The *bldA*-encoded tRNA is poorly expressed in the *bldI* mutant of *Streptomyces coelicolor* A3(2)

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The *bldA* gene, encoding a leucyl tRNA recognizing the UUA codon, is expressed at significantly lower levels in the *bldI* mutant, *Streptomyces coelicolor* J703, than in the parent *S. coelicolor* A3(2). Expression of a TTA-containing reporter gene was reduced in the *bldI* mutant, as was the mature, 87 nucleotide, form of the *bldA*-encoded tRNA. This reduced level of the tRNA was also seen when the *bldA* gene was introduced on a high-copy-number plasmid into the *bldI* mutant, suggesting that maximal *bldA* expression may require a *bldI*-dependent promoter.

Keywords: *Streptomyces coelicolor*, *bldA*, *bldI*, antibiotic production, morphological differentiation

INTRODUCTION

Streptomyces spp. are Gram-positive soil bacteria that have been the focus of much study because of two particular characteristics. They undergo a complex cycle of morphological differentiation that culminates in the production of spores on the tips of aerial hyphae and they produce a host of secondary metabolites, many of which are medically important antibiotics. Among morphological mutants of the genetically well-characterized strain *Streptomyces coelicolor* A3(2) are the aerial-mycelium-deficient *bld* (for bald) mutants, many of which are also blocked in antibiotic production. The study of such pleiotropic mutants holds promise for a greater understanding of global regulatory signals that coordinately control the two very different aspects of differentiation. The best-characterized of the *bld* genes, *bldA*, encodes a leucyl tRNA (Lawlor *et al.*, 1987) that recognizes the UUA codon, which is rare in *Streptomyces* mRNA because of the very high G + C (approx. 73 mol %) content of their DNA. The isolation of pleiotropic mutants blocked in the expression of a rare tRNA, together with the observation that the rare codon is confined largely to genes that would be expressed only late in growth, such as antibiotic resistance and regulatory genes, has led to the suggestion that the *bldA* gene might represent a translational regulatory mechanism controlling some antibiotic production and sporulation genes (Leskiw *et al.*, 1991). More recently, it has been shown, at least under some

growth conditions and for at least two *Streptomyces* spp. (Leskiw *et al.*, 1993; N. K. Trepanier & B. K. Leskiw, unpublished), that the *bldA*-encoded tRNA accumulates late in growth at a time that would be consistent with a regulatory role in morphological differentiation and antibiotic production.

We have been studying possible interconnections between other *bld* genes and expression of the *bldA*-encoded tRNA in an effort to determine if any of the *bld* genes are part of a regulatory pathway controlling *bldA* expression. To date little is known about other *bld* gene products: *bldD* and *bldG* have been cloned (B. K. Leskiw & R. Passantino, unpublished) but remain uncharacterized; and cloning and sequencing of *bldB* has failed to shed any light on a possible role in differentiation (Harasym *et al.*, 1990). We have tested many of the available classes of *bld* mutants for *bldA* expression, and based on the severely reduced *bldA* expression in the *bldI* mutant of *S. coelicolor*, suggest that *bldI* is required either directly or indirectly for *bldA* expression or function.

METHODS

Chemicals and enzymes. All electrophoresis chemicals were ultrapure grade. *Taq* DNA polymerase, and DNA-modifying enzymes, were from Boehringer Mannheim. Deep Vent DNA polymerase was from New England Biolabs. Nitrocefin was from Oxoid Unipath, thiostrepton was a gift from S. Lucania, Bristol-Myers Squibb, and viomycin was provided by Pfizer. All other chemicals were of reagent grade.

