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Growth Phase-Dependent Transcription of the *Streptomyces ramocissimus tuf1* Gene Occurs from Two Promoters

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The *str* operon of *Streptomyces ramocissimus* contains the genes for ribosomal proteins S12 (*rpsL*) and S7 (*rpsG*) and for the polypeptide chain elongation factors G (EF-G) (*fus*) and Tu (EF-Tu) (*tuf*). This kirromycin producer contains three *tuf* or *tuf*-like genes; *tuf1* encodes the regular EF-Tu and is located immediately downstream of *fus*. In vivo and in vitro transcription analysis revealed a transcription start site directly upstream of *S. ramocissimus tuf1*, in addition to the operon promoter *rpsL*p. Transcription from these promoters appeared to be growth phase dependent, diminishing drastically upon entry into stationary phase and at the onset of production of the EF-Tu-targeted antibiotic kirromycin. In surface-grown cultures, a second round of *tuf1* transcription, coinciding with aerial mycelium formation and kirromycin production, was observed. The *tuf1*-specific promoter (*tuf1*p) was located in the intercistronic region between *fus* and *tuf1* by high-resolution S1 mapping, in vitro transcription, and in vivo promoter probing. During logarithmic growth, the *tuf1*p and *rpsL*p transcripts are present at comparable levels. In contrast to *Escherichia coli*, which has two almost identical *tuf* genes, the gram-positive *S. ramocissimus* contains only *tuf1* for its regular EF-Tu. High levels of EF-Tu may therefore be achieved by the compensatory activity of *tuf1*p.

The polypeptide chain elongation factor Tu (EF-Tu), responsible for mediating the binding of aminoacyl-tRNA to the translating ribosome, is one of the most abundant proteins in the prokaryotic cell; in *Escherichia coli*, EF-Tu can constitute up to 10% of the total cellular protein under rapid growth conditions (35). Two unlinked, nearly identical copies of the *tuf* gene, which encodes EF-Tu, are present in *E. coli* (2, 19, 43) as well as in other gram-negative bacteria (13, 29). This *tuf* gene duplication has been suggested to be required to maintain the high levels of EF-Tu during rapid growth (2). In contrast, most gram-positive bacteria contain only a single copy of *tuf* (13, 29).

The *E. coli tufA* and *tufB* genes contribute about equally to the total EF-Tu concentration and are regulated coordinately under most growth conditions (35). The *tufA* gene is the promoter-distal gene in the *str* or S12 operon (27), which also includes the genes for ribosomal proteins S12 (*rpsL*) and S7 (*rpsG*) and for EF-G (*fus*). This operon organization is typical of the major *tuf* gene in eubacteria (reference 11 and references therein) and some archaea (20). The *str* operon is expressed from a promoter upstream of *rpsL*, via a polycistronic mRNA (19). Furthermore, weak secondary promoters were reported within the coding region of the *fus* gene (3, 44–46), which are exclusively used for transcription of *tufA*. The *tufB* gene is cotranscribed with four upstream tRNA genes (21) and is processed into separate tRNAs and a *tufB* mRNA (33).

The gram-positive streptomycetes are soil bacteria that undergo a complex process of morphological differentiation. The vegetative mycelium produces aerial hyphae which septate and differentiate into spores at their termini. The onset of morphological differentiation usually coincides with the production of a number of secondary metabolites, including a wide variety of antibiotics (9). In liquid cultures, antibiotic production is generally confined to the stationary phase.

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Streptomyces ramocissimus produces the antibiotic kirromycin, which immobilizes the EF-Tu–aminoacyl-tRNA complex on the ribosome and thus inhibits protein synthesis. *S. ramocissimus* EF-Tu is sensitive to kirromycin (34), which indicates that resistance of this organism against its own antibiotic is not due to alteration of the target protein EF-Tu. Recently it was shown that *S. ramocissimus* contains three strikingly divergent *tuf* genes (40). The *tuf1* gene is located in the *str* operon and encodes the kirromycin-sensitive elongation factor EF-Tu1. While *tuf1* is expressed at a very high level, no expression of *tuf2* or *tuf3* has been detected so far during normal growth, and apparently these genes do not contribute significantly to the total EF-Tu pool in *S. ramocissimus* (40).

