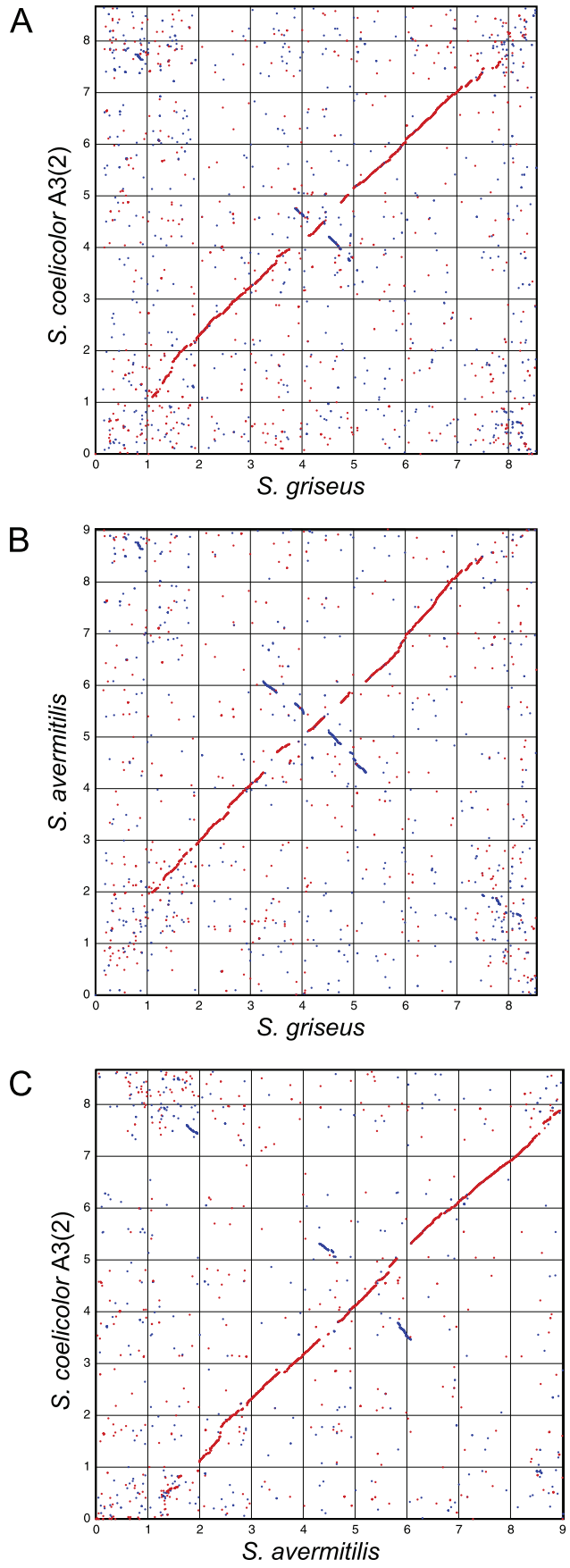


FIG. 2. Telomere of *S. griseus*. (A) Comparisons of the *S. griseus* telomere sequence with the typical telomere sequences of seven other *Streptomyces* species. Conserved nucleotides among eight telomeres of the seven *Streptomyces* species are highlighted in light blue. Palindromes are indicated by converging arrows. Nucleotides in the possible loop of the palindromes are colored red. L and R, left and right TIRs, respectively. (B) Secondary structure of the 3′-terminal 164 nucleotides of the *S. griseus* chromosome predicted by the Mfold program.

