JOURNAL OF BACTERIOLOGY, Dec. 2003, p. 7291–7296 Vol. 185, No. 24
0021-9193/03/$08.00+0 DOI: 10.1128/JB.185.24.7291–7296.2003

Colonial Differentiation in *Streptomyces coelicolor* Depends on Translation of a Specific Codon within the *adpA* Gene

Kien T. Nguyen,† Jennifer Tenor, Hansruedi Stettler, Lieu T. Nguyen, Liem D. Nguyen, and Charles J. Thompson*

Department of Molecular Microbiology, Biozentrum, Universität Basel, 4056 Basel, Switzerland

Received 28 August 2003/Accepted 4 September 2003

We identified *adpA* as an *araC*-like regulatory gene needed for colonial morphogenesis in *Streptomyces coelicolor* and showed that its activity depended on a unique TTA triplet corresponding to the leucyl-tRNA gene (*bldA*). These findings partially explained the dependence of aerial mycelium formation on a rare tRNA that is postulated to have developmental control functions.

Streptomyces species are multicellular bacteria that undergo a program of colonial development that may depend on a translational regulatory system. *bldA*, encoding the principal tRNA for translation of the TTA (leucine) codon, is dispensable for vegetative growth but is conditionally required at a later stage for the synthesis of aerial mycelium (in all species tested) and for the biosynthesis of some antibiotics (11, 12); for a recent analysis and additional citations, see reference 18. There is evidence that accumulation of the active form of *bldA* tRNA to maximal levels occurs later, at the onset of antibiotic biosynthesis and morphological differentiation (13, 19). The biosynthesis of the antibiotics actinorhodin (blue) and undecylprodigiosin (red) is *bldA* dependent (5, 20) and is activated by the pathway-specific activator genes *act*II-orfIV and *redZ*, both containing TTA codons. For *act*II-orfIV, it has been shown that replacement of the TTA codon by an alternative leucine codon relieves its dependence on *bldA* and thus allows actinorhodin biosynthesis in *bldA* mutants (5); comparable results have been obtained after mutagenesis of TTA codons in several antibiotic resistance genes (12). However, at least in some contexts and under certain nutritional conditions, TTA triplets are mistranslated and can provide active gene products in *bldA* mutants (6, 12, 18). The target gene (or genes) and corresponding TTA codon underlying the aerial mycelium defect in *bldA* mutants have not been identified.

In *Streptomyces griseus*, both aerial hypha formation and biosynthesis of streptomycin rely on *adpA*, a gene containing a TTA codon and encoding an AraC-like transcriptional regulator (17). This triplet is found at the corresponding position in all known *adpA* orthologs (*S. coelicolor* [1], *S. griseus* [17], and *S. avermitilis* [7]), suggesting that *adpA* may be a *bldA*-dependent regulator of aerial hypha formation conserved within *Streptomyces* species. This model was explored in the experiments described here (similar results have been obtained by E. Takano, M. Tao, F. Long, Maureen J. Bibb, L. Wang, W. Li, M. J. Buttner, Mervyn J. Bibb, Z. X. Deng, and K. F. Chater, personal communication).

***adpA*, a regulator of aerial hypha formation and antibiotic biosynthesis.** A 1.2-kb fragment encoding the *S. coelicolor* AdpA protein (Swiss-Prot/TrEMBL entry name Q9L062) was amplified by PCR with primers *adpA*-N-*Nde*I (GGGCTTAG CCATATGAGCCACGACTCCA; the engineered *Nde*I site containing the presumed ATG start codon is underlined) and *adpA*-C-*Bam*HI (GGATCCGAGCCGTCTGCTCACCTC ACG; the engineered *Bam*HI site is underlined) and cloned into pGEM-T-Easy (Promega), and its sequence was verified. To mutagenize the gene, an apramycin resistance gene cassette on an *Sma*I fragment (3) was introduced into the unique *Msc*I site in its central region. A 3.0-kb fragment carrying disrupted *adpA* was excised by *Eco*RI digestion, cloned into the same site in pSET151 (2), a vector carrying the thiostrepton resistance gene, and used to disrupt *adpA* in wild-type strain J1501 (by transformation) (10). An apramycin-resistant, thiostrepton-sensitive colony containing disrupted *adpA*, as confirmed by PCR and Southern blot analyses, was isolated. Inactivation of *adpA* dramatically slowed colonial development on several media (R2YE and MS [10]). Compared to parent strain J1501, the *adpA* mutant (Fig. 1A and 2) was impaired in its ability to form aerial mycelium (bald), and individual colonies produced more red pigment on R2YE medium. Possible effects on blue pigment synthesis could not be determined, since our isolate of parent strain J1501 produced very little actinorhodin under these conditions.