Bacterial strains, culture conditions and transformation procedures. *S. coelicolor* strains were J1501 [*hisA1 uraA1 strA1*

The GenBank accession number for the nucleotide sequence reported in this paper is U20761.

pgl, SCP1⁻ SCP2⁻; Chater *et al.* (1982)] and its derivatives J1681 [*ΔbldA*; Leskiw *et al.* (1993)], C103 [*bldG103*; Champness (1988)], C181 [*bldH181*; Champness (1988), provided by W. Champness] and C249 [*bld-249*, proposed to be a *bldI* mutant (Champness 1988); Harasym *et al.* (1990), provided by W. Champness] shown here to be a *bldB bldA* double mutant; J650 [*cysD18 mthB2 agaA1*, NF SCP2*; Merrick (1976)] and its derivatives J660 [*mthB2 cysD18 agaA1 bldC18*, NF SCP2*; Merrick (1976)], J669 [*mthB2 cysD18 agaA1 bldB43*, NF SCP2*; Merrick (1976)] and J703 [*mthB2 agaA1 bld-28*, NF SCP2*; Harasym *et al.* (1990), provided by J. Piret]; the J1508 derivative HU66 [*bisA1 uraA1 strA1 pgl bldD53*, NF SCP2*; Willey *et al.* (1993), provided by J. Willey]; and 166 [*bisD3 pheA1 strA1 bldF*, SCP1* SCP2*; Passantino *et al.* (1991)]. All strains were obtained from K. Chater (John Innes Centre, Norwich, UK) unless otherwise indicated. Yeast extract/malt extract (YEME) medium (Hopwood *et al.*, 1985) was used for *Streptomyces* liquid culture. Protoplasting and transformation of *Streptomyces* strains were as described by Hopwood *et al.* (1985). For RNA isolation, *Streptomyces* cultures were grown on cellophane discs (Leskiw *et al.*, 1993; Soliveri *et al.*, 1992) on the surface of R2YE agar (Hopwood *et al.*, 1985).

Phage and plasmid vectors. The *Streptomyces* plasmids pIJ584 and pAU2 were high-copy-number derivatives of pIJ486 (Ward *et al.*, 1986) containing *bldA* and *ampC*, respectively (Leskiw *et al.*, 1993). *Streptomyces* plasmids were maintained by selection for thiostrepton resistance [$10 \mu\text{g ml}^{-1}$; Hopwood *et al.* (1985)]. KC628 was a ϕ C31 derivative carrying *bldB* (Harasym *et al.*, 1990). Conditions for phage propagation were as described by Hopwood *et al.* (1985); phage integration to produce lysogens was scored as described by Piret & Chater (1985) by selecting for resistance to viomycin ($20 \mu\text{g ml}^{-1}$).

Preparation of cell extracts, and detection and assay of β -lactamase activity. Cell extracts were prepared from mycelium scraped from the surface of cellophane discs as previously described (Leskiw *et al.*, 1993). β -Lactamase activity was detected qualitatively by spraying colonies with the chromogenic cephalosporin nitrocefin (0.5 mg ml^{-1}) as a substrate, and quantitatively by spectrophotometric assay as described previously (Leskiw *et al.*, 1993). Specific β -lactamase activity is expressed as mU (mg protein^{-1}).

DNA procedures. Standard procedures were used for both *Escherichia coli* (Sambrook *et al.*, 1989) and *Streptomyces* (Hopwood *et al.*, 1985). Chromosomal DNA was isolated from *Streptomyces* strains using procedure 1 of Hopwood *et al.* (1985).

Sequence analysis of *bldA* genes from *Streptomyces* strains. The template for sequencing was generated by PCR amplification of a 297 bp fragment containing the *bldA* tRNA region. The template for PCR was chromosomal DNA ($1 \mu\text{g}$) from *S. coelicolor* J1501, J703 or C249, and the primers were a 28-mer synthetic oligonucleotide (5'-CATGGATCCACCCGGTAAC-TGATGCCACC-3') corresponding to a region upstream of the *bldA* promoter, and an 18-mer synthetic oligonucleotide (5'-GCCGCTGAGTCGCAACCA-3') corresponding to a sequence just downstream of the *SacII* site located 61 bp from the end of the tRNA. Two independent PCR reactions were performed. One reaction employed *Taq* polymerase (Boehringer Mannheim) while the other employed the proofreading enzyme, Deep Vent DNA polymerase (New England Biolabs). Both reactions were carried out according to the manufacturers' specifications and the amplified bands were purified from a 5% (w/v) polyacrylamide gel by the crush and soak method (Sambrook *et al.*, 1989). PCR products from both reactions were then directly sequenced using an ABI model 373A automated system (Department of Biological Sciences Sequencing Service, University of Alberta).

