Expression of the *melC* Operon in Several *Streptomyces* Strains Is Positively Regulated by AdpA, an AraC Family Transcriptional Regulator Involved in Morphological Development in *Streptomyces coelicolor*

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Dark brown haloes of melanin around colonies are an easily visualized phenotype displayed by many *Streptomyces* strains harboring plasmid pIJ702 carrying the *melC* operon of *Streptomyces antibioticus* IMRU3270. Spontaneous melanin-negative mutants of pIJ702 occur with a frequency of ca. 1%, and often mutation occurs in the *melC* operon, which removes the BglII site as part of an inverted repeat. Other melanin-negative mutations seem to occur spontaneously in *Streptomyces lividans*, resulting in white colonies from which intact, melanin-producing pIJ702 can be isolated by introduction into a new host. *S. lividans* ZX66 was found to be such a mutant and to have a secondary mutation influencing expression of the *melC* operon on the chromosome. A 3.3-kb DNA fragment was isolated from its progenitor strain, JT46, and a gene able to restore *melC* operon expression was found to encode a member of an AraC family of transcriptional regulators, which was equivalent to AdpA_5 in *Streptomyces coelicolor* and therefore was designated AdpA_5. Lack of *melC* operon expression was correlated with a single A-to-C transversion, which altered a single key amino acid residue from Thr to Pro. The transcription of the *melC* operon was found to be greatly reduced in the *adpA_5* mutant background. The counterpart gene (*adpA_5*) in the *S. antibioticus* strain in which the *melC* operon carried on pIJ702 originated was also isolated and was found to have an identical regulatory role. Thus, we concluded that the *melC* operon is under general direct positive control by AdpA family proteins, perhaps at the transcriptional level and certainly at the translational level via bldA, in *Streptomyces*.

Tyrosinase (EC 1.14.18.1) is a ubiquitous copper-containing monooxygenase that catalyzes both the O hydroxylation of monophenols and the oxidation of O-diphenols to O-quinones (29, 32) responsible for the biosynthesis of melanin pigment from tyrosine (29) in bacteria. In *Streptomyces antibioticus* (23) and *Streptomyces glaucescens* (16), the tyrosinase gene (*melC2*) is preceded by the *melC1* gene encoding a conserved protein essential for the expression of melanin (3, 23, 28) in a polycistronic operon. The MelC1 protein, which behaves like a molecular chaperone, has dual roles; it regulates copper incorporation, and it promotes secretion of apotyrosinase via a molecular chaperone, has dual roles; it regulates copper incorporation, and it promotes secretion of apotyrosinase via a molecular chaperone, has dual roles; it regulates copper incorporation, and it promotes secretion of apotyrosinase.

The *Streptomyces melC* operon has been used extensively as a phenotypic marker for *Streptomyces* plasmids (23) and for the construction of promoter-probe vectors for *Streptomyces* (36) and *Escherichia coli* (9). Investigation of the control of the *melC* operon revealed that a cloned gene of *Streptomyces lividans* 66, *cutR*, phenotypically suppresses defective *melC1* (44). The CutR protein resembles the response regulator OmpR of the osmoregulatory signal transduction system in *E. coli*. Next to *cutR* is *cutS*, which encodes the histidine protein kinase counterpart of OmpS. Thus, the putative *cutR-cutS* operon was postulated to regulate copper metabolism in *Streptomyces* (44).

In *Rhizobium leguminosarum* biovar phaseoli, transcription of a gene needed for melanin synthesis was found to be activated by *nifA* of *Rhizobium* and *Klebsiella pneumoniae* (15), which is an activator of *nifH* of *K. pneumoniae* in *E. coli* cells grown with low oxygen concentrations. Analysis of *Streptomyces griseus* mutants defective in melanogenesis showed that there was a close regulatory correlation between melanogenesis and morphological and physiological differentiation. Also, introduction of plasmids carrying the *melC* operon failed to confer melanin production in the melanin-negative mutants, and disruption of *melC2* barely affected melanin productivity, suggesting that another unknown enzyme is involved in melanogenesis in *S. griseus* (11).

Here we demonstrate unambiguously that expression of the *melC* operon in *S. lividans* and *Streptomyces coelicolor* and in its native host, *S. antibioticus*, is under strict positive control by a gene encoding a multifunctional regulatory protein (AdpA_5). A counterpart protein in *S. coelicolor* (AdpA_5) was shown to be the product of the *bldH* gene (47), which contains a rare leucine codon (TTA) controllable by *bldA* tRNA at the translational level, and to be closely associated with aerial mycelium development (41).