For complementation studies, a 1.6-kb fragment carrying the promoter and coding region of *adpA* was amplified by PCR with primers *adpA*-pro1-*Hin*dIII/*Bam*HI (GGATCCAAGCTT GGGAAAAAGCACCGGTCGACTGAC) and *adpA*-C-*Bam*HI (see above) and cloned into the *Bam*HI site of pIJ904 [pIJ904::*adpA*(TTA)]. Alternatively, to express *adpA* from the strong constitutive *ermE* promoter, the *adpA* coding region was PCR amplified with primers *adpA*-N-*Nde*I and *adpA*-C-*Bam*HI and cloned into pHM11A between the *Nde*I and *Bam*HI sites [pHM11A::*adpA*(TTA)]. In the *adpA* mutant, both of these plasmids suppressed the defect in aerial mycelium formation [the results obtained with pIJ904::*adpA*(TTA) are shown in Fig. 1A]. *adpA* overexpression by pHM11A::*adpA*(TTA) in the wild-type strain (J1501) induced rapid aerial hypha formation

* Corresponding author. Mailing address: Department of Molecular Microbiology, Biozentrum, Universität Basel, Klingelbergstr. 50/70, 4056 Basel, Switzerland. Phone: 41 61 267 2116. Fax: 41 61 267 2118. E-mail: Charles-J.Thompson@unibas.ch.

† Present address: Cubist Pharmaceuticals, 65 Hayden Ave., Lexington, MA 02421.

