Expression of the regulatory protein LndI for landomycin E production in Streptomyces globisporus 1912 is controlled by the availability of tRNA for the rare UUA codon

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Abstract

The gene lndl encodes the activator of landomycin biosynthesis. The utilization of LndI-EGFP fusions led us to investigate the temporal pattern of this gene expression and demonstrated the delay between lndl transcription and translation. The TTA codon in lndl is thought to be the reason for this delay. The replacement of TTA with CTC cancelled the pause between lndl transcription and the translation. The wild-type of the lndl gene is not expressed in the Streptomyces coelicolor bldA/C0 mutant strain, indicating the importance of the bldA tRNA in its mRNA translation.

Introduction

Over 70% of all known antibiotics are of streptomycete origin and their production is coordinated by morphological differentiation and culture growth (Chater, 1993; Kieser et al., 2000). Such regulation prevents the suicide of producer cells and operates within a complex network of intracellular and extracellular signals. The secondary metabolism is known to be affected by: hormone-like molecules, two-component signal transduction systems, the catabolism repression system, and the utilization of minor tRNAs (for reviews see Chater, 1993; Bibb, 2005). Extensive studies on antibiotic biosynthesis in various strains have shown that all the aforementioned systems and stimuli target (either directly or indirectly) the expression of specific gene(s) encoding transcriptional factors. These genes are usually clustered with antibiotic biosynthesis genes and activate or repress their expression. Most of the transcriptional factors encoded by these regulatory genes share a considerable homology and are grouped into the SARP (for Streptomyces antibiotics regulatory proteins) or LAL (large ATP-binding regulators of LuxR family) families (Wietzorreck & Bibb, 1997; Bibb, 2005). Manipulations of the SARP genes have been shown to increase the production of antibiotics by streptomycetes (Stutzman-Engwall et al., 1992; Gromyko et al., 2004). Therefore, a deep understanding of the signals which tune the expression of regulatory proteins is of practical interest.

The presence of rare leucine TTA codons within the coding region of many SARP genes is well documented (Fernandez-Moreno et al., 1991; Wright & Bibb, 1992). The availability of the respective tRNA for the translation of such a codon can be a limiting factor in regulatory protein expression. The Streptomyces coelicolor gene bldA, which encodes tRNAUUA, shows a growth-phase dependent pattern of expression (Leskiw et al., 1993). A maximum accumulation of tRNAUUA is reached late in its growth. S. coelicolor bldA– mutants are deficient in sporulation and antibiotic production (Lawlor et al., 1987). The effects of bldA mutations are suggested to result from an inefficient expression of the regulatory genes triggering sporulation and antibiotic production. However, unequivocal data on how the bldA product (tRNAUUA) exerts its function are still absent, even for the model strain Streptomyces coelicolor. Moreover, recent studies on the regulatory gene caar showed its efficient mistranslation in bldA mutants of Streptomyces clavuligerus and S. coelicolor (Trepanier et al., 2002). Therefore,
caution should be taken when interpreting the role of the rare TTA codon in the regulation of streptomycete gene expression.

The scope of our research is genetic control of the landomycin group of polyketides. Landomycins (La) are glycosylated angucyclines with an interesting spectrum of anticancer activities. For instance, anthracycline-resistant tumour cell lines have been shown not to be cross-resistant to LaA and E (Fig. 1a) (Panchuk et al., 2004). La derivatives are thought to be potential antitumor drugs, and genes for La production have been harnessed for use in the rational design of novel polyketide antibiotics (Luzhetskyy et al., 2004; Ostash et al., 2004). Pathway-specific regulatory genes of the SARP family, controlling La and E production in *Streptomyces globisporus* 1912 and *Streptomyces cyanogenus* S136 respectively, were found within the respective biosynthesis gene clusters and studied via gene expression and disruption (Pankevych et al., 2001; Rebets et al., 2003). Following on from sequence analyses, the LndI and LanI proteins are thought to have the structural features of transcriptional regulators, which attracted our interest and prompted us to study them in more detail. LndI was shown to be a DNA binding protein regulating the transcription of its own gene as well as the structural genes for LaE (landomycin E) biosynthesis (Rebets et al., 2005). However, the signals which switch the *lndI* gene expression are not known. Gene *lndI* contains a TTA codon in the 8th position of the coding region and *lanI* in the 40th position. We wanted to determine whether *lndI* gene expression depends on the availability of *bldA* tRNA for the TTA codon. Analysis of the expression of the wild-type and mutant LndI protein translationally fused with EGFP (green fluorescence protein), in addition to evidence from heterologous expression experiments, support the suggestion that the *bldA* gene is essential for expression of the positive activator of LaE biosynthesis.