**MATERIALS AND METHODS**

**Bacterial strains and plasmids.** *Streptomyces* and *E. coli* strains used in this study are listed in Table 1.

**General methods and techniques.** The general growth media and conditions used for *E. coli* and *Streptomyces* strains and the standard methods used for handling *E. coli* and *Streptomyces* in vivo and in vitro, such as preparation of

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the sequencing gel was examined with an ABI 377 autosequencer. The sequence subjected to cycle sequencing with a DNA thermal cycler (Perkin-Elmer) before Ready Reaction mixture and the appropriate primer, and the total volume was ing. About 200 to 400 ng of template DNA was mixed with 4 μl of a terminator Erase-a-Base system (Promega). The double-stranded plasmid DNA used as the template was purified by polyethylene glycol 8000 precipitation before sequenc-

**TABLE 1. Strains and plasmids used in this study**

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant phenotype and/or characteristics</th>
<th>Reference or source</th>
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<tr>
<td><strong>S. coelicolor A3(2) strains</strong></td>
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<tr>
<td>M145</td>
<td>Wild type</td>
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<td>ZD2</td>
<td>M145-derived <em>adpA</em> disruptant, <em>aac(3)IV</em></td>
<td>This study</td>
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<td><strong>S. lividans 66 strains</strong></td>
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<td>1326</td>
<td>Wild type, Dnd&lt;sup&gt;+&lt;/sup&gt;/φHAU3&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>JT46</td>
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<td>HXY1</td>
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<td>Same as JT46 but <em>adpA</em></td>
<td>This study</td>
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<td>ZD1</td>
<td>ZNX6 derivative with an autonomous pJ702 but integrative, pJTU1452, *aac(3)IV/Mel&lt;sup&gt;+&lt;/sup&gt;</td>
<td>This study</td>
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<td>IMRUS720</td>
<td>Wild type, Mel&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>IMRUS720-derived <em>adpA</em> disruptant, <em>aac(3)IV</em></td>
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<td>GIBCO BRL</td>
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<td><strong>Plasmids</strong></td>
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<td>pSET152</td>
<td><em>aac(3)IV lacZ rep&lt;sub&gt;II&lt;/sub&gt; att&lt;sub&gt;ES&lt;/sub&gt; oriT</em></td>
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<td>pOJ260</td>
<td><em>apr oriT rep&lt;sub&gt;II&lt;/sub&gt; lacZ</em></td>
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<td>pMD18-T</td>
<td>pUC18 derivative</td>
<td>TaKaRa</td>
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<td>pJTU1452</td>
<td>ca. 3.5-kb PCR fragment (obtained by using pSET152-FP and pSET152-RP as primers and ZD1 DNA as the template) inserted into the EcoRV site of pSET152</td>
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<td>Religation of pJTU1452 after digestion with BamHI and Bg/II, <em>AdpA&lt;sup&gt;+&lt;/sup&gt;</em></td>
<td>This study&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>pJTU1455</td>
<td>Religation of pJTU1452 after partial digestion with ApaI, <em>AdpA&lt;sup&gt;+&lt;/sup&gt;</em></td>
<td>This study&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>pJTU1457</td>
<td>Religation of pJTU1452 after digestion with ApaI, <em>AdpA&lt;sup&gt;+&lt;/sup&gt;</em></td>
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<td>pJHU1448</td>
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<td>This study</td>
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<td>pJHU1461</td>
<td>ApaI fragment (1.7 kb) of ZNX6 containing complete <em>adpA</em> inserted into the Apal site of Bluescript SK&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>pJHU1465</td>
<td>ApaI fragment (1.5 kb) of IMRUS3720 containing complete <em>adpA</em> inserted into the Apal site of Bluescript SK&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>pJHU1466</td>
<td>958-bp PCR fragment (obtained by using A1296 and pSET152-FP as primers and pJHU1465 as the template) internal to <em>adpA</em> inserted into the EcoRV site of the pMD18-T vector</td>
<td>This study</td>
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<td>pJHU1467</td>
<td>HindIII-EcoRI fragment carrying, the 958-bp PCR fragment of pJHU1466 inserted into the corresponding sites of pOJ260</td>
<td>This study</td>
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<sup>a</sup> *mel*, tyrosinase gene for melanin production; *oriT*, origin of transfer of plasmid RK2; *tr*, thiostrepton resistance gene; *aac(3)IV*, apramycin resistance gene; Cm<sup>R</sup>, chloramphenicol resistance.<br>