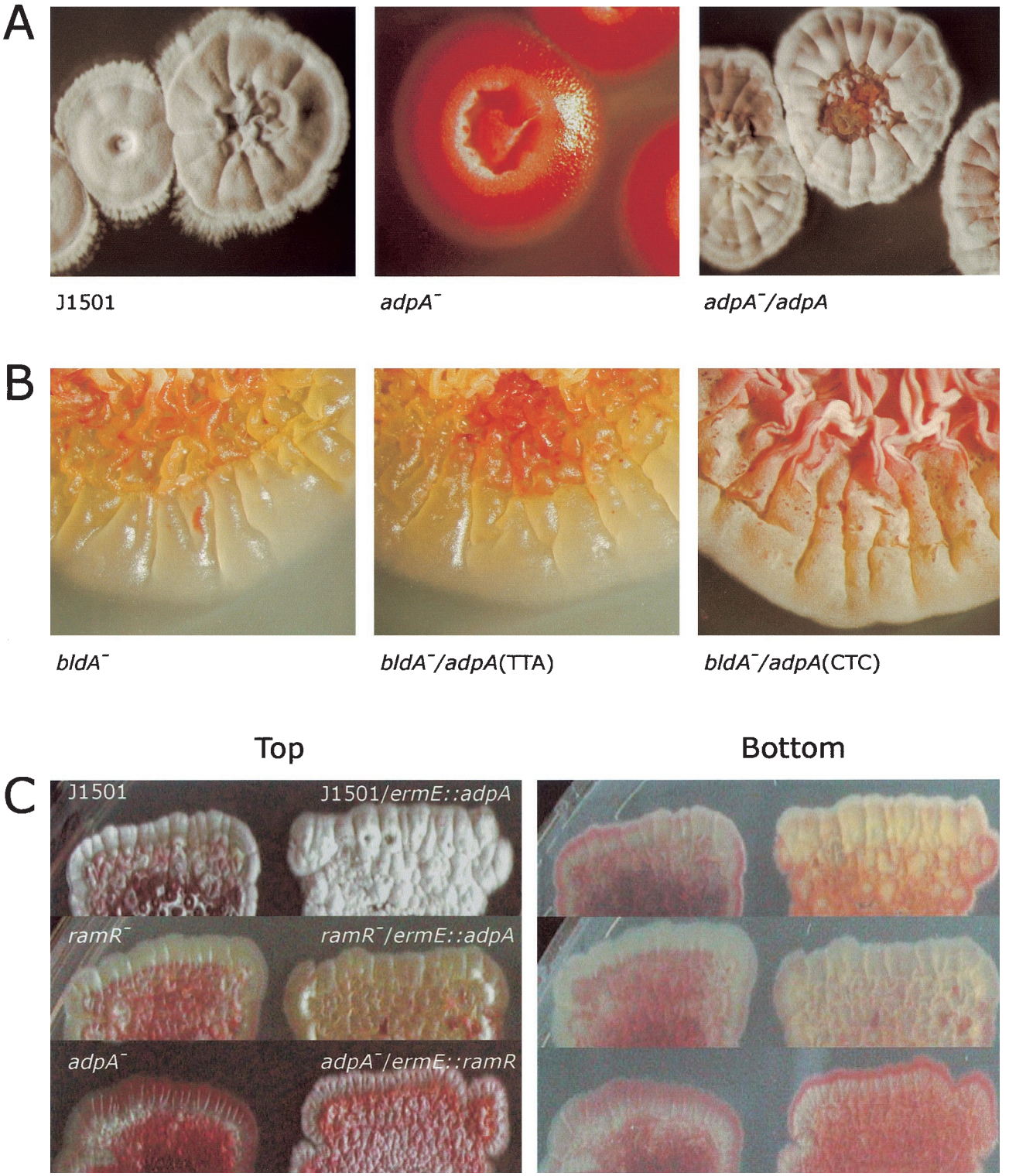

FIG. 1. *bldA*-dependent translation of *adpA* (*bldH*) is required for colonial morphogenesis. (A) The wild type (J1501) (left panel), the *adpA* mutant (middle panel), or the *adpA* mutant transformed with plasmid pIJ904::*adpA*(TTA) carrying the intact wild-type gene (right panel) was grown on R2YE medium at 30°C for 5 days. Colonies produced no diffusible blue pigment; the darker appearance of the agar reflects photographic conditions that were adjusted for the optimal exposure of colonies having different levels of brightness. (B) The *bldA39* mutant strain (left panel) or the *bldA39* mutant strain containing a wild-type (middle panel) or a mutant (right panel) allele cloned in pIJ904 was grown on R2YE medium at 30°C for 2 weeks. (C) Strains that were wild type (J1501) or defective for aerial mycelium formation (*ramR* or *adpA* mutants) (left panels) were transformed with plasmids containing wild-type *ramR* (*ermE*::*ramR*) or *adpA* (*ermE*::*adpA*) genes cloned into vector pHM11A to allow expression from the strong constitutive *ermE* promoter (right panels). Cultures were grown on R2YE medium at 30°C for 1 week and photographed from the top or bottom.

