Expression of the *Streptomyces alboniger pur* cluster in *Streptomyces lividans* is dependent on the *bldA*-encoded tRNA^{Leu}

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Abstract ***Streptomyces lividans* 1326-9, a *bldA*$^+$ strain, and its *bldA39* mutant derivative J1725 were transformed with a cosmid containing the *pur* cluster, which determines the puromycin biosynthetic pathway from *Streptomyces alboniger*. *bldA*$^+$ transformants produced puromycin in typical amounts, whereas *bldA39* transformants did so at drastically decreased levels. Transformation of low producers with the wild-type *bldA* gene reverted this phenotype to normal production. These data, in addition to the presence of a TTA codon in the amino-terminal coding region of the *pur10* and *pur6* genes of the *pur* cluster, suggest that the puromycin biosynthetic pathway is translationally dependent on the *bldA* gene product, a tRNA^{Leu}.**

Key words: *pur* cluster; Puromycin biosynthesis; *bldA* gene; *pur* regulation; *Streptomyces*

1. Introduction

The *bld* mutants of *Streptomyces coelicolor* are deficient in the formation of aerial mycelium despite their apparent normality in vegetative growth. In addition, most are blocked in the synthesis of a variety of antibiotics [1–3]. This establishes a link between the morphological and physiological differentiation of these organisms and is therefore the subject of studies to find the mechanisms regulating both processes. *bldA* encodes the single tRNA^{Leu} which recognizes the UUA codon [4,5]. This codon is extremely rare in *Streptomyces* mRNAs, given the high content of G/C in their DNA (approximately 73%). The presence of TTA codons is almost restricted to those genes which are implicated in the processes of either production of secondary metabolites or morphological differentiation. It has thus been suggested that the *bldA* gene may act as a translational regulator of this type of genes [6]. The existence of *bldA* mutants in *Streptomyces lividans* and *S. griseus* [5,7] sharing similar phenotypes to those found in *S. coelicolor* suggests that translational regulatory mechanisms by *bldA* homologues may be a general feature of *Streptomyces*.

The *pur* cluster, which determines the biosynthetic pathway of puromycin from *Streptomyces alboniger*, has been cloned in the heterologous host *S. lividans*, a puromycin non-producing organism. In these transformants, puromycin biosynthesis was regulated during growth in a similar manner to that in *S. alboniger* [8]. This finding suggests that either a regulatory system is included in *pur* or it is present in *S. lividans*. However, the *pur* cluster, which was completely characterized (Fig. 1), lacks any apparent regulatory gene [9]. Therefore, its regulation should be dependent on common mechanisms within *S. lividans* and *S. alboniger*. In this respect, two genes of this cluster, *pur10* and *pur6* (Fig. 1) contain a TTA codon within their amino-terminal coding regions (Leu3 for *pur6* and Leu29 for *pur10*) [9]. This suggests that both genes, and consequently puromycin biosynthesis, may be regulated/dependent by/on the *bldA* product. Moreover, the *pur10* gene product (Pur10) appears to catalyze the first step of the pathway, which makes it a most likely target for translational control. In the present work, the expression of the *pur* cluster is studied in *S. lividans bldA*$^+$ and *bldA39* mutant strains. The results indicate that in this organism puromycin biosynthesis is indeed dependent on the *bldA* product.