<sup>b</sup> See Fig. 2.
PCR primers and conditions and Southern and colony hybridizations. Oligonucleotide primers pSET152-FP and pSET152-RP were used to recover by PCR amplification a ca. 3.5-kb DNA fragment that included adpA, cloned and integrated into the ZD1 chromosome before it was inserted into the EcoRV site of pSET152 to obtain pJTU1452. The primers were synthesized commercially. PCRs were performed by using a HYBAID PCR machine. A typical PCR mixture (50 μl) consisted of 1.5 mM MgCl2, 5 ng of genomic DNA as the template, 5% dimethyl sulfoxide, each deoxynucleoside triphosphate at a concentration of 100 μM, and 25 pmol of the required primers in 1× PCR buffer. After addition of 5 U of Pfu polymerase (13), the DNA template was denatured at 95°C for 3 min. Amplification was carried out by 30 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 55°C, and extension for 4 min at 72°C, followed by extension for 5 min at 72°C. PCR products were then purified from 0.8% agarose gels by using a Gene Recovery kit (12). For Southern hybridization experiments, DNA was cleaved with restriction enzymes, separated in 0.8% agarose gels, and transferred onto a Hybond-N+ nylon membrane (Amersham-Pharmacia). α-[32P]dCTP-labeled radioactive probes and a Random Priming kit (Roche) were used for both Southern blotting and in situ colony hybridization.

Cloning of adpA was performed by first generating an adpA mutant of S. lividans JT46 (ZD2), an adpA mutant of S. coelicolor M145 (ZD3), and an adpA mutant of S. antibioticus IMRU3720 (ZD4) by targeted gene disruption. The PCR product mediating disruption of adpA was constructed by PCR amplification of a ca. 3.3-kb DNA fragment covering adpA from the S. coelicolor M145 chromosome by using oligonucleotide primers 1458-FP (5′-GACAGCCCCGCAACCTC-3′) and 1458-RP (5′-GCGTCTGCTCACAAGGC-3′), followed by digestion with Alul to generate a 921-bp internal fragment for insertion into the EcoRV site of E. coli plasmid pOJ260. Similarly, vector pJTU1459 mediating disruption of adpA was constructed by recovery of a 921-bp DNA fragment after Alul digestion of pJTU1452 for insertion into the EcoRV site of pOJ260. Vector pJTU1467 mediating disruption of adpA was constructed by cloning a 958-bp PCR fragment covering part of adpA from pJTU1465 by using oligonucleotide primers pSET152-FP (5′-CCAGTCGACGACGTTGTAAAACGA-3′) and pSET152-RP (5′-GGCCGTCTGCTCGAGTCAC-3′) into the EcoRV site of the pMD18-T vector to generate a 921-bp internal fragment to pJTU1467.

Reverse PCR for transcript analysis. Total RNAs from JT46 and ZD3/ pJTU1467 were isolated by using an RNeasy mini kit (QUIAGEN) and were treated with DNAse I. Reverse transcription (RT)-PCR was performed by using the SuperScript First-Strand Synthesis System (QUIAGEN). The cycling parameters used were 30 min at 50°C and 15 min at 95°C. The reverse transcription step, followed by 30 PCR cycles of amplification (30 s at 94°C, 30 s at 55°C, and 30 s at 72°C). Primers melF (5′-GACACCTGTCGACGTTGTAAAACGA-3′) and melMR (5′-GAGCTTTCACATCCGGTGA-3′) were used as the forward and reverse primers for amplification of a 395-bp fragment internal to the melC1 and melC2 genes from S. antibioticus (23) into the EcoRV site of the pMD18-T vector to produce pJTU1466, from which an EcoRI-HindIII fragment was recovered for insertion into the corresponding site of pOJ260 to obtain pJTU1467.

Reverse PCR for transcript analysis. Total RNAs from JT46 and ZD3/ pJTU1467 were isolated by using an RNeasy mini kit (QUIAGEN) and were treated with DNase I. Reverse transcription (RT)-PCR was performed by using the SuperScript First-Strand Synthesis System (QUIAGEN). The cycling parameters used were 30 min at 50°C and 15 min at 95°C. The reverse transcription step, followed by 30 PCR cycles of amplification (30 s at 94°C, 30 s at 55°C, and 30 s at 72°C). Primers melF (5′-GACACCTGTCGACGTTGTAAAACGA-3′) and melMR (5′-GAGCTTTCACATCCGGTGA-3′) were used as the forward and reverse primers for amplification of a 395-bp fragment internal to the melC1 and melC2 genes from S. antibioticus (23) into the EcoRV site of the pMD18-T vector to produce pJTU1466, from which an EcoRI-HindIII fragment was recovered for insertion into the corresponding site of pOJ260 to obtain pJTU1467.