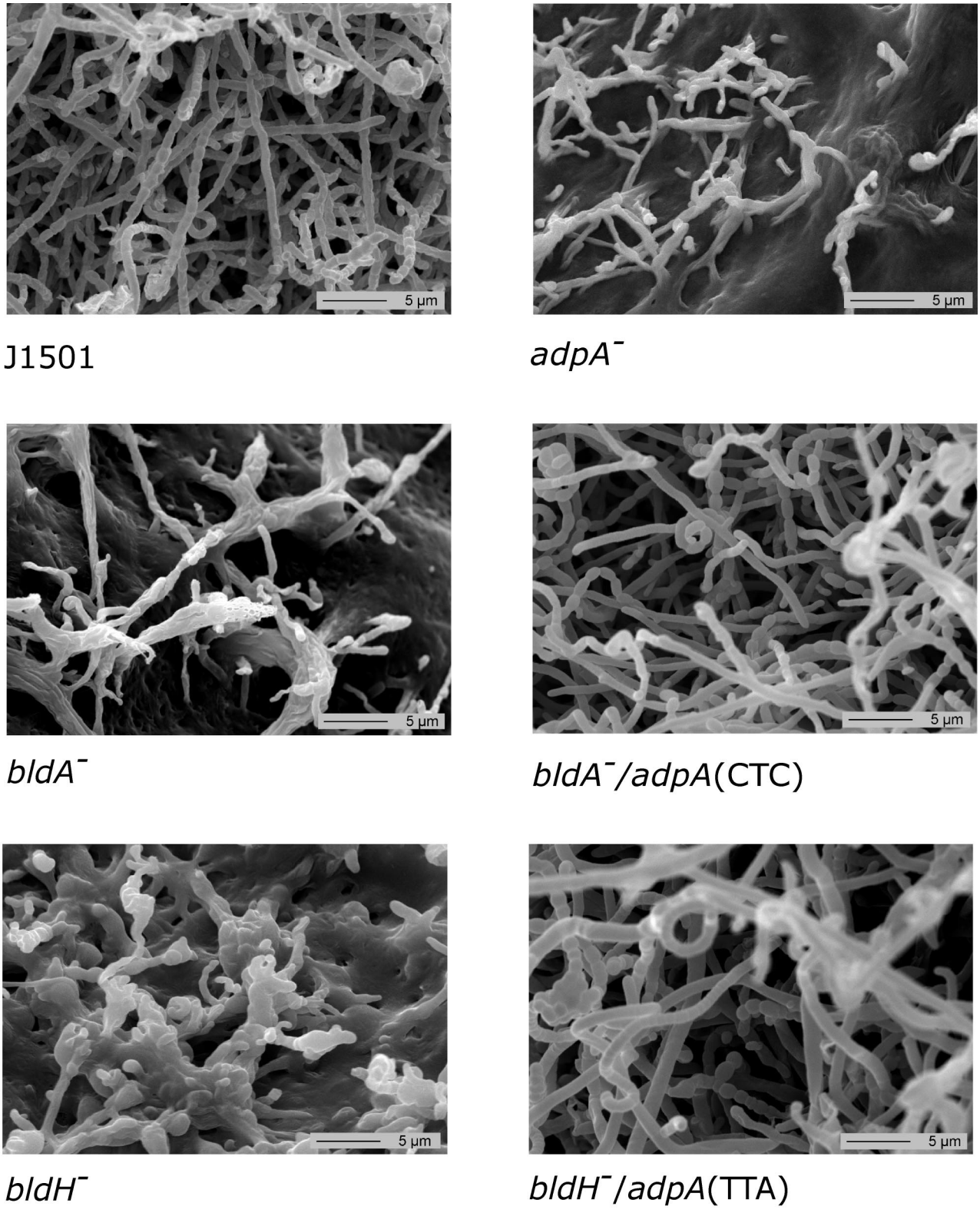

FIG. 2. *bldA*-dependent translation of *adpA* (*bldH*) is required for aerial mycelium formation. Scanning electron microscopic views (14) of colonial surface cultures grown on R2YE medium for 10 days at 30°C are shown. *adpA* and *bldH* mutant strains were transformed with vector pIJ904 carrying the indicated *adpA* alleles [*adpA*(CTC), *adpA* allele with the CTC mutation; *adpA*(TTA), *adpA* allele with wild-type TTA].