proteins using the BLASTCLUST program with a threshold of 60% identity plus 70% length coverage (Table 3). Despite these strict conditions, 3,204 *S. griseus* proteins (45% of the total proteins), 3,280 *S. coelicolor* A3(2) proteins (42%), and 3,240 *S. avermitilis* proteins (43%) were classified into 3,039 clusters that are commonly present among these three species. Because these three species are taxonomically distant from one another within the genus *Streptomyces* (see Fig. S1 in the supplemental material), it is probable that most of these proteins are also common in any other *Streptomyces* species. Thus, 45% of the proteins encoded on the *S. griseus* chromosome are likely to be "common *Streptomyces* proteins" that have essential or important functions in the biology of *Streptomyces*. We then searched for *Streptomyces*-specific proteins among the 3,204 *S. griseus* proteins. These proteins were compared with the proteins of nine other actinobacteria, i.e., *Nocardia farcinica* IFM 10152 (21), *Mycobacterium tuberculosis* H37Rv (8), *Corynebacterium glutamicum* ATCC 13032 (24), *Rhodococcus jostii* RHA1 (30), *Propionibacterium acnes* KPA 171202 (5), *Frankia alni* ACN14a (32), *Thermobifida fusca* YX (28), *Saccharopolyspora erythraea* NRRL23338 (35), and *Salinispora tropica* CNB-440 (39), using the BLASTCLUST program with a threshold of 30% identity plus 50% length coverage. In total, 240 *S. griseus* proteins were not clustered with any of the proteins in these nine actinobacteria. We carried out BLAST analyses of each of these 240 *S. griseus* proteins and selected 24



S. griseus proteins (≥60 aa) that were highly conserved among only the three *Streptomyces* species (≥75% identity); their significant homologs were never found in any other organisms (Table 4). The functions of all of these 24 *S. griseus* proteins and their *S. coelicolor* A3(2) and *S. avermitilis* homologs are not yet known. However, their high-level conservation and their unique distribution among the *Streptomyces* species suggest that these proteins have some important functions in the nature of the genus *Streptomyces*.

We also found 941 clusters that are present in both *S. coelicolor* A3(2) and *S. avermitilis* but absent in *S. griseus* by the BLASTCLUST program with a threshold of 60% identity plus 70% length coverage (Table 3). *S. griseus* lacks the *whiE* gene cluster (SCO5320 to SCO5214, SAV2836 to SAV2841) that is involved in the biosynthesis of an aromatic-polyketide spore pigment (10). Consistent with this, a type III polyketide synthetase (PKS), RppA (13), and a cytochrome P-450 monooxygenase, P-450 mel (12), are involved in the biosynthesis of a different spore pigment (hexahydroxyperylenequinone [HPQ] melanin) in *S. griseus*. Concerning developmental genes, the *bldK* genes (SCO5112 to SCO5116) (31), which encode a peptide transporter involved in morphological development in *S. coelicolor* A3(2), are also present in *S. avermitilis* (two copies, SAV3176 to SAV3172 and SAV3150 to SAV3154) but not in *S. griseus*. *S. griseus* contains at least six putative peptide transporters. The lack of the BldK transporter might be compensated for by one of these transporters in *S. griseus*. Alternatively, the BldK system involved in morphological development by means of a putative extracellular signaling peptide may not be used by *S. griseus*. Putative gene clusters for gas vesicle synthesis are duplicated in *S. coelicolor* A3(2) (SCO0649 to SCO0658 and SCO6499 to SCO6508) and triplicated in *S. avermitilis* (SAV599 to SAV591, SAV1890 to SAV1881, and SAV2356 to SAV2347) but are never found in *S. griseus*. Although the role of gas vesicle proteins in the complex biology of *Streptomyces* has been discussed (40, 42), these proteins do not seem to be essential in *Streptomyces*.

We also compared *S. griseus*, *S. coelicolor* A3(2), and *S. avermitilis* proteins using the BLASTP program with two different thresholds (E values, <0.1 and $<10^{-20}$) (Table 3). With the relatively strict conditions under which the E value was $<10^{-20}$, 1,420 *S. griseus*, 1,506 *S. coelicolor* A3(2), and 1,483 *S. avermitilis* proteins did not show sufficient similarity to any proteins of the other two strains. The number of strain-specific proteins greatly decreased under the relaxed condition where the E value was <0.1; 219 *S. griseus*, 234 *S. coelicolor* A3(2), and 153 *S. avermitilis* proteins are classified as strain-specific proteins. In *S. griseus*, these include biosynthesis proteins for some specific secondary metabolites, such as streptomycin (SGR5918, SGR5933, SGR 5935, and SGR5936), a putative enediyne compound (SGR604 and SGR606), and a putative lantibiotic compound (SGR4418), in addition to many hypothetical proteins. A putative restriction

FIG. 3. Ortholog plots for *S. griseus* versus *S. coelicolor* A3(2) (A), *S. griseus* versus *S. avermitilis* (B), and *S. avermitilis* versus *S. coelicolor* A3(2) (C). Dots represent reciprocal best matches (by BLAST comparison) between orthologs. Matches on the same strand are in red, and those on the opposite strand are in blue.