Reversal of the melC operon expression was achieved in JT46 (right) but not in ZD4 (left). Both strains were transformed with pIJ702 carrying melC1 and melc2 and were grown on MTT containing thiostrepton.

**RESULTS**

**ZX66/pIJ702**

**JT46/pIJ702**

FIG. 1. melC operon is expressed in JT46 (right) but not in ZX66 (left). Both strains were transformed with pIJ702 carrying melC1 and melc2 and were grown on MTT containing thiostrepton.
ZD1 as the template. A 3.5-kb DNA fragment was amplified, and reinsertion of this fragment into pSET152 (resulting in pJTU1452) and introduction by transformation into ZX66 could complement full expression of the melC operon (data not shown).

Localization of a gene complementing melC expression deficiency. The 3.5-kb DNA insert from pJTU1452 was sequenced. BLAST searches immediately revealed strong similarity or even identity to a region (nucleotides 3045346 to 3048682) of the sequenced S. coelicolor genome which contained three complete ORFs, SCO2790, SCO2791, and SCO2792 (Fig. 2). Two incomplete ORFs (SCO2789 and SCO2793) (Fig. 2) seemed to be irrelevant to the control of melC expression and thus were not included in further analysis. One of the three complete ORFs (SCO2792; nucleotides 3047135 to 3048331) was clearly identified as an arac family transcriptional regulator, a potential candidate for controlling melC expression and thus were not included in further analysis.

To localize the responsible gene(s), religation of pJTU1452 after digestion with BamHI (flanking one side of the multiple cloning sites of the pSET152 vector) and BglII yielded pJTU1454; this removed SCO2790 (Fig. 2). In a similar way, religation of pJTU1452 after digestion with ApaI produced pJTU1455; this removed SCO2792 completely but left SCO2790 and SCO2791 intact (Fig. 2). Meanwhile, the 1.7-kb ApaI fragment carrying intact SCO2092 was inserted into the corresponding site in pSET152, resulting in pJTU1457, which carried only SCO2792 (Fig. 2).

When the three pJTU1452 derivatives (pJTU1454, pJTU1455, and pJTU1457) were introduced by transformation into ZX66, black colonies were observed only when inserts carried SCO2792 alone (pJTU1457) or in combination with SCO2791 (Fig. 2). This experiment unambiguously demonstrated that SCO2792 is a required positive regulator of melanogenesis.

The locus that positively controls melC expression is equivalent to adpA (adpA₁) in S. coelicolor, and analysis of the adpA₁ mutant allele in ZX66. The gene that positively controls melC operon expression in S. lividans, SCO2792, is an adpA₁ homolog that has been reported to contain a rare leucine codon (TTA) whose translation depends on bldA tRNA and is involved in aerial mycelium development in S. coelicolor (41). We used the subscripts c and l, as used by Takano et al. (41), to distinguish between the genes of S. coelicolor and S. lividans, respectively. adpA₁ in S. coelicolor and adpA₁ in S. lividans are 99% identical at the nucleotide level, and four of the different nucleotides resulted in only one variation at the amino acid level. Similarly, the N-terminal portion of AdpA₁ (and AdpA₂) has sequence homology with a group of proteins in the Thl–Ppi–DJ-1 family, which is widely distributed in bacteria, archaea, and eukaryotes, and has structural similarity to the type I glutamine amidotransferase domain (19), although the amino acid sequences of the members of this family were not dramatically conserved. This potential AraC family transcriptional regulator has a typical conserved DNA-binding domain with two helix-turn-helix motifs at the C terminus and a sequence...
FIG. 3. Overall characteristics of the adpA \(_\alpha\) gene and alignment of adpA \(_\alpha\) (from S. coelicolor M145), adpA \(_\beta\) (from S. lividans ZX64), and adpA \(_\gamma\) (from S. lividans ZX66) and the protein sequences encoded by these genes. Nucleotides are numbered from the ATG start codon. L is the rare leucine (TTA) codon. HTH\_AraC is a region with a helix-turn-helix motif for the typical AraC family of bacterial regulatory proteins, and the shaded region (with two alternative sequences) has the potential to form a stem-loop structure. Only the regions with differences at the nucleotide (nt) and amino acid (aa) levels between alleles are shown, and mutant positions are indicated by triangles.