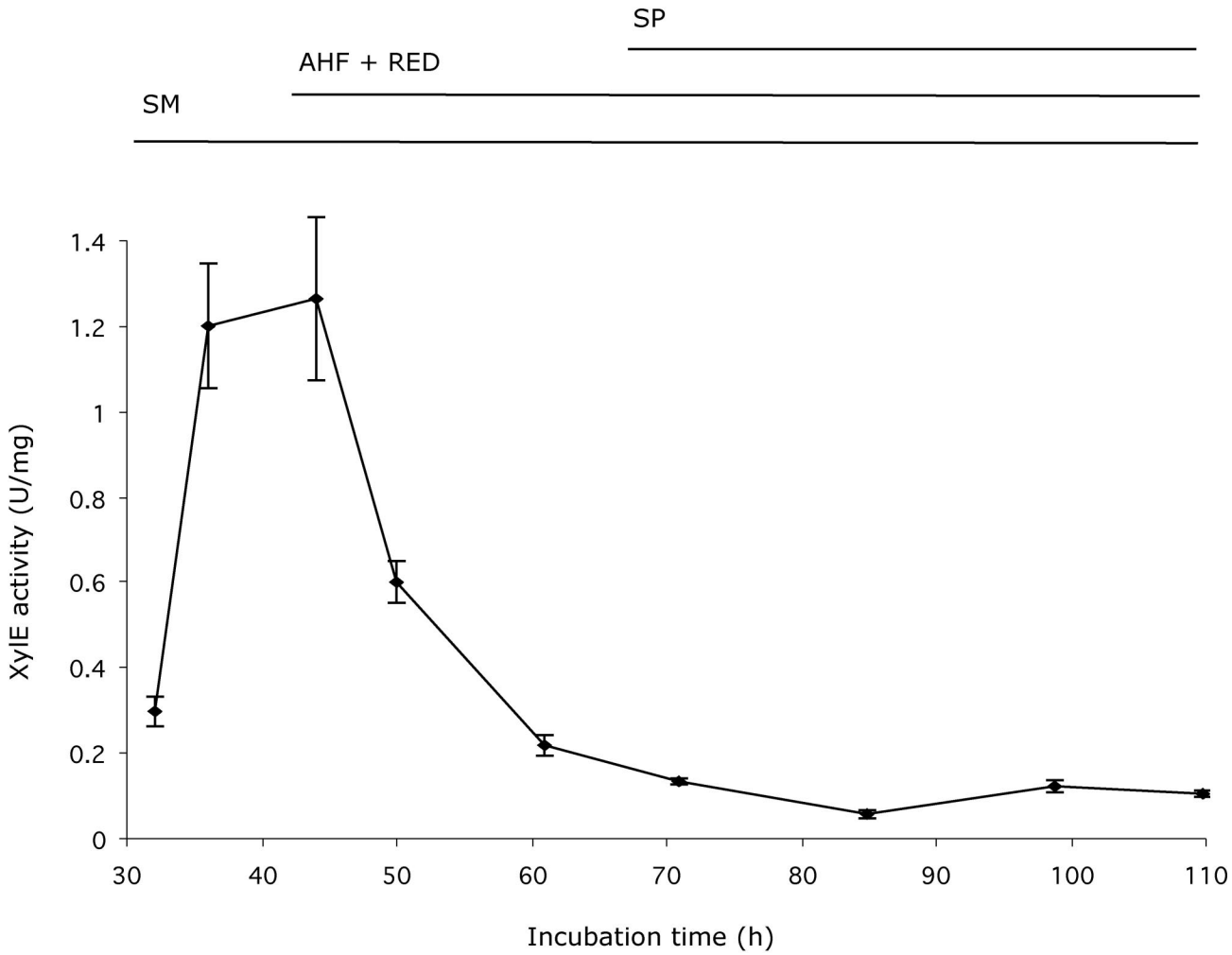

FIG. 3. *adpA* promoter activity is developmentally regulated. Pregerminated spores (10^6) of wild-type strain J1501/pXE4 and strain J1501/pXE4::p*adpA* were plated on thiostrepton-containing R2YE medium, covered with cellophane membranes, and incubated at 30°C. Extracts were prepared as described previously (15), and catechol dioxygenase activity was calculated as the rate of change in the optical density at 375 nm per minute, expressed as specific activity (units per milligram of protein; standard deviation bars are based on assays of three biological replicate samples). The XylE activity of the control strain carrying only vector pXE4 was insignificant (<0.005 U/mg) (data not shown). SM, substrate mycelium; AHF, aerial hypha formation; RED, undecylprodigiosin production; SP, sporulation.

but permanently suppressed pigmentation (Fig. 1C). These results, along with the enhanced red pigmentation of *adpA* mutant colonies, showed that *adpA* was needed to activate morphological differentiation and could suppress synthesis of the red-pigmented antibiotic (undecylprodigiosin) in *S. coelicolor*. However, the inverse relationship between aerial mycelium formation and pigment biosynthesis related to *adpA* expression may be indirect, representing competing processes that may or may not have biological relevance.