**Materials and methods**

**Bacterial strains and culture conditions**

Strains *Streptomyces globisporus* 1912-2 (LaE producer, wild type) and *S. globisporus* 12-1 (*lndI* LaE nonproducing mutant) were used for the study of *lndI*-EGFP gene expression (Rebets et al., 2003). *Streptomyces coelicolor* strain J1681 *bldA* was used to test the mistranslated *lndI*-EGFP (a gift from Prof. K. Chater, JIC, Norwich, UK). *Escherichia coli* DH5 (Life Technologies) was used for routine subcloning. *E. coli* ET12567 [dam-13::Tn9(Cmr), dcm-6, hsdM] harbouring the conjugative plasmid pUB307 (a gift from C.P. Smith, UMIST, Manchester, UK) was used to perform the intergeneric conjugation from *E. coli* to *Streptomyces globisporus*.
Vector pIJ8660 carrying the promoter-less EGFP reporter gene was used to study \textit{indl} gene expression (a gift from Dr Sun and Prof. K. Chater, JIC, Norwich, UK).

The \textit{E. coli} strains were grown at 37 °C in LB, or TB (Sambrook & Russell, 2001) for the routine applications. \textit{S. globisporus} strains were grown at 30 °C. Solid oatmeal medium (OM) (Kieser \textit{et al.}, 2000) was used for \textit{S. globisporus} sporulation and plating of the \textit{E. coli} – \textit{Streptomyces} matings. Growth dynamics, EGFP expression analysis and LaE production of the \textit{S. globisporus} 1912-1 strains was examined in TSB medium (using 120 h incubation) (Rebets \textit{et al.}, 2005). For total and plasmid DNA isolation, the \textit{S. globisporus} strains were grown for 48 h in YEME (Kieser \textit{et al.}, 2000). Where required, the \textit{S. globisporus} and \textit{E. coli} strains were grown in the presence of ampicillin, apramycin, spectinomycin, kanamycin, chloramphenicol, or nalidixic acid at previously specified concentrations (Kieser \textit{et al.}, 2000; Sambrook & Russell, 2001). The chromogenic substrates 5-Br-4-Cl-3-indolylgalactopyranoside (X-Gal) and isopropyl-\(\beta\)-D-thiogalactopyranoside (IPTG) were used for the blue-white selection.

DNA manipulations

Genomic and plasmid DNA from \textit{Streptomyces} and plasmid DNA from \textit{E. coli} was isolated using standard protocols (Kieser \textit{et al.}, 2000; Sambrook & Russell, 2001). \textit{E. coli} transformation and intergeneric \textit{E. coli} – \textit{Streptomyces} matings were performed as previously described (Kieser \textit{et al.}, 2000; Luzhetskyy \textit{et al.}, 2001). The restriction endonucleases, Klenow fragment, bacterial alkaline phosphatase, T4 DNA ligase, Taq polymerase and T4 nucleotide kinase were all purchased from standard commercial sources (MBI Fermentas, NEB, Amersham Biosciences) and used according the manufacturer’s instructions. PCR reactions were performed using an iCycler thermal cycler (BioRad). The PCR mixtures (50 μL) were loaded with 10 ng of template DNA (pSI2-9), \textit{Pfu} polymerase (1 U), 0.5 μM of (each) primer and deoxynucleoside triphosphate (25 μM of each). Products of the PCR were verified by sequencing. DNA sequencing was performed by the Sanger method using a DTCs Quick Start Kit on a CEQ2000 sequencer (Beckman Coulter). Sequences were analyzed using DNASIS software (version 2.1, Hitachi Software Engineering). Other molecular biological techniques (DNA purification, DIG DNA hybridization) were performed according to standard procedures (Sambrook & Russell, 2001).