TABLE 3. Comparative analysis of ORFs encoded on the chromosomes of three *Streptomyces* species

Distribution class[a]	No. of proteins classified by[b]:									
	BLASTP with threshold of indicated E value						BLASTCLUST[c]			
	<0.1			<10^{-20}						
	SGR	SCO	SAV	SGR	SCO	SAV	Clusters[d]	SGR	SCO	SAV
SGR(+) SCO(+) SAV(+)	6,586	7,203	7,004	4,944	5,191	5,111	3,039	3,204	3,280	3,240
SGR(+) SCO(+) SAV(−)	180	190		455	454		469	487	493	
SGR(+) SCO(−) SAV(+)	153		173	319		304	302	310		307
SGR(−) SCO(+) SAV(+)		196	253		672	685	941		976	967
SGR(+) SCO(−) SAV(−)	219			1,420			2,998	3,137		
SGR(−) SCO(+) SAV(−)		234			1,506		2,973		3,074	
SGR(−) SCO(−) SAV(+)			153			1,483	2,992			3,069

[a] + and – indicate the presence and the absence of ORFs, respectively.
[b] A total of 7,138 *S. griseus*, 7,823 *S. coelicolor* A3(2), and 7,583 *S. avermitilis* proteins are classified.
[c] Proteins were clustered by the BLASTCLUST program with a threshold of 60% identity plus 70% length coverage.
[d] The number of clusters, not proteins, is shown.

enzyme (a SacI isoschizomer, SGR3359) was also found in these strain-specific proteins. Just upstream of the SacI isoschizomer gene, a putative cytosine-specific DNA methylase (SGR3358) is encoded. It is likely that the SGR3358 protein has SacI-methylase activity because DNA extracted from *S. griseus* IFO 13350 is resistant to SacI digestion. Interestingly, a transmissible-plasmid-like segment is integrated just upstream of SGR3358. *S. griseus* may acquire this restriction/modification system by virtue of the transmissible integrating plasmid.

Gene clusters for secondary metabolites. The biosynthetic gene cluster for streptomycin has been analyzed previously (11). We found that the cluster (*str* and *sts*, SGR5914 to SGR5940) is located 560 kb interior to the right end of the conserved core region. We previously analyzed three other gene clusters for secondary metabolites, specifically, grixazone (*gri*, SGR4238 to SGR4250) (15), a carotenoid (*crt*, SGR6824 to SGR6830) (26), and the HPQ melanin (P-450*mel-rppA*, SGR6619 to SGR6620) (12, 13). Genomic sequencing revealed a further 30 gene clusters or genes for putative secondary metabolites (Table 5). Of the total 34 clusters, 18 occur outside the conserved core region of the chromosome (Fig. 1, line iv), supporting the idea that the variable subtelomeric regions of linear chromosomes contribute to the metabolic diversity of *Streptomyces* (3, 20).

In addition to the *crt* cluster described above, another *crt* cluster (SGR54t to SGR60t or SGR7079t to SGR7085t) is found in the TIR sequence. Thus, *S. griseus* has three copies of *crt* clusters on the chromosome. However, *S. griseus* is unable to produce carotenoids under any conditions thus far examined (26; H. Ikeda, unpublished data), representing an example of