***adpA* and *bldH109* are mutant alleles of the same gene.** While an *adpA* ortholog is present in the *S. coelicolor* genome, a corresponding *adpA* mutant allele has not been found among known developmental genes. Several lines of evidence suggested that *bldH*, one of the few unidentified mutant genes, may be such an allele. Intercellular complementation analyses placed *bldH* and *bldA* alleles in the same group, suggesting that they are required for the same developmental functions (21). Consistent with this suggestion, *bldA* tRNA may be needed to translate the *S. coelicolor adpA* gene, since it contains a rare TTA codon (1). Initial evidence that *bldH* does indeed correspond to *adpA* was provided by experiments showing that the cloned *adpA* gene [pIJ904::*adpA*(TTA)] was able to complement the *bldH109* mutant (Fig. 2). Finally, when the *adpA* locus from the *bldH109* mutant was amplified by three independent PCRs, cloned separately, and sequenced, each was found to contain an additional G residue inserted at position +221 with respect to the annotated coding frame. We conclude that *bldH109* is a frameshift mutation in the *adpA* gene.

Transcription and translation of *adpA*. To monitor the promoter activity of the *adpA* upstream sequence, it was fused to the reporter gene, *xylE*, in vector pXE4 (pXE4::p*adpA*). A 0.5-kb fragment covering the *adpA* promoter region was amplified by PCR with primers *adpA*-pro1-*Hin*dIII/*Bam*HI and *adpA*-pro2/*Bam*HI (GGATCCCAGCACCGCGACGATCTC CTTG) and cloned into the *Hind*III and *Bam*HI sites of pXE4 (10), generating a fusion with *xylE* (8). XylE enzyme activity

increased markedly just before and during aerial hypha formation and then gradually decreased (Fig. 3). Thus, the *adpA* promoter was apparently under developmental control, and the timing of its expression was coordinated with aerial hypha formation.

To test whether the translation of *adpA* mRNA was dependent on *bldA*, perhaps related to the accumulation of an active form of *bldA* tRNA (13, 19), the unique TTA codon in *adpA* was changed to CTC. The mutant allele was generated by a two-stage PCR mutagenesis approach (15) with primers *adpA*-pro1-*Hin*dIII/*Bam*HI and *adpA*-C-*Bam*HI together with primers *adpA*/CTC1 (ACAGGTCTCTCCCGGAGGAGATCG; the mutated codon is underlined) and *adpA*/CTC2 (TCTCCT CCGGGAGAGACCTGTCGA; the mutated codon is underlined). The PCR product was cloned into pGEM-T-Easy, and its sequence was verified. The insert was removed by *Bam*HI digestion and cloned into the *Bam*HI site of pIJ904 [pIJ904::*adpA*(CTC)]. In the *bldA* mutant background, the *adpA* allele with the CTC mutation (Fig. 1B) was able to induce aerial hypha formation (Fig. 1B). In contrast, the cloned wild-type TTA-containing allele had no apparent effect on the *bldA* mutant (Fig. 1B). Scanning electron microscopic inspection (Fig. 2) of the colony surface showed that pIJ904::*adpA*(CTC) allowed the *bldA* strain to form some aerial hyphae and spores having a wild-type appearance, while the wild-type construct [pIJ904::*adpA*(TTA)] had no effect (data not shown). The fact that aerial hypha formation in the pIJ904::*adpA*(CTC)-complemented *bldA* strain was significantly delayed and reduced compared to that in the wild-type strain could have been due to defective transcriptional control of the cloned gene. However, it may indicate the requirement for additional *bldA* targets that facilitate aerial hypha formation, including TTA-containing genes outside the *adpA* locus. Finally, it is also possible that, in the *bldA* mutant background, the mutant CTC codon facilitated translation of the cloned *adpA* copy but translational arrest at the native TTA-containing chromosomal copy had a polar effect on the expression of other developmental genes located downstream.

***adpA* activity requires *ramR*.** *ramR* is a response regulator gene within a cluster of genes needed for aerial hypha formation in both *S. coelicolor* (15, 16) and *S. griseus* (*amfR*) (17). In *S. coelicolor*, its overexpression accelerates aerial mycelium formation in wild-type strains and restores aerial hypha formation in most defective mutants, including *bldA* and *bldH* mutants. *ramR* promoter activity is dependent on several developmental genes, including the *bldA* and *bldH* genes (9). Thus, *ramR* apparently serves as one of the final steps of the regulatory cascade leading to aerial hypha formation (15).