Plasmid constructions

For \textit{indl}-EGFP fusion generation

To create the fusion between \textit{indl} and \textit{EGFP}, a PCR protocol was followed. \textit{indl} was amplified from pSI2-9 (Rebets \textit{et al.}, 2003) with the primers FlndI (TGTTCCTCGGTCTATAGGCCG) and RlndI (AGGCGCATATGCAGAACATC) (recognition sites for EcoRV and Ndel are underlined). The PCR product was cloned into pT7Blue (Novagen) T-vector (Novagen) to yield pT7Blueindl-1. The resulting plasmid was digested with Ndel, and the \textit{indl} coding region was cloned into the Ndel treated vector pIJ8660 in appropriate orientation to create pIJindl. In this way the \textit{indl}-EGFP fusion system was generated. Transcription of this hybrid gene was provided by \textit{indl} promoter.

Creation of the mutant CTC form of the \textit{indl} gene

To study the influence of the TTA codon on \textit{indl} gene expression it was modified on a synonymous leucil CTC codon. The \textit{indl} promoter region and 5’ terminus was amplified from pSI2-9 with FlndI and Rctc (TGGTCGAAGTATGAGAGTTTG) primers carrying EcoRV and BstBI sites, respectively, and cloned into pT7Blue (Novagen) using a T/A-cloning procedure, resulting in pT7CTC1 (Fig. 1). The reverse primer, corresponding to the region where the TTA codon is located, contains changes that result in a substitution of the TTA with a synonymous leucil CTC codon, which is common in streptomycete genes (Wright & Bibb, 1992; Kieser \textit{et al.}, 2000). The 3’ region of the \textit{indl} gene was amplified with Fcct (CTCTACTTCTGAACACCTGATG) and RndI primer pairs and cloned into the same vector to yield pT7CTC2. The mutant \textit{indl}(CTC) gene was reconstructed via subcloning of the EcoRV–BstBI fragment from pT7CTC1 into the respective sites of pT7CTC2 (Fig. 1). The resulting plasmid pT7indlICTC was used to retrieve the mutant CTC \textit{indl} gene, along with its promoter as an Ndel fragment which was subsequently cloned into Ndel-treated pIJ8660. The final construct pIJindlICTC was thus generated carrying \textit{indl}(CTC), translationally fused to the EGFP gene.

For \textit{indl} and \textit{indl}(CTC) expression in \textit{Streptomyces coelicolor}

An \textit{S. coelicolor} bldA\textsuperscript{−} mutant was used to study \textit{indl} gene expression. The plasmids pKEALnldl and pKEALndlICTC were constructed for this purpose. EcoRV–EcoRI fragments containing the \textit{indl} gene and its CTC variant fused to the EGFP coding region from pIJindl and pIJindlICTC respectively were cloned into the EcoRV–EcoRI digested streptomycyes expression vector pKC1218Ea (Rebets \textit{et al.}, 2005) in an orientation that provided the expression of fusion genes from the erythromycin resistance gene promoter \(P_{\text{ermE}}\).

Protein analysis

Protein concentrations were determined using the BCA protein assay (PIERCE) with bovine serum albumin (BSA)
as a standard. Growth dynamics of the *S. globisporus* strains were studied by measuring the total protein concentration in samples taken from the culture broth every 12 h of growth. A 1 mL sample of the liquid culture was washed twice with deionized water and resuspended in 10% trichloroacetic acid for 60 min at 37 °C. The cells were centrifuged and incubated in 0.4 N NaOH for 12 h.

**Western blot analysis**

*Streptomyces globisporus* cultures containing plasmids for *lndI*-EGFP and *lndICTC*-EGFP expression washed twice in deionized water and suspended in SDS-PAGE sample buffer (50 mM Tris-HCl [pH 6.8], 100 mM DTT, 2% w/v SDS, 0.1% w/v bromophenol blue, and 10% v/v glycerol) and heated in boiling water for 7 min. Total proteins were separated in SDS–12% polyacrylamide gel according to Laemmli’s methods (Sambrook & Russell, 2001). Proteins were transferred to a pre-wetted membrane (Bio-Rad) using a Mini Trans-Blot apparatus (Bio-Rad) at 100 V for 60 min in transfer buffer (25 mM Tris-HCl, 192 mM glycine, 20% methanol, pH 8 ± 1). The filters were blocked for 3 h with PBS containing 5% bovine serum (BS) and then incubated for 2 h with a 1 : 1000 dilution of anti-EGFP (anti-green fluorescence protein) polyclonal antibody (Sigma-Aldrich) in 25 mL PBS-Tween 20 containing 5% BS. Following incubation, the filters were washed twice for 5 min with 20 mL PBS-Tween 20. Following this, anti-mouse secondary antibody was added to 25 mL PBS-Tween 20 containing 5% BS, and the filters were incubated for 1 h and then washed twice with 20 mL PBS-Tween 20 containing 5% BS. Alkaline phosphatase detection was performed following standard procedures (Sambrook & Russell, 2001).