RNA manipulations. RNA was isolated at various intervals from mycelium grown on cellophane discs on the surface of R2YE agar, and Northern analysis was performed as previously described (Leskiw *et al.*, 1993) except that $10 \mu\text{g}$ of each total RNA sample was loaded per lane. In all cases the membranes were first hybridized to an end-labelled oligonucleotide, 5'-CCAGGGACTCGAACCCCG, complementary to the *lysT* tRNA (Sedlmeier & Schmieger, 1990), and then after stripping were hybridized to an end-labelled oligonucleotide, 5'-TTAAGCTCGCCGTGTCT, complementary to the *bldA* tRNA (Leskiw *et al.*, 1993). In some cases RNA accumulation was quantified by densitometry of the exposed X-ray films with a BioImage XRS 3cx scanner.

The contribution of plasmid copy number variations to tRNA accumulation was determined after isolation of total DNA and RNA from the same cultures. Total nucleic acid was extracted and precipitated by the method of Kirby *et al.* (1967) as described by Hopwood *et al.* (1985), and then either DNA or RNA was selectively purified from half of the sample after treatment with RNase ($40 \mu\text{g ml}^{-1}$, Sigma) or DNase ($0.14 \text{ U } \mu\text{l}^{-1}$; RNase-free, Boehringer Mannheim), respectively. Northern and Southern blots of RNA and DNA samples, respectively, were then probed as described above.

Dot-blot analysis of *ampC* transcripts. Total RNA ($10 \mu\text{g}$) was denatured as described by Ward *et al.* (1986), and applied to a Hybond-N nylon filter with a dot-blot apparatus. Hybridization was performed as previously described (Ward *et al.*, 1986); the probe was a 410 bp *HindIII*-*XbaI* fragment internal to *ampC*, and was labelled with [α - ^{32}P]dATP by the random primer method. All samples were treated with DNase-free RNase to ensure that the signals resulted from RNA transcripts.

RESULTS

Some *bld* mutants are unable to translate UUA codons efficiently

In an effort to determine if other classes of *bld* genes might play a role in regulating the presence of a functional *bldA* tRNA, perhaps by involvement in transcription of the *bldA* gene, 5' or 3' processing of the primary transcript, base modification, or charging by aminoacyl tRNA synthetase, the ability of *bldB*, -C, -D, -F, -G, -H and -I mutants to translate UUA codons was tested. A high-copy-number plasmid derivative, pAU2, containing the *ampC* reporter gene (Leskiw *et al.*, 1993) was introduced into each of the mutant strains by protoplast transformation. The *ampC* gene has seven TTA codons; its expression can be detected by spraying colonies with the chromogenic substrate nitrocefin, or it can be assayed spectrophotometrically using the same substrate. Although pIJ101-based vectors like pAU2 are incompatible with resident SCP1 plasmids (Schauer *et al.*, 1991), as is the case in SCP1⁺ as well as NF strains (that have SCP1 integrated into the chromosome), pAU2 was maintained, albeit at a much lower copy number, in HU66, 166, and J650 and its derivatives by selection for thiostrepton resistance (data not shown). Preliminary screening of *bld* mutants involved qualitative assay for *ampC* β -lactamase activity. Transformants, including J1501/pAU2 and J1681/pAU2 as positive and negative controls respectively, were sprayed with nitrocefin and monitored for substrate cleavage by the appearance of a red/brown halo. All of the mutants tested were positive