2. Materials and methods

2.1. Strains, plasmids, media and DNA methodology

The isogenic strains wild-type *S. lividans* 1326-9 [10] and its *bldA39* mutant derivative *S. lividans* J1725 [5] were provided by Dr. B.K. Leskiw (Edmonton, Canada). *Escherichia coli* DH5 was described [11]. The *Streptomyces-E. coli* cosmid pPB5.13, a pKC505 derivative construct [12], which carries the *pur* cluster [8], *Streptomyces* vectors pIJ486 [13] and its *bldA*-containing pIJ584 plasmid [14] have been described. Plasmid DNA from *Streptomyces* and *E. coli* was prepared as described [15]. *Streptomyces* were grown in solid media R5 [15]. Puromycin production liquid medium for *Streptomyces* was S medium containing starch as a carbon source [8]. *Streptomyces* were grown at 30°C. Liquid and agar media for *E. coli* were Luria-Bertani and Luria-Bertani plus 2% agar respectively [16]. Transformation of *E. coli* and *Streptomyces* was performed as described [15]. *Streptomyces* transformants were selected on R5 plates in the presence of 25 μg/ml apramycin and/or 10 μg/ml thiostrepton [12,15]. The presence of cosmid DNA was confirmed by transforming *E. coli* DH5 with DNA isolated from *S. lividans* transformants. *E. coli* transformants were selected on LB plates containing 100 μg/ml apramycin. Plasmid structures were examined by restriction endonuclease analyses of isolated plasmid DNA.

2.2. Puromycin production

To determine production profiles, transformants were grown in flasks containing 100 ml S medium, supplied with the required antibiotic(s), and puromycin concentration was quantitated in culture filtrates by the Pac assay [8].

2.3. Enzymic activities

Pac (puromycin-*N*-acetyltransferase) and NapH (*N*-acetylpuromycin-*N*-acetylhydrolase) activities were determined in cell-free extracts and in cultures filtrates [8,17], respectively, from samples taken at different intervals during the growth curves of *Streptomyces* strains.

3. Results

3.1. Puromycin production in S. lividans bldA$^+$ and bldA39 mutant containing pur

To study the effect of *bldA* on puromycin production, the *bldA*$^+$ strain *S. lividans* 1326-9 and its *bldA39* mutant deriva-

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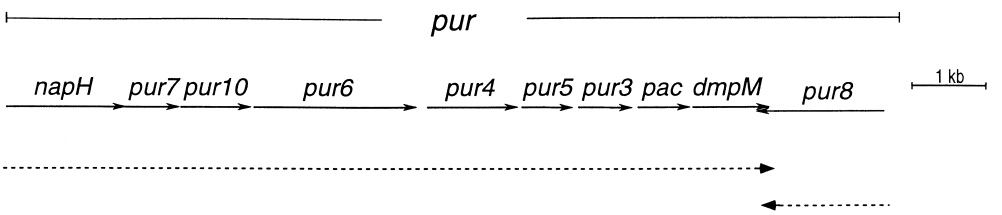

Fig. 1. Gene organization of the *pur* cluster. This figure is modified from [9]. *NapH*, *pac*, *dmpM* and *pur8* encode *N*-acetylpuromycin-*N*-acetylhydrolase, puromycin-*N*-acetyltransferase, 0-demethylpuromycin-0-methyltransferase and a transmembrane protein which confers puromycin resistance, respectively ([9], and references therein). *pur7*, *pur10*, *pur6*, *pur4*, *pur5* and *pur3* encode putative NTP pyrophosphohydrolase, oxidoreductase, tyrosinyltransferase, aminotransferase, methyltransferase and monophosphatase activities, respectively [9]. Size of the genes is indicated by continuous arrows. Dotted arrows indicate the length and direction of transcription of *pur* transcripts [9].

tive *S. lividans* J1725 were transformed with the *pur*-containing cosmid pPB5.13. Puromycin production profiles were studied during the growth curve of a representative of both types of transformants. As Fig. 2 shows, the production of puromycin by *S. lividans* 1326-9(pPB5.13) reached maximum values (7.5 nmol per ml) and displayed a profile similar to those found previously for *S. lividans* 1326(pPB5.13) and *S. alboniger* [8]. In contrast, the puromycin production levels by *S. lividans* J1725(pPB5.13) were drastically reduced (0.18 nmol per ml, at the highest). These results suggest that *bldA* affects puromycin biosynthesis in some way.