Like the overexpression of *ramR*, the overexpression of *adpA* in wild-type *S. coelicolor* induced rapid aerial mycelium formation (Fig. 1C). The fact that this effect was not significant in the *ramR* mutant (Fig. 1C) suggested that *adpA* activity was dependent on *ramR*. In the complementary experiment, the overexpression of *ramR* in either *adpA* (Fig. 1C) or *bldH* (15) mutants was able to accelerate aerial hypha formation. These results provide additional evidence that *ramR* functions downstream of *adpA* or *bldH*.

Concluding remarks. We have presented evidence suggesting that *adpA* transcription is activated during aerial hypha formation and that its TTA triplet is required for aerial mycelium formation. However, we reemphasize that these and other studies of *bldA* do not prove that the activity of a specific tRNA reflects a bona fida regulatory system (18) or that it is necessarily peculiar to developmental systems in *Streptomyces*. Interestingly, a recent comparison of the levels of charged tRNAs that carry the same amino acid (isoacceptors) relative to the nonrandom distributions of their corresponding codons in *Escherichia coli* genes suggested that the selective use of alternative isoacceptors can regulate certain functions (4). The model predicts that under amino acid starvation conditions, charged tRNA isoacceptors corresponding to preferred codons will be depleted first and that those corresponding to rare codons will remain relatively abundant. Selective codon usage in genes encoding enzymes involved in amino acid biosynthesis or their corresponding regulatory elements (attenuators) suggests that it may provide translational regulatory functions during starvation. By analogy, developmental genes containing the rare TTA codon in *Streptomyces* may be preferentially translated during starvation responses associated with antibiotic biosynthesis and colonial morphogenesis.

This work was supported by Swiss National Science Foundation grants 31-59156.99 and 3100AO-100293/1.

We thank D. Fink, S. P. Salvatore, and K. Chater for sharing information prior to publication, Marcus Dürrenberger and Marcel Dueggelin for electron micrographs, and Urs Jenal for critical reading of the manuscript.

REFERENCES

1. **Bentley, S. D., K. F. Chater, A. M. Cerdeno-Tarraga, G. L. Challis, N. R. Thomson, K. D. James, D. E. Harris, M. A. Quail, H. Kieser, D. Harper, A. Bateman, S. Brown, G. Chandra, C. W. Chen, M. Collins, A. Cronin, A. Fraser, A. Goble, J. Hidalgo, T. Hornsby, S. Howarth, C. H. Huang, T. Kieser, L. Larke, L. Murphy, K. Oliver, S. O'Neil, E. Rabbinowitsch, M. A. Rajandream, K. Rutherford, S. Rutter, K. Seeger, D. Saunders, S. Sharp, R. Squares, S. Squares, K. Taylor, T. Warren, A. Wietzorrek, J. Woodward, B. G. Barrell, J. Parkhill, and D. A. Hopwood.** 2002. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature **417:**141–147.
2. **Bierman, M., R. Logan, K. O'Brien, E. T. Seno, R. N. Rao, and B. E. Schoner.** 1992. Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces spp*. Gene **116:**43–49.
3. **Blondelet Rouault, M. H., J. Weiser, A. Lebrihi, P. Branny, and J. L. Pernodet.** 1997. Antibiotic resistance gene cassettes derived from the omega interposon for use in *E. coli* and *Streptomyces*. Gene **190:**315–317.
4. **Elf, J., D. Nilsson, T. Tenson, and M. Ehrenberg.** 2003. Selective charging of tRNA isoacceptors explains patterns of codon usage. Science **300:**1718–1722.
5. **Fernandez-Moreno, M. A., J. L. Caballero, D. A. Hopwood, and F. Malpartida.** 1991. The *act* cluster contains regulatory and antibiotic export genes, direct targets for translational control by the *bldA* tRNA gene of *Streptomyces*. Cell **66:**769–780.
6. **Gramajo, H. C., E. Takano, and M. J. Bibb.** 1993. Stationary-phase production of the antibiotic actinorhodin in *Streptomyces coelicolor* A3(2) is transcriptionally regulated. Mol. Microbiol. **7:**837–845.
7. **Ikeda, H., J. Ishikawa, A. Hanamoto, M. Shinose, H. Kikuchi, T. Shiba, Y. Sakaki, M. Hattori, and S. Omura.** 2003. Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis*. Nat. Biotechnol. **21:**526–531.
8. **Ingram, C., M. Brawner, P. Youngman, and J. Westpheling.** 1989. *xylE* functions as an efficient reporter gene in *Streptomyces* spp.: use for the study of *galP1*, a catabolite-controlled promoter. J. Bacteriol. **171:**6617–6624.
9. **Keijser, B. J., G. P. Van Wezel, G. W. Canters, and E. Vijgenboom.** 2002. Developmental regulation of the *Streptomyces lividans ram* genes: involvement of RamR in regulation of the *ramCSAB* operon. J. Bacteriol. **184:**4420–4429.
10. **Kieser, T., M. J. Bibb, M. J. Buttner, K. F. Chater, and D. A. Hopwood.** 2000. Practical Streptomyces genetics. The John Innes Foundation, Norwich, United Kingdom.
11. **Lawlor, E. J., H. A. Baylis, and K. F. Chater.** 1987. Pleiotropic morphological and antibiotic deficiencies result from mutations in a gene encoding a tRNA-like product in *Streptomyces coelicolor* A3(2). Genes Dev. **1:**1305–1310.
12. **Leskiw, B. K., E. J. Lawlor, J. M. Fernandez-Abalos, and K. F. Chater.** 1991. TTA codons in some genes prevent their expression in a class of develop-