TABLE 4. Twenty-four *S. griseus* proteins selected based on BLAST analyses

S. griseus protein (aa)	*S. coelicolor* A3(2)		*S. avermitilis*		Other hit (non-*Streptomyces* protein)	
	Protein (aa)	E value	Protein (aa)	E value	Source (aa)	E value
SGR1707 (212)	SCO5814 (204)	1.1e−83	SAV2451 (211)	2.4e−86	*Roseiflexus castenholzii* (111)	8.9e−01
SGR2042 (244)	SCO5475 (265)	1.3e−111	SAV2770 (268)	4.7e−114	*Alcanivorax borkumensis* (982)	2.2e−02
SGR3064 (148)	SCO4402 (166)	1.1e−62	SAV3847 (166)	1.0e−68	*Aspergillus terreus* (419)	4.0e−01
SGR3164 (69)	SCO4335 (62)	2.5e−22	SAV3896 (60)	1.6e−21	*Pyrobaculum arsenaticum* (157)	2.0e+00
SGR3380 (216)	SCO3624 (221)	5.4e−102	SAV4548 (216)	2.6e−104	*Gloeobacter violaceus* (223)	7.5e−03
SGR3428 (106)	SCO3662 (111)	8.0e−45	SAV4512 (111)	1.5e−46	*Roseovarius nubinhibens* (350)	6.2e−05
SGR3657 (70)	SCO3924 (70)	1.3e−26	SAV4269 (70)	1.1e−27	*Saccharopolyspora erythraea* (492)	1.8e−01
SGR3786 (155)	SCO3799 (156)	1.1e−72	SAV4391 (154)	9.7e−72	*Chlamydomonas reinhardtii* (1216)	9.0e−01
SGR3920 (102)	SCO4131 (102)	6.0e−45	SAV4098 (102)	5.8e−48	*Pseudomonas aeruginosa* (667)	9.1e−01
SGR4040 (165)	SCO4269 (166)	2.5e−79	SAV3953 (166)	3.4e−81	*Aspergillus clavatus* (664)	3.9e+00
SGR4191 (145)	SCO4467 (145)	4.1e−78	SAV4793 (145)	4.6e−77	*Lactobacillus plantarum* (603)	2.0e+00
SGR4388 (325)	SCO3116 (335)	6.6e−158	SAV3557 (328)	1.4e−163	*Clostridium thermocellum* (566)	9.7e−08
SGR4514 (215)	SCO3022 (204)	1.1e−94	SAV5054 (204)	1.0e−100	*Nocardia farcinica* (699)	2.4e−01
SGR4720 (113)	SCO2820 (115)	2.8e−42	SAV5240 (121)	7.3e−43	*Stigmatella aurantiaca* (431)	9.0e−01
SGR4722 (539)	SCO2809 (547)	0.0e+00	SAV5242 (531)	0.0e+00	*Rhodobacter sphaeroides* (651)	3.3e−02
SGR4723 (335)	SCO2808 (342)	1.2e−130	SAV5243 (346)	5.0e−132	*Psychromonas ingrahamii* (920)	1.2e−05
SGR4972 (77)	SCO2574 (77)	1.7e−39	SAV5484 (77)	1.7e−39	*Aedes aegypti* (887)	2.7e+00
SGR5248 (60)	SCO2261 (85)	1.4e−14	SAV5936 (60)	5.2e−17	*Pseudomonas stutzeri* (97)	7.6e+00
SGR5318 (66)	SCO2195 (71)	7.0e−17	SAV6008 (65)	1.8e−20	*Salinispora tropica* (77)	1.6e+00
SGR5784 (87)	SCO1721 (89)	4.7e−29	SAV6584 (89)	1.7e−31	*Mus musculus* (3291)	1.6e+00
SGR6060 (187)	SCO1474 (183)	2.9e−73	SAV6876 (180)	7.8e−71	*Rhodococcus jostii* RHA1 (193)	4.0e−06
SGR6119 (472)	SCO1413 (472)	0.0e+00	SAV6933 (472)	0.0e+00	*Anaeromyxobacter dehalogenans* (690)	3.2e−03
SGR6277 (75)	SCO1249 (75)	4.3e−22	SAV7086 (79)	5.2e−28	*Prochlorococcus marinus* (227)	2.1e+00
SGR6836 (166)	SCO7249 (165)	2.0e−81	SAV1239 (165)	4.2e−79	*Aeromicrobium erythreum* (3574)	2.7e−01

TABLE 5. Secondary metabolite-biosynthetic gene clusters (or genes) of *S. griseus* IFO 13350