3.2. Reversion of the puromycin non-producing phenotype by *bldA*

To prove that the *bldA* gene played a role in the expression of *pur*, the *S. lividans* 1326-9(pPB5.13) and *S. lividans* J1725(pPB5.13) strains were transformed with the *bldA*-containing pIJ584 plasmid, which is a derivative of vector pIJ486. Puromycin production was again determined during the growth curves of a typical representative of both types of transformants. Both types showed a similar profile of puromycin production (Fig. 2). As a control, an *S. lividans* J1725(pPB5.13) transformed with the pIJ486 vector, did not produce puromycin (data not shown). Therefore, the reversion of the non-producing phenotype in the relevant strain was due exclusively to *bldA*.

3.3. Study of the Pac and NapH activities

To gain additional information on the dependence of *bldA* by *pur*, the Pac and NapH enzymic activities, which catalyze specific steps of the puromycin biosynthetic pathway [8,17], were determined during the growth curves of *S. lividans* 1326-9(pPB5.13) and *S. lividans* J1725(pPB5.13). The genes encoding these enzymes lack TTA codons. Table 1 shows that in the *bldA39*(pPB5.13) strain NapH and Pac activities were produced, although at a lower rate of one and two order of magnitude, respectively, relative to the *bldA*$^+$(pPB3.15) strain. This result indicates that the expression of TTA-lacking genes of the *pur* cluster is also affected by the *bldA* gene.

4. Discussion

This work has addressed aspects of the mechanisms which affect the biosynthesis of the aminonucleoside antibiotic puromycin. The results clearly show that in *S. lividans*, a heterologous host, the *bldA* gene plays a key role on the expression of the *pur* cluster from *S. alboniger*. Thus, whereas a *bldA* mutant strain almost does not produce puromycin, the *bldA*$^+$ strain is able to express *pur*. Moreover, the non-producing phenotype is reverted by the *bldA* gene. Because the puromycin production in *S. lividans* (*pur*) is regulated during growth in a similar way to that in *S. alboniger* [8], it may be inferred that in the latter organism, expression of *pur* is also dependent on the *bldA* gene. Indeed, two genes of *pur*, *pur6* and *pur10*, contain a TTA codon at their amino-terminal ends [9], which suggests that their coding sequences can not be translated in the absence of a *bldA* wild-type gene. In addition, we have shown that the expression in the *S. lividans bldA* mutant strain of two other *pur* genes, *napH* and *pac*, which lack TTA codons, is reduced to different extents with respect to that in the *bldA*$^+$ strain. This finding suggests that the lack of translation of coding sequences which carry TTA codons affects expression of additional genes of *pur*. In fact, this result might be expected considering that all the structural biosynthetic genes of *pur* are transcribed in a single polycistronic mRNA and, therefore, they should be regulated as a unit [9] (Fig. 1). The *napH* gene is the first to be translated whereas *pac* is located downstream of *pur10* and *pur6* [9] (Fig. 1). It is thus to be expected that in the *bldA39* strain, similarly to what happens with antibiotics which prevent aminoacyl-tRNA binding to the ribosomal A site (i.e. tetracyclin) [18], UUA codons at this ribosomal site would stop translation because of a lack of the cognate tRNA^{Leu}. Consequently, this will freeze the ribosomes and prevent new rounds of translation as well as the ribosomes from reaching the genes located downstream of UUA codons. Under these conditions, some ribosomes should detach from mRNA and translation of *pur* would occur at low levels [18,19]. We would, therefore, expect a slowdown of the rate of translation of genes (i.e. *napH*; Fig. 1) which are located upstream of genes encoding UUA codons (*pur10* and *pur6*) and very low translation rate of genes located downstream of these genes (i.e. *pac*; Fig. 1), as it has been found experimentally (Table 1).