**Landomycin E production analysis**

*Streptomyces globisporus* culture broths were extracted three times with ethylacetate, dried in *vacuo* and dissolved in methanol. The extract was subjected to TLC (thin layer chromatography). The TLC was performed according to methods (Luzhetskyy et al., 2001; Ostash et al., 2004) on Alugram SIL G/UV254 plates (Macherey Nagel). The fraction corresponding to LaE was scraped from the TLC plate and twice extracted from the silica gel with 300 μL of methanol (Merck). The LaE concentrations in the solutions was determined spectrophotometrically on a Helios MT13 machine (Heraeus) by measuring absorption values at 442 nm and plotting them against an LaE calibration curve. The levels of LaE production in our results were referred back to equal amounts of total protein in the different strains. The measurements were done in triplicate and averaged.

**Sample preparation and confocal microscopy**

For the GFP production analysis mycelia grown in the TSB media were washed with deionized water and applied to glass slides. A Fluoroview confocal system (Olympus) with an Olympus OL BX50 microscope and an argon laser (providing excitation at 488 nm) was used to study the *S. globisporus* strains with GFP expression. Fluorescein-isothiocyanate (FITC) (emission at 506–535 nm) filters were used to observe the green fluorescence. The green fluorescence and transition images were obtained simultaneously using separate detectors. To ensure a high reliability in the quantitative analysis of the captured images, the same operational parameters were used for each sample at the same time point. The confocal images were saved as TIFF files and the image analysis was done using Fluoview 2.1 software.

**Results and discussion**

**Studying the temporal pattern of *LndI* expression using EGFP fusion**

C-terminal translational fusion of the *lndI* gene to the EGFP reporter gene was created in order to study the temporal pattern of its coupled transcription and translation. The EGFP variant used in the pIJ8660 reporter vector does not contain TTA codons, thus giving rise to the absence of any artefacts relative to the purpose of our study (Sun et al., 1999). pIJlndI (Fig. 1b) provides *lndI*-EGFP fused gene expression from the *lndI* promoter and allows us to study both the transcription and translation of *lndI*. pIJlndI+ transconjugants of *Streptomyces globisporus* 1912-2 were obtained and shown to carry two copies of plasmid-integrated site-specificity as was the case in our previous experiment using the pSET152 vector (Luzhetskyy et al., 2001). *Streptomyces globisporus* 1912-2 (pIJlndI) grown on TSB media for 60 h produced LaE at the level of a parental strain, or a little more, while the expression of *lndI* on the pSET152 vector in *S. globisporus* was shown to increase LaE production 11-fold (Gromyko et al., 2004). Such a difference in LaE production stimulation when using the same vector system can be attributed to a lost of efficiency of the LndI-EGFP fused protein in binding DNA in comparison to LndI alone, resulting in a weaker activation of antibiotic production. Plasmid pIJlndI was introduced into an *lndI* disrupted LaE nonproducing mutant, *S. globisporus* I2-1, to ensure that the LndI-EGFP fusion protein was functional. LaE production was restored in I2-1 (pIJlndI) transconjugants. Thus, the C-terminal translational fusion of LndI to EGFP did not interrupt LndI activity, but only decreased its ability to activate LaE production. The LndI DNA-binding region probably resides in the C-terminal portion of the protein; however the possibility of its influencing fusion on the total protein structure can not be excluded.
Protein extracts from the *S. globisporus* wild-type and pIJlndI-harbouring strains grown in TSB media, were analysed by Western blot to determine whether pIJlndI directs the production of a LndI-EGFP fused protein in *S. globisporus* cells. Hybridization signals corresponding to a 28 kDa protein (the monomeric form of EGFP) were seen in the case of protein extracts from *Escherichia coli* (pGLO) and *S. globisporus* 1912-2 (pIJ8660 H) cultures (Fig. 2) (Rebets et al., 2005). Both strains express native GFP and served as positive controls in our study. One signal at 56 kDa was detected in the *S. globisporus* 1912-2 (pIJlndI) protein extract, while no signals were seen at the same position in the wild-type extracts (data not shown). No signals at 28 kDa were detected in the *S. globisporus* 1912-2 (pIJlndI) extracts. We conclude that the LndI-EGFP fusion protein is expressed efficiently from the *lndI* gene promoter. For all future experiments mentioned herein, crude protein extracts of *S. globisporus* cultures grown for 12, 24 and 48 h were examined for LndI-EGFP production (Fig. 2). In the case of 1912-2 (pIJlndI) extracts, positive hybridization signals were obtained for protein extracts prepared from 24 h and 48 h old culture. In case of 12 h old culture, no signals were observed, indicating the absence of a fused protein in the early stages of growth.