Gene cluster[a]	Product and/or type
Known	
SGR5914-SGR5940	Streptomycin (aminoglycoside)
SGR4238-SGR4250	Grixazone (phenoxazinone)
SGR6824-SGR6830	Isorenieratene (carotenoid)
SGR6619-SGR6620	HPQ melanin (by type III PKS)
SGR470-SGR472	Alkylresorcinol (by type III PKS)
SGR1268-SGR1269	2-Methylisoborneol (by terpene cyclase)
Predicted	
Terpene	
SGR54t-SGR60t	Carotenoid
SGR7085t-SGR7079t	Carotenoid
SGR962-SGR966	Hopanoid
SGR2079	Terpene
SGR6065	Terpene
SGR6839	Germacradienol/geosmin
PKS and NRPS	
SGR443-SGR455	NRPS for siderophore
SGR574-SGR593	NRPS
SGR653-SGR656	NRPS
SGR895-SGR901	NRPS
SGR2586-SGR2598	NRPS
SGR6709-SGR6717	NRPS for siderophore
SGR6730-SGR6742	NRPS for siderophore
SGR6071-SGR6083	Type I PKS
SGR6177-SGR6183	Type I PKS
SGR6360-SGR6387	Type I PKS
SGR604-SGR611	Enediyne PKS
SGR278-SGR283	PKS-NRPS hybrid
SGR810-SGR815	PKS-NRPS hybrid
SGR2482-SGR2489	Type I PKS, NRPS
SGR6776-SGR6786	Type I PKS, PKS-NRPS hybrid
SGR3239-SGR3288	Type II PKS, NRPS
Others	
SGR4747-SGR4751	Nocardamine (siderophore)
SGR527-SGR528	Melanin
SGR2446-SGR2447	Melanin
SGR3845-SGR3849	Lantibiotic
SGR4408-SGR4421	Lantibiotic
SGR5285-SGR5295	Unknown

[a] The range of clusters for unknown compounds was predicted on the basis of possible gene function, putative transcriptional units, synteny, and/or our transcriptome data.

gene clusters for secondary metabolism that are cryptic. SGR962, encoding a putative squalene-hopene cyclase, seems to be a member of the hopanoid biosynthesis gene cluster (*hopABCDE*, SGR962 to SGR966), which is also found in *S. coelicolor* A3(2) (37) and *S. avermitilis* (36). Four putative terpene cyclases containing a metal binding motif (SGR1269, SGR2079, SGR6065, and SGR6839) are found in the *S. griseus* genome. According to the homology with the germacradienol/geosmin synthases SCO6073 (23) and SAV2163 (6), SGR6839 seems to be involved in the biosynthesis of germacradienol/geosmin; germacradienol/geosmin production by *S. griseus* has already been confirmed (H. Ikeda, unpublished data). Recently, SGR1269 has been identified as a 2-methylisoborneol synthase (25a). SGR2079 and SGR6065 seem to be sesquiterpene cyclases (25a), but their products remain to be elucidated. *S. griseus* has no mevalonate pathway, which sometimes occurs in a flanking region of a biosynthesis gene cluster for terpenoids such as furaquinocin A and terpentecin (9). *S. griseus* has a type III PKS (SGR472) other than RppA. SGR472 has been demonstrated to be an alkylresorcinol synthase (13a). Like *S. coelicolor* A3(2) and *S. avermitilis*, *S. griseus* has putative melanin-biosynthetic gene clusters (*mel*, SGR527 to SGR528 and SGR2446 to SGR2447) and a gene cluster (SGR4747 to SGR4751) for nocardamine (desferrioxamine E). Nocardamine production by *S. griseus* has been confirmed (44).

Similar to other actinomycete strains, *S. griseus* has many gene clusters that contain a putative PKS, nonribosomal peptide synthetase (NRPS), and PKS-NRPS hybrid genes. An NRPS gene cluster (SGR6709 to SGR6717) is homologous with SCO499 to SCO491 of *S. coelicolor* A3(2). The gene organization of an enediyne gene cluster (SGR604 to SGR611) is similar to that of many enediyne gene clusters found in several actinomycete strains (45). The other 14 PKS and/or NRPS gene clusters seem to be specific to *S. griseus*. Therefore, these clusters probably direct the synthesis of some novel compounds. Three NRPS gene clusters (SGR443 to SGR455, SGR6709 to SGR6717, and SGR6730 to SGR6742) appear to be involved in the biosynthesis of siderophores because they contain some genes for a siderophore-dependent iron transport system. The presence of IdeR (iron-dependent transcription regulator) box-like sequences (29) in the upstream regions of many genes within these three gene clusters (data not shown) also supports this idea.