Table 1. Expression of *ampC* β -lactamase in extracts from cells of selected *S. coelicolor* strains

Strain	β -Lactamase activity (mU mg ⁻¹)	Genotype
J1501/pAU2	47 $\pm$ 8.6	<i>hisA1 uraA1 strA1 pgl</i> , SCP1 ⁻ SCP2 ⁻
J1681/pAU2	0.11 $\pm$ 0.01	<i>hisA1 uraA1 strA1 pgl</i> Δ <i>bldA</i> , SCP1 ⁻ SCP2 ⁻
J650/pAU2	17.3 $\pm$ 1.6	<i>cysD18 mthB2 agaA1</i> , NF SCP2*
J703/pAU2	0.86 $\pm$ 0.24	<i>mthB2 agaA1 bld-28</i> , NF SCP2*
C249/pAU2	0.11 $\pm$ 0.01	<i>hisA1 uraA1 strA1 pgl bld-249</i> , SCP1 ⁻ SCP2 ⁻

Fig. 1. Dot-blot analysis of *ampC* transcripts in RNA samples (10 μ g) isolated from the *bldI* mutant J703, and its parent strain J650. Controls included RNA isolated from the SCP1⁻ strain C249, and RNA samples treated with DNase-free RNase.

with the exception of C249 and J703, both reported to be *bldI* mutants, which showed no and severely reduced *ampC* expression, respectively. To rule out the possibility that reduced expression resulted from a change in the plasmid DNA, pAU2 was isolated from both J703 and C249 and used to transform J1501. In neither case was any defect seen in the expression of *ampC*. The *ampC*-specified β -lactamase was then assayed quantitatively in cell extracts of C249 and J703 and their respective parent strains (Table 1). As with the qualitative assay, J1681/pAU2, the *bldA* deletion mutant containing pAU2, served as a negative control. Two independent transformants were tested, and to account for differences in growth rates of the strains, mycelia were harvested at three times (between 30 and 72 h); the highest activities are reported. Activities shown in Table 1 are means of values in duplicate extracts from the two independent transformants. As with the qualitative assay, C249 and J703 showed strikingly reduced β -lactamase activity. To show that the reduced expression of *ampC* did not result from a defect in *ampC* transcription, RNA isolated from C249, J703 and J650 was subjected to dot-blot analysis with an internal fragment of *ampC* as probe. The loss of signal after samples were digested with DNase-free RNase verified that the signals came from hybridization to RNA transcripts. RNA samples from both J650 and J703 showed identical levels of *ampC* transcripts, and, as expected due to the higher copy number of pAU2 in SCP1⁻ strains, C249 showed abundant *ampC* transcripts (Fig. 1). These results suggest that the reduced β -lactamase activity seen in C249 and J703 is not attributable to a lack

Fig. 2. (a) Northern analysis of the *bldA* tRNA in RNA samples (10 μ g) isolated from surface-grown *S. coelicolor* *bldB*, *-C*, *-D*, *-F*, *-G*, *-H*, and *-I* mutants. Controls included parent strains from which the mutants were derived (see Methods) and a *bldA* deletion mutant, *S. coelicolor* J1681. (b) The *lysTα* tRNA was probed as a control for RNA loading levels. RNA was isolated at 48 h post-inoculation. Probes were end-labelled oligonucleotides complementary to the *bldA* and *lysTα* tRNAs (see Methods).

of *ampC* transcription, and furthermore that these strains have mutations that affect the availability of translation-proficient *bldA* tRNA.