In our previous studies we found that *S. globisporus* 1912-2 mycelia did not possess a level of fluorescence that would interfere with the EGFP expression analysis (Rebets et al., 2005). Strain *S. globisporus* 1912-2 (pIJlndI) was examined for green fluorescence. The 48 h old culture showed green fluorescence, but it was not as strong as that observed in from *S. globisporus* (pIJ8660 H).

The expression of *lndI*-EGFP was monitored by observing fluorescence using laser-scanning confocal microscopy. No fluorescence was observed after 12 h of growth of *S. globisporus* 1912-2 (pIJlndI) culture (Fig. 3). At 24 h, a very weak fluorescence was seen, indicating the start of LndI expression. The fluorescence level reached a maximum at between 48 and 60 h of culture growth. At this time, which corresponded to the most active LaE production, the green fluorescence was concentrated in some regions of the mycelia. We therefore suggest that LndI actively binds to the promoters of *lnd*-genes in the chromosome of *S. globisporus*, leading to an accumulation of green fluorescence dots in the area of the nucleoids. A similar effect was observed in the case of cephamycin C and the clavulanic acid biosynthesis regulator CcaR in *S. clavuligerus* (Kyung et al., 2001).

After 60 h of incubation the level of fluorescence decreased significantly showing that the culture had reached the late stationary phase of growth and partial autolysis had started (Fig. 3). In this phase *lndI* expression and LaE production had decreased, as was shown in our previous studies on *lndI* gene transcription (Rebets et al., 2005).

In the case of *S. globisporus* 1912-2 harbouring the plasmid pIJ8660 H with *lndI* promoter transcriptionally fused to EGFP reporter, fluorescence was observed for 12 h after inoculation (Rebets et al., 2005). In contrast, in strain 1912-2 expressing the LndI-EGFP translational fusion, both fluorescence and fused protein production appeared exactly after 24 h of growth. We suggest that a gap exists between *lndI* mRNA production and its translation (Fig. 3). This might be caused by the regulatory role of *bldA* tRNA for the TTA codon which is present in the *lndI* gene, and its translation may be a limiting factor for LndI production.

**Mutant allele of the *lndI* gene with a replaced TTA codon is expressed without a delay in its mRNA translation**

A mutant *lndI* gene lacking the TTA codon has been constructed to verify our idea. For this purpose a PCR procedure was used with two sets of primers that result in the replacement of a TTA codon on synonymous CTC
within the lndI coding region. pIJlndICTC was generated, carrying lndI(CTC) translationally fused to the EGFP gene. An S. globisporus wild-type strain carrying pIJlndICTC plasmid was obtained and analyzed as described above for the S. globisporus 1912-2 (pIJlndI) strain. A Western blot analysis showed that protein extracts prepared from even a 12 h old culture of 1912-2 (pIJlndICTC) contained the fused protein (Fig. 2). An S. globisporus 1912-2 (pIJlndICTC) strain had been generated and shown to produce LaE, meaning that the mutant LndI(CTC) protein retained its ability to activate LaE biosynthesis structural genes. Confocal microscopy confirmed the results of the Western blot analysis, and showed a weak fluorescence of the 12 h old 1912-2 (pIJlndICTC) culture, which became brighter after 24 h (Fig. 3). Between 48 and 60 h incubation the fluorescence intensity reached a maximal level, indicating the highest level of LndI expression. After 60 h, fluorescence decreased as was observed in the S. globisporus 1912-2 (pIJlndI) and S. globisporus 1912-2 (pIJ8660 H) cultures.