mental, antibiotic-negative, *Streptomyces* mutants. Proc. Natl. Acad. Sci. USA **88:**2461–2465.
13. **Leskiw, B. K., R. Mah, E. J. Lawlor, and K. F. Chater.** 1993. Accumulation of *bldA*-specified tRNA is temporally regulated in *Streptomyces coelicolor* A3(2). J. Bacteriol. **175:**1995–2005.
14. **Müller, T., R. Guggenheim, M. Düggelin, and C. Scheidegger.** 1991. Freeze-fracturing for conventional and field emission low-temperature scanning electron microscopy: the scanning cryo unit SCU 020. J. Microsc. **161:**73–83.
15. **Nguyen, K. T., J. M. Willey, L. D. Nguyen, L. T. Nguyen, P. H. Viollier, and C. J. Thompson.** 2002. A central regulator of morphological differentiation in the multicellular bacterium *Streptomyces coelicolor*. Mol. Microbiol. **46:** 1223–1238.
16. **O'Connor, T. J., P. Kanellis, and J. R. Nodwell.** 2002. The *ramC* gene is required for morphogenesis in *Streptomyces coelicolor* and expressed in a cell type-specific manner under the direct control of RamR. Mol. Microbiol. **45:**45–57.
17. **Ohnishi, Y., S. Kameyama, H. Onaka, and S. Horinouchi.** 1999. The A-factor regulatory cascade leading to streptomycin biosynthesis in *Streptomyces griseus*: identification of a target gene of the A-factor receptor. Mol. Microbiol. **34:**102–111.
18. **Trepanier, N. K., S. E. Jensen, D. C. Alexander, and B. K. Leskiw.** 2002. The positive activator of cephamycin C and clavulanic acid production in *Streptomyces clavuligerus* is mistranslated in a bldA mutant. Microbiology **148:** 643–656.
19. **Trepanier, N. K., G. D. Nguyen, P. J. Leedell, and B. K. Leskiw.** 1997. Use of polymerase chain reaction to identify a leucyl tRNA in *Streptomyces coelicolor*. Gene **193:**59–63.
20. **White, J., and M. Bibb.** 1997. *bldA* dependence of undecylprodigiosin production in *Streptomyces coelicolor* A3(2) involves a pathway-specific regulatory cascade. J. Bacteriol. **179:**627–633.
21. **Willey, J., J. Schwedock, and R. Losick.** 1993. Multiple extracellular signals govern the production of a morphogenetic protein involved in aerial mycelium formation by *Streptomyces coelicolor*. Genes Dev. **7:**895–903.