Two putative gene clusters (SGR3845 to SGR3849 and SGR4408 to SGR4421) for lantibiotics are found in *S. griseus*. SGR3848 and SGR4418 seem to be lantibiotic precursors because these small proteins contain many Cys, Thr, and Ser residues in their C-terminal regions, most of which could be modified to produce lanthionine and methyllanthionine bridges (43). We annotated SGR5285 to SGR5295, containing a putative 5-aminolevulinic acid synthase gene (SGR5295), as a gene cluster for an unknown secondary metabolite. A similar gene cluster (CV0801 to CV0808) is found in *Chromobacterium violaceum* (4).

Genes possibly under the control of A-factor. In *S. griseus*, A-factor induces morphological development and secondary metabolite formation (16, 17). We have revealed the A-factor regulatory cascade in which AdpA functions as a central transcriptional regulator that has multiple target genes. Although we have already identified 14 AdpA target genes, many other genes are expected to be targets of AdpA. Furthermore, the transcription of a much greater number of genes seems to be activated or repressed indirectly by AdpA. For the first step in analyzing global gene regulation by A-factor in *S. griseus*, we compared the transcriptomes of the wt and Δ*adpA* mutant strains. The total RNA of both strains was extracted from the cells in the early stationary phase in liquid culture because AdpA begins to trigger the transcription of target genes in the middle of the exponential growth phase. To evaluate the accuracy of our DNA microarray analysis, we randomly selected 17 regulator genes and analyzed their transcription using quantitative RT-PCR. There was a good correlation between the ratios measured using the DNA microarray and quantitative RT-PCR analyses (Fig. 4B). Therefore, we regarded genes with an increase in expression of more than twofold and a P value of <0.05 when analyzed with a t test ($n = 4$) as genes with significant variation in transcriptional level. When this threshold was used, 639 and 373 genes were transcriptionally upregu-

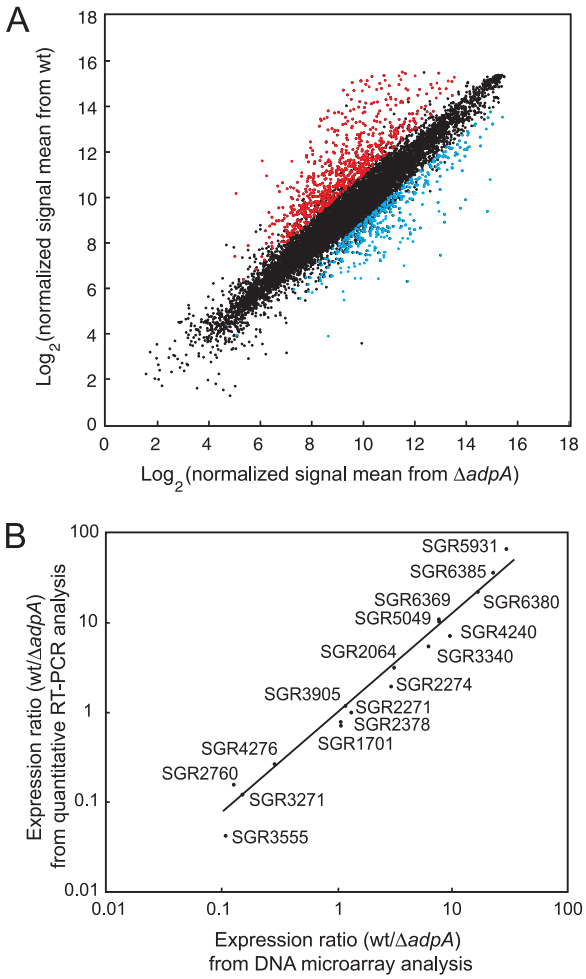


FIG. 4. DNA microarray analysis of *S. griseus* gene expression. (A) Scatter plot for DNA microarray analysis of *S. griseus* gene expression affected by the deletion of the *adpA* gene. The scatter plot shows the averages of normalized signal means from the wt strain and those from the Δ*adpA* mutant ($n = 4$). The red dots indicate genes that were >2-fold upregulated ($P < 0.05$), and the blue dots indicate genes that were >2-fold downregulated ($P < 0.05$) in the wt strain. (B) Comparison of results obtained by DNA microarray and quantitative RT-PCR analyses. The ratios of gene expression (wt strain to Δ*adpA* mutant) are plotted. The results obtained by the two methods show a high level of correlation ($r^2 = 0.93$).