We could find no delay between lndI transcription and its translation for the mutant (CTC) gene. The fluorescence intensity of S. globisporus 1912-2 (pIJlndICTC) mycelia was 40–50% higher than that of the S. globisporus 1912-2 (pIJlndI) culture, but still less bright than for the pIJ8660H-harbouring strain (Fig. 4). The bldA encoded leucine tRNA should act at the stage of lndI mRNA transcription. If leucil tRNA/CTC is not available in the early stages of S. globisporus growth (e.g. during the first 12–24 h of incubation), the TTA codon within lndI would cause a delay between transcription and translation of this gene, as the analysis of lndI-EGFP transcriptional and translational fusions showed. Replacement of the rare TTA codon with a more efficiently used CTC encoding the same amino acid cancelled the delay between lndI gene transcription and translation, as was evident from the results of both Western blotting and microscopy. In addition, TTA substitution led to a higher fluorescence intensity, meaning that utilization of the TTA codon affected both the temporal pattern of gene expression and the amount of LndI-EGFP accumulation in mycelia. We could therefore tentatively assume that the availability of tRNA for rare the TTA codon was one of the regulatory mechanisms governing LndI protein production.

**Wild-type lndI gene is not expressed in Streptomyces coelicolor bldA mutant**

As a complementary approach to confirm the role of the TTA codon in regulation of LndI production, both wild-type and mutant lndI genes fused to EGFP were expressed in a Streptomyces coelicolor bldA mutant. Because this strain is apramycin resistant, pKCEalndI and pKCEalndICTC plasmids were constructed to provide essentially the same copy number in S. coelicolor as does pIJ8660 in S. globisporus, and expression of fused constructs is driven from P_{ermE}. Both plasmids were introduced by mean of intergeneric conjugation into both S. coelicolor bldA and wild-type strain M145. The expression of both variants of the lndI-EGFP fusion genes was monitored by fluorescence measurements during their growth in TSB media. For S. coelicolor M145 harbouring either pKCEalndI or pKCEalndICTC, a bright green fluorescence of the mycelia was observed after 48 h of incubation. Strong fluorescence was also detected when examining S. coelicolor bldA (pKCEalndICTC). However, for S. coelicolor bldA harbouring the wild-type lndI-EGFP fusion, fluorescence was not detected, even after 48–60 h of growth, implying that LndI-EGFP was not produced (data not shown). These results show that bldA tRNA is indeed important for lndI gene expression and that the TTA codon is not mistranslated in lndI mRNA in the S. coelicolor bldA mutant at least under conditions tested.

It is known that bldA tRNA affects secondary metabolism, morphological differentiation, spore formation, antibiotic resistance, alternative substrate utilization and other processes in Streptomyces. Mutations in the bldA gene result in the absence of aerial mycelia, spore formation and the production of both pigmented antibiotics by S. coelicolor.
(Lawlor et al., 1987). The gene bldA exerts its influence on secondary metabolism through the control of pathway-specific regulatory genes, of which almost all are known to contain TTA codon(s). However in some cases the mistranslation of this codon has been seen. The increase of copies of actII-ORF4 in the bldA mutant of S. coelicolor restored the production of the respective antibiotics (Panchuk et al., 2004). The TTA codon is efficiently mistranslated in both cephemycin C and the clavulanic acid producer S. clavuligerus (Trepanier et al., 2002). The same was also observed for several other TTA-containing antibiotic resistance and reporter genes in Streptomyces lividans and S. coelicolor (Leskiw et al., 1991; Kataoka et al., 1999).

We showed that the availability of leucyl tRNA$^{\text{UA}}$ in S. coelicolor and S. globisporus is a limiting factor for lnld gene expression. The presence of a rare TTA codon within lnldI caused the delay between the gene transcription and translation of its mRNA. Replacement of this rare codon with a more efficiently used CTC codon encoding the same amino acid resulted in the absence of such a delay in lnld gene expression. This replacement also led to an increase in fusion protein production, indicating that the mutant lnldI(CTC) can be used in approaches towards the improvement of La production.

Unlike lnldI(CTC), the wild-type lnldI gene is not expressed in the bldA mutant of S. coelicolor. We did not observe lnldI mistranslation. In most of the cases studied, mistranslation of TTA codon was shown to depend on experimental conditions. Additional copies of actII-ORF4 restore actinorhodin production by the bldA mutant growing on R2YE medium but not on SMMS medium (Passanti-no et al., 1991). Additional experiments on the cloning of the bldA gene from S. globisporus 1912-2 and a study of the lnldI expression in the S. globisporus bldA mutant of this strain are still required in order to understand the bldA-mediated mechanism of lnldI gene expression regulation.

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