The aim of this work was to study in detail the transcription of the *S. ramocissimus tuf1* gene and to assess the relationship between *tuf1* transcript levels, growth phase, and kirromycin production. Our results indicate that, in addition to the major promoter for the *str* operon, another transcription start site, located in the *fus-tuf1* intergenic region, makes an important contribution to the high EF-Tu1 level in the cell. Transcript levels of the promoters varied greatly during growth, perhaps reflecting the growth phase-dependent synthesis of different σ factors (7). The consequences of the additional promoter *tuf1*p in relation to the presence of only one active major *tuf* gene in *S. ramocissimus* is discussed.

MATERIALS AND METHODS

Bacterial strains, plasmids, and DNA manipulations. *E. coli* JM101 (28) and ET12567 (23) were used as hosts for pUC18 derivatives (42). *S. ramocissimus* B7 and *S. coelicolor* M145 were obtained from Gist-brocades NV, Delft, The Netherlands, and the John Innes Centre, Norwich, United Kingdom, respectively.

pISRT1*xylE*-1, pISRT1*xylE*-2, and pISRT1*xylE*-3 were made by cloning fragments of the *tuf1*-containing plasmids pUSRT1 or pUSRT1-1 (40) via pUC18 into the *xylE*-based promoter probe vector pIJ4083 (10). Inserts were *Bgl*II/*Nru*I (−1180 to −180) in pISRT1*xylE*-1, *Nru*I/*Bcl*I (−180 to +280) in pISRT1*xylE*-2, and *Sma*I/*Bcl*I (−70 to +280) in pISRT1*xylE*-3 (Fig. 1).

All DNA manipulations were performed according to standard protocols (28). Transformation of *S. coelicolor* M145 was performed as described elsewhere (17).

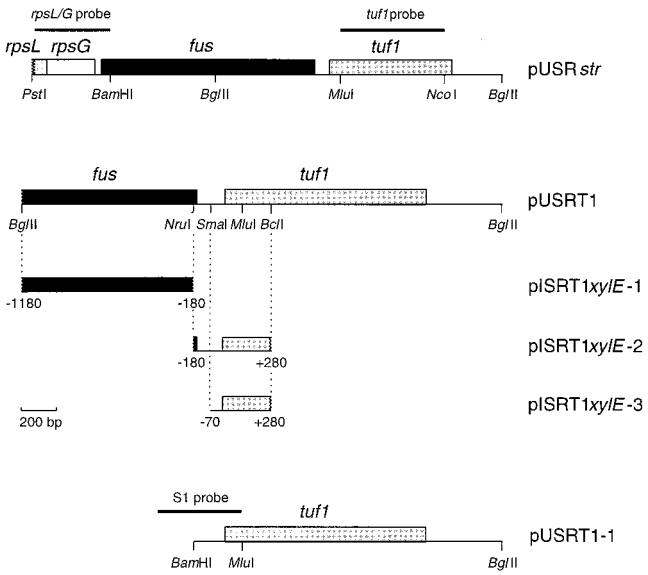

FIG. 1. *S. ramocissimus fus-tuf1* constructs. Only the plasmid inserts are shown. *fus* and *tuf1* are part of the *str* operon and were cloned in pUC8 to give pUSRT1 (40, 42). The pISRT1*xylE* constructs were made by inserting fragments into the multiple cloning site of pIJ4083 and were used for promoter probing. Numbers under inserts correspond to nucleotide positions relative to the translation start site of *tuf1*. Only the important *Sma*I and *Nru*I sites are shown. The scale applies to construct pUSRT1 and derivatives.

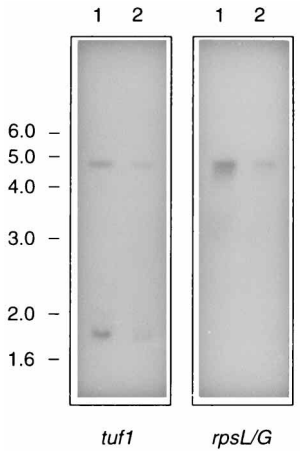

FIG. 2. Northern blot analysis of total RNA from *S. ramocissimus*, isolated during exponential growth. Filters were probed with the *tuf1* probe (left panel) and the *rpsL*/*G* probe (right panel) indicated in Fig. 1. Lane 1 and 2 contained 5 and 2 μg of total RNA, respectively. The 1-kb ladder (Gibco BRL) was used as DNA size marker (sizes are given in kilobases).