lated and downregulated, respectively, in the wt strain, in comparison with the Δ*adpA* mutant (Fig. 4A). These genes are dispersed over the whole chromosome (Fig. 1, line iii). This result indicates that the disruption of *adpA* had a great influence on the transcription of many genes in *S. griseus*, as expected. Of 14 known AdpA target genes, six genes (*strR* [change, 29.3-fold increase], *sgiA* [14.2-fold], *orf1-AdBS2* [SGR6559] [4.19-fold], *sprD* [3.77-fold], *adsA* [3.52-fold], and *orfA-AdBS3* [SGR4617] [2.57-fold]) were detected to be upregulated. A similar DNA microarray analysis using RNA from cells grown on a solid medium indicated that all of the 14 known AdpA target genes were upregulated in the wt strain (details will be published elsewhere).

In conventional nutrient-rich liquid medium, at least four secondary metabolite gene clusters (SGR443 to SGR455 [NRPS], SGR574 to SGR593 [NRPS], SGR6360 to SGR6387 [type I PKS], and SGR6709 to SGR6717 [NRPS]), as well as the streptomycin-biosynthetic gene cluster, were upregulated in the wt strain (Fig. 1, lines iii and iv), indicating that these four gene clusters were activated directly or indirectly by AdpA. A-factor probably induces the production of some peptide and polyketide compounds that are synthesized by these gene clusters. In contrast, one large gene cluster (SGR3239 to SGR3288 [type II PKS and NRPS]) was downregulated in the wt strain (Fig. 1, lines iii and iv). To confirm the apparent repression of this gene cluster by AdpA, further experiments are required. We are now analyzing the changes in the transcriptome triggered by the exogenous addition of A-factor to an A-factor-deficient mutant using the same DNA microarray. The overall picture of genome-wide gene regulation by A-factor will be determined through a combination of several DNA microarray analyses.

Concluding remarks. A striking feature of the *S. griseus* genome is the unique telomere sequence. The complete genome sequence revealed the absence of conserved Tpg and Tap in their functional forms and allowed us to find a novel pair of Tpg and Tap proteins encoded in the TIRs. Further analysis of the novel pair of Tpg and Tap proteins will provide insights into the evolution of the linear chromosome of *Streptomyces*. Comparisons of the *S. griseus* genome with the genomes of *S. coelicolor* A3(2) and *S. avermitilis* clearly showed a common feature of the *Streptomyces* linear chromosome—a conserved internal core region of approximately 6.4 Mb with a couple of variable subtelomeric regions of 1 to 2 Mb. It also allowed us to find 24 *Streptomyces*-specific proteins. *S. griseus* has at least 34 gene clusters (or genes) for the production of known or unknown secondary metabolites, although a considerable number of these gene clusters (or genes) are presumably transcriptionally inactive under the conventional culture conditions. Novel secondary metabolic enzymes or pathways can be found through studies of these gene clusters (or genes); the SGR470-to-SGR472 and SGR1268-to-SGR1269 proteins have already been demonstrated to be responsible for the biosynthesis of alkylresorcinol and 2-methylisoborneol, respectively. We constructed a DNA microarray that allowed us to analyze the transcriptome of *S. griseus*. For the first step of the analysis of global gene regulation by A-factor, we compared the transcriptomes of the wt strain and the Δ*adpA* mutant. The transcription of approximately 1,000 genes was affected by *adpA* disruption, demonstrating a large effect of A-factor in the biology of *S. griseus*. Importantly, we found that four secondary metabolite gene clusters for unknown peptide and polyketide compounds were activated by A-factor. Thus, the genome sequence will greatly facilitate future studies of the molecular genetics of *S. griseus*.

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