with the potential to form a stem-loop structure 21 bp downstream of the stop codon of AdpA \(_\alpha\) or AdpA \(_\gamma\) (Fig. 3). A rare leucine codon (TTA) was found at the same position in both proteins (AdpA \(_\alpha\) and AdpA \(_\beta\)) (Fig. 3), which must be the target for bldA regulation. When pIJ702 was introduced into an S. coelicolor M145-derived bldA mutant strain (J1700), none of the many independent thiostrepton-resistant transformants produced a black pigment, as expected.

A 1.7-kb ApaI fragment carrying a hypothetical mutant adpA \(_\alpha\) locus corresponding to SCO2792 in ZX66 was cloned into the corresponding site of pBluescript SK(+) by colony hybridization by using the 1.7-kb ApaI fragment carrying the JT46 adpA \(_\alpha\) gene as a probe and sequenced. A transversion (A to C) was found at nucleotide 214, which changed a codon from ACC (for Thr) to CCG (for Pro) (Fig. 3). Indeed, alignment of the nucleotide (and amino acid) sequences of adpA \(_\alpha\) and adpA \(_\beta\), as well as other sequenced adpA genes (and the corresponding proteins) in Streptomyces avermitilis (adpA \(_\alpha\)) and S. griseus (adpA \(_\beta\), adpA \(_\gamma\)) revealed that none of the adpA genes has any variation at nucleotide 214 (A is part of the ACC codon encoding Thr).

Expression of the melC operon is under the same positive control by AdpA \(_\alpha\) in S. coelicolor M145 and by AdpA \(_\gamma\) in its native host, S. antibioticus. pIJ702 was introduced into ZD2 (an M145 derivative with a mutation in adpA \(_\alpha\)) and ZD3 (a JT46 derivative with a mutation in adpA \(_\gamma\)). No black pigment was secreted from ZD2 or ZD3 carrying pIJ702 or from ZD4 (an S. antibioticus IMRU3720 derivative with a mutation in adpA \(_\gamma\)), while a black pigment was clearly observed with M145 and JT46 carrying the same plasmid, as well as with the wild-type strain S. antibioticus IMRU3720 on the same plates (Fig. 4), suggesting that the melC operon is under the same positive control in all three organisms. Meanwhile, a sharp increase in the amount of black pigment was observed when an extra copy of adpA \(_\alpha\) was added by integrating pJTU1457 into the attB sites of either S. coelicolor or S. lividans strains carrying pIJ702 or S. antibioticus IMRU3720, from which the melC operon carried on pIJ702 originated (Fig. 4), by site-specific recombination through the attP site of φC31 on pSET152.

AdpA \(_\alpha\) affects colony phenotypes and transcriptionally interacts with the promoter region of the melC operon. Constructed gene disruptants with disruptions internal to adpA \(_\alpha\) in M145 (ZD2), adpA \(_\alpha\) in JT46 (ZD3), and adpA \(_\alpha\) in S. antibioticus IMRU3720 (ZD4) were compared in order to examine their colony phenotypes along with those of the corresponding parental strains. All of the mutants appeared to be obviously bald, as reported previously for the bldA mutant of S. coelicolor M145 (41), while all of the parental strains sporulated well.

An attempt was also made to detect the possible interaction of AdpA \(_\alpha\) with the promoter region of the melC operon by a gel mobility shift assay. As shown in Fig. 5, the total proteins isolated with AdpA \(_\alpha\) from E. coli strain BL21(DE3) carrying pJTU1464 (a pET15b derivative carrying the adpA \(_\alpha\) gene) obviously bound to the 295-bp DNA fragment carrying the promoter region of the melC operon radioactively labeled with \(\gamma\)\(^32\)P\)ATP and T4 polynucleotide kinase, while the total proteins without AdpA \(_\alpha\) from E. coli strain BL21(DE3) carrying pET15b lacked specific binding. Additionally, the band in lane 3, in which 0.02 \(\mu\)g of total proteins was added, was obviously more intense than the band in lane 2, in which only 0.01 \(\mu\)g of total proteins was added. The same experiments were repeated by using various concentrations of the same protein preparations with and without AdpA \(_\alpha\) but with an unrelated 318-bp labeled probe fragment as an additional control, and no shifted band, like that in lanes 2 and 3, was detected on the gel (data not shown).
FIG. 4. (A) Lack of mel operon expression in adpA mutants of S. coelicolor ZD2, S. lividans ZD3, and S. antibioticus ZD4 compared with their progenitors, M145 (left), JT46 (middle), and IMRU3720 (right), respectively. (B) Dosage effect of adpA. The top half of each plate contained wild-type S. antibioticus IMRU3720, S. lividans JT46 and ZX64 carrying pIJ702, or S. lividans ZX64 and ZX66 carrying pIJ702, while the bottom half of each plate contained strains corresponding to the strains in the top half but with an additionally integrated copy of adpA, (carried on pJTU1457) except that the two patches for the same strain (ZX64/pIJ702::pJTU1457) are shown in the bottom half of the left plate. For the strains at the bottom there was both increased production of black pigment and enhanced sporulation compared with the strains at the top.