Culture conditions. *E. coli* JM101 and ET12567 were cultured in LB medium (28). *S. coelicolor* M145 was grown in YEME and on R2YE (17). High-titer spore suspensions of *S. ramocissimus* B7 were obtained from SFM plates (8). Reproducibly dispersed growth (doubling time of 2.5 h) was obtained when spores were pregerminated in 2× YT medium (4) for 8 h, subsequently inoculated in NMMP containing 1% (wt/vol) glucose (17), and grown at 28°C with vigorous shaking (300 rpm). Estimates of growth were made by measuring optical density at 450 nm (OD_{450}). Kirromycin production was determined by extraction of the filtrate with ethyl acetate and analysis by thin-layer chromatography. Surface-grown cells of *S. ramocissimus* B7 were cultured on AMMAT medium, containing, per liter, 375 mg of $NaNO_3$, 375 mg of K_2HPO_4, 1.725 mg of *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), 337.5 mg of $CaCl_2$, 3.75 mg of $CuSO_4$, 7.5 mg of $FeSO_4$, 127.5 mg of $MgCl_2$, 30 mg of $MnSO_4$, 7.5 mg of $ZnSO_4$, 337.5 mg of Tyr, 337.5 mg of Met, 337.5 mg of Leu, 1% (wt/vol) mannitol, 0.1% (vol/vol) trace element solution (17), and 7.5 g of plant tissue agar (pH 7.1). Spores were plated on cellophane disks to facilitate harvesting of the mycelium. Morphology of the surface-grown cultures was determined by phase-contrast microscopy, while kirromycin secretion into the agar was detected by using *E. coli* JM101 as the indicator strain.

Promoter-probe experiments. The *xylE* gene from *Pseudomonas putida* was used as a reporter gene (47) for in vivo detection of promoter activity. Fragments containing various parts of the *tuf1* upstream region were cloned upstream of the promoterless *xylE* gene of pIJ4083 (10). Transformants containing pISRT1*xylE*-1, pISRT1*xylE*-2, and pISRT1*xylE*-3 were grown on R2YE agar plates in the presence of 25 μg of thiostrepton (a gift from Squibb, Princeton, N.J.) per ml. Plates were sprayed with 0.5 M catechol after 2, 3, 4, and 5 days of growth, and the amount of catechol converted into yellow 2-hydroxymuconic semialdehyde by catechol 2,3-dioxygenase was assessed visually.

RNA isolation. RNA was isolated as described by Hopwood et al. (17) from cultures of *S. ramocissimus* B7 grown in NMMP liquid medium containing 1% glucose and from surface-grown *S. ramocissimus* B7 cultured on AMMAT medium. RNA concentrations were determined spectrophotometrically, and the quality of the preparations was checked by gel electrophoresis.

Northern blotting. RNA samples were glyoxylated and run in 1% agarose in 15 mM NaH_2PO_4-Na_2HPO_4 buffer (pH 6.5). After electrophoresis, the RNA was transferred onto Hybond N^+ nylon membranes (Amersham), using 25 mM NaH_2PO_4-Na_2HPO_4 (pH 6.5) as the blotting buffer. The filters were hybridized for 16 to 20 h at 65°C in 10 ml of hybridization solution consisting of 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.6% sodium dodecyl sulfate, 1% blocking reagent (Boehringer), and DNA probes ^{32}P labelled by random priming (12). DNA probes (10 to 50 ng) were the 1.0-kb *Mlu*I/*Nco*I fragment from the *S. ramocissimus tuf1* gene (designated *tuf1*) and the 0.75-kb *Pst*I/*Bam*HI fragment containing part of the *S. ramocissimus rpsL* gene and the entire *rpsG* gene (designated *rpsL*/*G*). Filters were washed twice in 2× SSC–0.1% sodium dodecyl sulfate at 65°C for 30 min.

Nuclease S1 protection studies. Hybridization of 10 or 40 μg of RNA with the appropriate DNA probe was performed as described previously (26) in sodium trichloroacetate-based buffer (32). All subsequent steps were carried out as described previously (15, 30) under conditions of probe excess. The 490-bp *Pvu*II/*Mlu*I fragment from pUSRT1-1, ^{32}P end labelled at the 5′ end of the *Mlu*I site, was used for mapping *tuf1* transcripts. The *tuf2* and *tuf3* genes (40) have no homology with the noncoding region of this probe and differ sufficiently in the nucleotide sequence of the *tuf* coding region to exclude the possibility that these mRNAs contribute to the protection pattern. Products were analyzed on denaturing 6% polyacrylamide gels, using ^{32}P end-labelled *Hpa*II fragments of pBR322 as size markers. The endpoints of the RNA-protected fragments were determined by sequencing pUSRT1 with an 18-nucleotide (nt) oligomer (JIC4), the 5′ end of which corresponds to the 5′ end of the *Mlu*I site.