Expression of *bldA*-encoded tRNA in *bldI* mutants

Inability to express *ampC* efficiently due to limited translation of its UUA codons could result either from a failure to transcribe the *bldA* gene or from a defect in processing or modification of the *bldA* transcript. To investigate the possibility that the level of the *bldA* tRNA might be reduced in C249 and J703, total RNA samples isolated from 48 h surface cultures of all of the *bld* mutant and parent strains described above were subjected to Northern analysis. The probes were end-labelled oligonucleotides complementary to the *bldA* tRNA and, as a control for RNA loading levels, *lysTα* tRNA sequences. The Northern analysis was performed on RNA isolated from at least two independent transformants; representative results are shown in Fig. 2. As expected, the *bld* strains with no impairment in *ampC* activity had *bldA* tRNA levels comparable to those in the parent strains. However, the *bldA* tRNA could not be detected in C249, and was present at very low levels in J703 [the faint band

Fig. 3. DNA sequence of the *bldA* region. Primers used for PCR amplification of a 297 bp fragment are shown. **, the previously determined location of the *bldA* transcription start point (Leskiw *et al.*, 1993).

in Fig. 2 was more easily visualized after prolonged exposure to X-ray film (data not shown)]. Prolonged exposure failed to reveal any band corresponding to the *bldA* tRNA in RNA samples from C249. Densitometric scanning of two independent autoradiograms from long exposures gave 11-fold higher tRNA levels in the parent strain J650 than in the *bldI* mutant J703.

Sequence analysis of the *bldA* gene in C249

The lack of any detectable tRNA, together with the complete absence of *ampC* activity, in C249 mirrored the pattern in the *bldA* mutant, J1681, and contrasted with the low but detectable tRNA levels and *ampC* activity in J703. These observations suggested that C249 might carry a *bldA* mutation. To investigate this possibility a fragment of DNA containing *bldA* was amplified from chromosomal DNA of C249 by PCR, and sequenced directly using the same primers as were used for the amplification (Fig. 3; see Methods). Controls included PCR-amplified *bldA* fragments from both J1501(*bldA*⁺) and J703. PCR amplifications were performed independently with *Taq* polymerase and with Deep Vent polymerase and both of the resulting amplified fragments were sequenced. The sequencing (Fig. 4) revealed a mutation changing the pseudouridine (ψ) stem of the *bldA* tRNA in C249. No mutations were found within the amplified sequence from J703.

Complementation of the *bld* phenotype in C249

The finding of a *bldA* mutation in C249, together with the fact that the *bldA*-249 mutation mapped to the *bldB* region (Champness, 1988), suggested that C249 might represent a *bldA bldB* double mutant. To verify this, C249 containing pIJ584, a high-copy-number plasmid derivative of *bldA* (Leskiw *et al.*, 1993), was lysogenized with a ϕ C31 phage vector, KC628, carrying the *bldB* gene. Lysogens resistant to both viomycin and thiostrepton, the selectable markers present on the phage and the plasmid, respectively, were isolated. All lysogens expressing both resistance markers were able to sporulate and produce the pigmented antibiotics characteristic of wild-type *S. coelicolor*, confirming that C249 represents a double mutant.

Fig. 4. Deduced secondary structure of the *bldA* tRNA showing the position of the pseudouridine stem mutation in *S. coelicolor* C249.