FIG. 5. (A and B) Gel mobility shift assay (B) for detection of the binding of AdpA, to a γ-32P-labeled 295-bp DNA fragment (A) covering the promoter region of the melC operon. The solid arrowhead in panel B indicates the position when 0.01 µg (lane 2) or 0.02 µg (lane 3) of total proteins isolated from E. coli BL21(DE3) carrying pJTU1464 was added to the radioactively labeled probe fragment (open arrowhead). Addition of 0.02 µg of total proteins isolated from E. coli BL21(DE3) carrying pET15b to the same probe was used as a negative control (lane 1). (C) Alignment of the three regions upstream of the melC operon (A) with the consensus AdpA-binding sequence (51) shown at the top (5'-TGGCSNGWY-3'; where S is G or C, W is A or T, Y is T or C, and N is any nucleotide).
DISCUSSION

The tyrosinase (mel) gene, which is responsible for melanin synthesis, has been sequenced from at least nine Streptomyces strains (S. coelicolor, S. avermitilis, S. lincolnensis, S. galbus, S. castaneoglobisporus, S. tanashiensis, S. griseus, S. venezuelae, S. antibioticus, and S. glaucescens). Both the gene sequences and the protein sequences of all of these organisms are similar, with similar sizes and with the genes organized as a polycistrionic operon (melC) consisting of two separate ORFs (melC1 and melC2), but little was known about the regulation of tyrosinase production at the molecular level in Streptomyces.

The isolation of many melanin-negative mutants of S. griseus revealed a close association among melanogenesis, the ability to produce streptomycin and A-factor, and the ability to reveal a close association among melanogenesis, the rosinase production at the molecular level in Streptomyces. and melC1 consisting of two separate ORFs (24) acts as a chemical signaling molecule that triggers morphological differentiation and secondary metabolism (6) at an extremely low concentration. A-factor, a molecule that triggers morphological differentiation and secondary metabolism (6) at an extremely low concentration. A-factor acts as a chemical signaling molecule that triggers morphological differentiation and secondary metabolism (6) at an extremely low concentration. A-factor activates transcription of free AdpA g to activate a transcription factor involved in multiple biological processes. Several direct targets activated by AdpA have been identified, and these targets include strR, a pathway-specific transcriptional activator for streptomycin biosynthetic pathway genes in S. griseus (34); sgmA, a gene encoding a metalloendopeptidase that appears to affect the rate of development in S. griseus (22); adsA (bldN in S. coelicolor [4]), a gene encoding an RNA polymerase extracytoplasmic-function sigma factor needed for normal mycelial development in S. griseus (48); ssgA, which encodes a small acidic protein important for aerial mycelial septation in S. coelicolor (45) and S. griseus (21, 49); and amfR, a gene encoding a transcriptional activator of the amf operon needed for aerial mycelium formation in S. griseus (50). A direct link among melanogenesis, its absolutely required activation by the AdpA homolog, and its clear dosage effect either on melanin production of antibiotics on most media (27, 33). As adpA also has a rare TTA codon like that found in adpA and adpA, it was not surprising to find that the mel gene was not expressed in a bldA mutant and hence was likely to be controlled at the translational level via bldA RNA.

Comparative analysis of a single mutation resulted in identification of a critical nucleotide (A at position 214), whose change (to C) led to a transversion in the amino acid sequence (from Thr to Pro at position 27). Conceivably, this amino acid must be at the active site of the AdpA protein to activate the transcription of the melC operon and to affect sporulation, but whether it has a similar role in activating other biological process, such as antibiotic production, still needs to be examined closely.

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