RESULTS

Transcription of *tuf1* occurs from two promoters. In *E. coli*, *tufA* is the most distal gene in the transcription unit comprising the genes *rpsL*, *rpsG*, *fus*, and *tuf* (19). Since EF-TuA is synthesized in three- to fivefold larger amounts than the other proteins of the *str* operon, additional promoter activity within the *fus* gene has been suggested and indeed observed (3, 44–46).

To investigate transcription of the *S. ramocissimus tuf1* gene, we carried out Northern blotting experiments using RNA isolated from exponentially growing cultures harvested 17 h after inoculation of liquid medium (NMMP–1% glucose) with pregerminated spores (see below for details). Using the *S. ramocissimus* 1.0-kb *Mlu*I/*Nco*I fragment of pUSR*str* (32a) as a probe (Fig. 1, *tuf1* probe), we identified two major in vivo *tuf1* transcripts, hybridizing to similar extents (Fig. 2). The large transcript of approximately 5 kb was also visualized when the 0.75-kb *Pst*I/*Bam*HI fragment of pUSR*str* was used as a probe (Fig. 1, *rpsL*/*G* probe), indicating that this transcript includes at least *rpsG* and *fus* in addition to *tuf1*. The strongly hybridizing *tuf1* transcript of approximately 1.8 kb suggested the presence of a prominent *tuf1*-specific promoter. It is unlikely that this *tuf1* transcript is a processing product of the large transcript, since only one band was found with the *rpsL*/*G* probe.

To determine the presence and approximate location of any *tuf1*-specific promoter(s), *S. coelicolor* M145 was transformed with various derivatives of the multicopy vector pIJ4083, each containing a different *S. ramocissimus tuf1* upstream fragment cloned in front of the promoterless *xylE* gene. The origin of

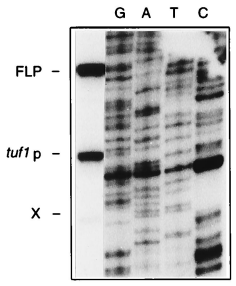

FIG. 3. S1 nuclease protection analysis of the transcription start sites of *tuf1*. FLP, full-length protection of the *fus-tuf1* region of the *tuf1* probe indicative of transcription from *rpsL*p; *tuf1*p, transcript initiated at *tuf1*p; X, putative transcript or processed product. Lanes G, A, T, and C show the *tuf1* sequence ladder generated with oligomer JIC4.

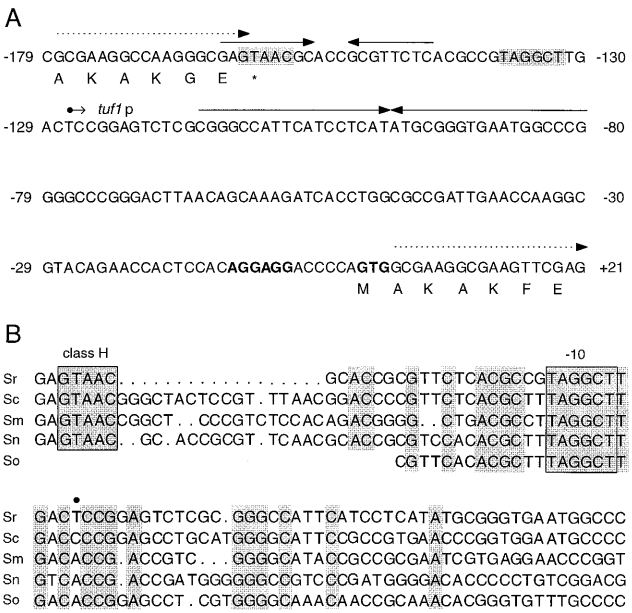

FIG. 4. (A) Nucleotide sequence of the *fus-tuf1* intergenic region. The transcription start site for *tuf1* is shown by a black dot, and the direction of transcription is indicated by an arrow. The putative promoter sequences are shaded. Converging arrows indicate the inverted repeats between *fus* and *tuf1*; the direct repeat is shown by dotted arrows. The asterisk denotes the *fus* translation stop codon. The likely ribosome binding site and *tuf1* translation initiation codon are in boldface. (B) Sequence alignment of streptomycete promoter regions upstream of the *tuf1* translation start site. The first 100 nt of the *fus-tuf1* intergenic spacers of *S. ramocissimus* (Sr) (40), *S. coelicolor* (Sc) (36), *S. mobaraense* (Sm) (32a), *S. netropsis* (Sn) (32a), and *S. collinus* (So) (partial sequence [24]) are shown. The consensus sequence is indicated by shading when the nucleotide is conserved among all species. The location of the transcription start site for the *S. ramocissimus tuf1* gene is shown by a black dot. The putative polymerase recognition elements are indicated by boxes.