Introduction of a high-copy-number plasmid containing *bldA* into J703

To rule out the possibility that reduced *bldA* tRNA levels in J703 resulted from a mutation in the promoter region of the *bldA* gene (the location of the sequencing primers shown in Fig. 3 did not allow the entire promoter region to be sequenced), the *bldA* gene from strain J1501 (*bldA*⁺) was introduced into J650 and J703 on the high-copy-number plasmid derivative, pIJ584. RNA was isolated at various times from two independent transformants of each strain and Northern analysis was performed as described above. Controls included J1501, J1681 and C249 containing pIJ584, as well as pAU2-containing transformants of those strains to show the single-copy expression levels for the *bldA* tRNA. Typical results are shown in Fig. 5. J703 showed reduced expression of the *bldA* tRNA, even when the *bldA* gene was present on a high-copy-number plasmid. Densitometric analysis of the signals from undiluted RNA samples taken at 53 h (from a time-course of samples isolated at 20, 29 and 53 h) and from 72 h cultures (from a time-course of samples isolated at 24, 48 and 72 h) of J703/pIJ584, and serially diluted RNA samples from J650/pIJ584, isolated from the same time-courses, showed 6.5- and 6.9-fold increases in the *bldA* tRNA levels in J650 as compared to J703 (data not shown). Comparisons of densitometry values for signals from all of the time-course points are not reported because the signals were too low to be detected from the J703 samples. To verify the structural stability of the plasmid, pIJ584 was also isolated from both J703 and C249 and transformed into the *bldA* deletion mutant J1681. In both cases high levels of tRNA were seen in the J1681 transformants (Fig. 5). To rule out plasmid copy number differences as a possible explanation for the differences in *bldA* tRNA expression, total DNA samples from the cultures used for RNA isolation were also subjected to

Fig. 5. (a) Northern analysis of the *bldA* tRNA in RNA samples (10 µg) isolated from surface-grown *S. coelicolor bldI* mutants containing *bldA* on a high-copy-number plasmid (pIJ584). Controls included RNA isolated from: parent strains from which the mutants were derived (see Methods); the *bldA* deletion mutant *S. coelicolor* J1681; all parent and mutant strains with the same high-copy-number replicon containing the *ampC* gene (pAU2) in place of pIJ584; and in the two right-hand lanes, strain J1681 after transformation with plasmid pIJ584 from *S. coelicolor* C249 and J703 (strains from which the plasmids were isolated for transformation into J1681 are shown in parentheses). (b) The *lysTα* tRNA was probed as a control for RNA loading levels. RNA was isolated at 48 h post-inoculation unless otherwise indicated. Probes were end-labelled oligonucleotides complementary to the *bldA* and *lysTα* tRNAs (see Methods).

Fig. 6. Southern analysis for comparison of pIJ584 (a high-copy-number plasmid derivative carrying the *bldA* gene) levels in total DNA samples isolated from the *bldI* mutant, J703, and its parent strain, J650. DNA samples isolated at 24 h and 48 h are shown in (a); DNA samples isolated from a separate time-course at 20 h and 29 h are shown in (b). The probe was the *bldA*-specific, end-labelled oligonucleotide also used to detect the *bldA* tRNA in Figs 2 and 5. The arrowhead indicates the CCC form of the plasmid DNA. Slower-migrating bands most likely represent relaxed circle and linear forms of the plasmid.

Southern analysis. Both strains gave similar results, with markedly higher copy numbers at later times (Fig. 6). Densitometry of the bands corresponding to CCC plasmid DNA in Fig. 6(a) showed no difference between the two strains in plasmid levels in the 24 h DNA samples, and only a 1.4-fold difference at 48 h. Likewise the samples isolated at 20 and 29 h (Fig. 6b) showed virtually no

differences in plasmid levels between strains. Additional, slower-migrating bands, most likely corresponding to linear and relaxed circle forms of the plasmid, are also visible on the blot; however, virtually no differences were seen in the levels of these bands between the strains.

DISCUSSION

Previous studies on *bldA* transcription indicated that the gene was transcribed from a promoter that was active at all times during growth, and that the 5' processed form of the tRNA showed increased accumulation only in older cultures (Leskiw *et al.*, 1993). These results suggested that the temporal appearance of the mature form of the tRNA was not principally regulated at the transcriptional level, but rather at the level of processing of the primary transcript to give a mature 5' end. To further our understanding of regulation of the appearance of a translation-proficient form of the *bldA*-encoded tRNA, we undertook a study of the expression of the *bldA* gene in other classes of *bld* mutants of *S. coelicolor*.