Table 1
Values of Pac and NapH activities at different intervals of the growth curves of two different *Streptomyces* strains

Optical density[a]	*S. lividans* 1326-9(pPB5.13)		*S. lividans* J1725(pPB5.13)	
	NapH[b]	Pac[c]	NapH[b]	Pac[c]
2.0	19.2	7.5	1.3	0.06
3.0	43.3	18.1	3.2	0.09
4.5	50.3	26.6	3.8	0.19

[a]Determined at 660 nm.
[b]Determined as nmol of hydrolyzed *N*-acetylpuromycin per min per mg of protein.
[c]Determined as nmol of acetylated puromycin per min per mg of protein.

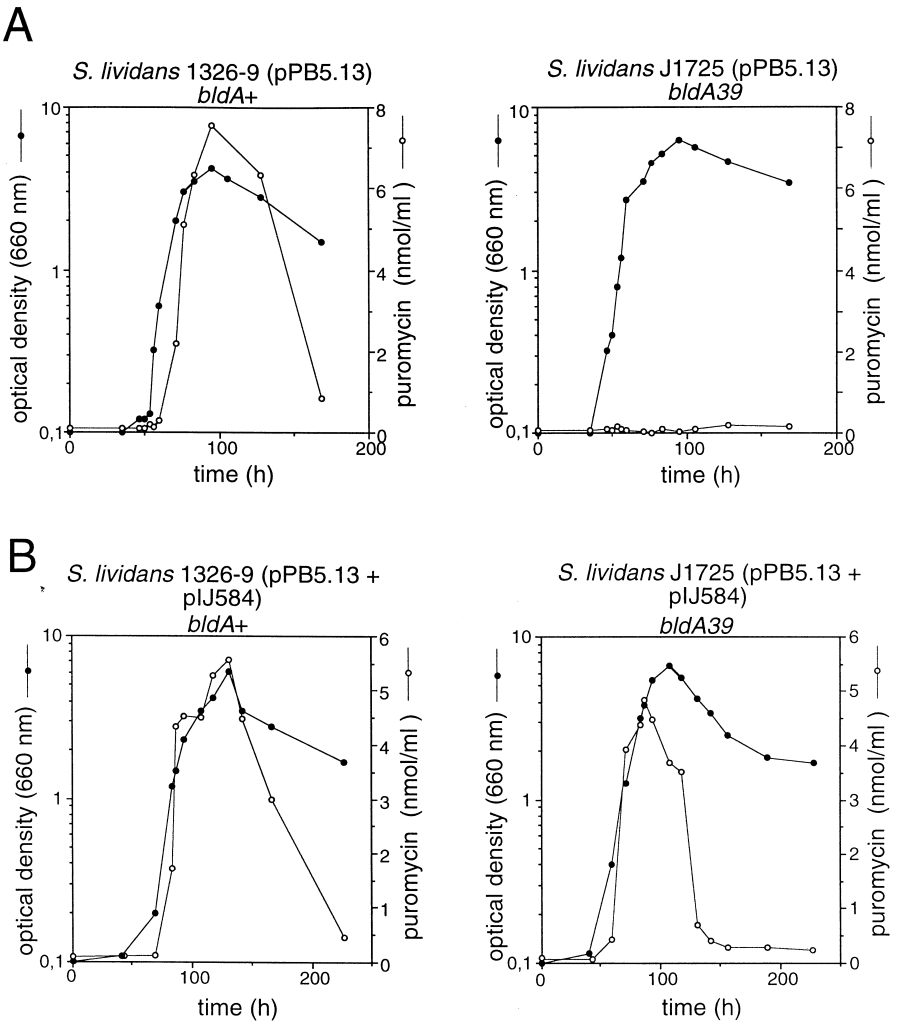

Fig. 2. A: Puromycin production during growth of *S. lividans* 1326-9 (*bldA*$^+$) and J1725 (*bldA39*) each carrying the *pur* cluster-containing pPB5.13 cosmid [8]. B: As in A except that both strains were transformed with the *bldA*-containing pIJ584 plasmid [14]. Cells were grown on liquid S medium at 30°C. At the indicated intervals, the optical density at 660 nm was determined and samples of the cultures were removed immediately to quantitate puromycin by the enzymic Pac assay from the relevant filtrates.

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