When expression of the TTA-containing reporter gene *ampC* was assayed in cell extracts prepared from *bldB*, -C, -D, -F -G, -H and -I mutants, only *bldI* mutants showed a defect in *ampC* activity. Of the two proposed *bldI* strains, C249 proved to be a *bldA bldB* double mutant, and will not be discussed further. A defect in secretion of the *ampC*-specified β -lactamase could not account for the reduced activity in the *bldI* mutant J703 because the enzyme is not secreted in *Streptomyces* spp. (Häggström & Jaurin, 1985). Furthermore, the reduced *ampC* activity was not attributable to a defect in *ampC* transcription, suggesting that the *bldI* mutant is impaired in expression of the *bldA* tRNA. Indeed, when Northern analysis was used to assess mature *bldA* tRNA levels, J703 showed much lower tRNA levels than its parent strain J650, and the strikingly reduced tRNA levels were also seen when a high-copy-number plasmid containing *bldA* was introduced into J703. Since the *bldA* tRNA is required for morphological differentiation in *S. coelicolor*, these results suggest that the *bld* phenotype and the *bldA* tRNA deficiency in J703 are aspects of the same mutation. However, it is clear that the *bldI* mutation has manifestations separate from those concerned with *bldA* tRNA expression, since introducing the high-copy-number derivative of *bldA* into J703 increased the tRNA to a level similar to that in the parent strain J650, but this increase did not abolish the defect in aerial mycelium formation. (It should be noted that our experiments did not address the possibility that the *bldI* gene product plays a role in modification of the tRNA into a functional, translation-proficient form. Although this explanation would account for the inability to restore aerial mycelium after over-expression on a high-copy-number plasmid, it would not account for the reduced levels of the processed, 87 nt form of the tRNA in J703 relative to the parent J650.) That *bldI* may have effects unrelated to *bldA* expression is not entirely surprising in view of the differences in phenotypes between these *bld* mutants. *bldA* mutants show carbon-source-dependent restoration of aerial my-

celium formation, whereas the *bldI* mutant phenotype is unaffected by the carbon source (Harasym *et al.*, 1990).

The low level of mature *bldA* tRNA expression in J703, even under conditions where *bldA* was present on a high-copy-number plasmid, suggest that the *bldI* mutant may lack a positive transcriptional activator that is required for maximal *bldA* expression. The *bldI* gene product might itself interact directly with the *bldA* promoter region, or it could be required for expression of an as yet unidentified transcription factor. Whether the effect is direct or indirect, the requirement of a functional *bldI* product for maximal *bldA* expression suggests an additional level in the regulatory cascade controlling some antibiotic production and sporulation genes. Since a low level of the *bldA* tRNA is expressed in J703, the increased accumulation of the tRNA in older cultures of wild-type *S. coelicolor* may well represent transcription from a second, perhaps *bldI*-dependent, promoter. Although previous S1 nuclease analysis of *bldA* transcripts failed to reveal a second promoter, an unexplained series of bands 21–23 nt upstream of the beginning of the tRNA was present in those analyses (Leskiw *et al.*, 1993) and it is conceivable that they represent transcripts initiating at a separate promoter. Alternatively, as is the case with most tRNAs in *E. coli*, transcripts arising from a second promoter may be processed so quickly that precursor tRNA species are not seen [except, for example, in the case where the tRNA has mutations which alter the secondary or tertiary structure (King & Schlessinger, 1987)]. This very rapid processing to the mature tRNA appears to be the case for *bldA* transcripts produced late in growth (Leskiw *et al.*, 1993). As an alternative to an effect of *bldI* on *bldA* transcription, the reduced tRNA levels in the *bldI* mutant might be explained by an inability to process the primary transcript to the mature 5' processed form. Since bands corresponding to tRNA precursors were not detected by Northern analysis in this or in previous studies (Leskiw *et al.*, 1993), this possibility has not been ruled out. These questions will most easily be answered by determining the nature of the *bldI* gene product.

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