each of these fragments is shown in Fig. 1. Transformants containing pISRT1*xylE*-2, which has the region from −180 to +280 (relative to the *tuf1* translation start site) of *tuf1* in front of *xylE*, yielded colonies with bright-yellow aerial hyphae when sprayed with catechol after at least 3 days growth on R2YE agar plates. M145 transformants containing pISRT1*xylE*-1 or pISRT1*xylE*-3 displayed no or hardly any yellow coloring, respectively, upon spraying with catechol, reducing the *tuf1*-specific promoter region to the *Nru*I/*Sma*I fragment (−180 to −70).

To map precisely possible transcription start sites directly upstream of *tuf1*, S1 nuclease protection experiments were carried out with RNA isolated from exponentially growing *S. ramocissimus*, harvested 17 h after inoculation of liquid medium (NMMP–1% glucose) with pregerminated spores (see below for details). The 490-bp *Pvu*II/*Mlu*I fragment from pUSRT1-1 (Fig. 1, S1 probe), uniquely ^{32}P labelled at the 5′ end of the *Mlu*I site, was used as a probe; the 200-bp nonhomologous pUC18-derived extension allowed discrimination between full-length RNA-protected fragments (transcription read-through from *fus*) and reannealed probe. A protected fragment corresponding in size to the *Nru*I/*Mlu*I segment of the probe (Fig. 3, FLP) indicated transcription of *tuf1* from a promoter upstream of, or within, *fus* (most likely *rpsL*p). An additional transcription start site was identified at nt −127 (Fig. 3, *tuf1*p). Figure 3 also shows a possible minor transcription start site (band X), located at nt −97. We cannot exclude the possibility that this S1-protected fragment is a processing product of the *tuf1*p-derived mRNA. Transcripts derived from *tuf1*p and the read-through promoter *rpsL*p were present in almost equal amounts, whereas the additional transcript (band X in Fig. 3) was barely detectable. The location of the *tuf1*p transcription start site is depicted in Fig. 4A.

To confirm that the RNA-protected fragment assigned to *tuf1*p, which was identified by the in vivo analyses described above, represents a transcription start site rather than a processing event, the 290-bp *Bam*HI/*Mlu*I fragment of pUSRT1-1 was used as a template for in vitro transcription assays using different fractions of partially purified RNA polymerase holoenzymes isolated from transition-phase cultures of *S. coelicolor* M145. A runoff transcript of approximately 240 nt corresponded to the expected size for *tuf1*p, as was predicted from the S1 nuclease mapping data (data not shown).

Evidence that this additional *tuf1* transcription start site is not unique for *S. ramocissimus* was obtained by comparison of homologous DNA sequences from *S. coelicolor*, *S. mobaraense*, *S. netropsis*, and *S. collinus* (Fig. 4B). The first 80 nt of the *fus-tuf1* intergenic region were only 39% conserved between *S. ramocissimus* and the other streptomycetes. However, the putative recognition sequences for transcription were 100% conserved. Sequences immediately upstream of the translation start site (−64 to −1) were highly conserved among the five species (83%) (data not shown). The significance of the conservation of this part of the untranslated leader mRNA is not known.

Growth-phase-dependent transcription of *tuf1* in liquid cultures. To study the growth phase dependence of *tuf1* transcription and to assess the relationship between growth rate, *tuf1* transcription, and kirromycin production, we developed conditions which gave reproducible, dispersed growth of *S. ramocissimus* mycelia. In NMMP–1% glucose, exponential growth was obtained with a doubling time of about 2.5 h and the culture entered stationary phase (OD_{450} of 2.2) usually about 22 h after inoculation (Fig. 5A). At the same time, the pH of the medium dropped from about 6.6 to 5.0, and small amounts of kirromycin were detected about 4 h later. The doubling times and onset of antibiotic production were highly reproducible. In S medium, an almost identical growth curve was observed, but kirromycin production was significantly increased (data not shown).

To establish the level and timing of *tuf1* transcription from *tuf1*p and *rpsL*p, S1 nuclease protection experiments were carried out with RNA isolated from *S. ramocissimus* NMMP cultures at different time points, between 13 h (OD_{450} of 0.6, corresponding to early exponential phase) and 34 h (OD_{450} of 2.2, late stationary phase) after inoculation of NMMP–1%

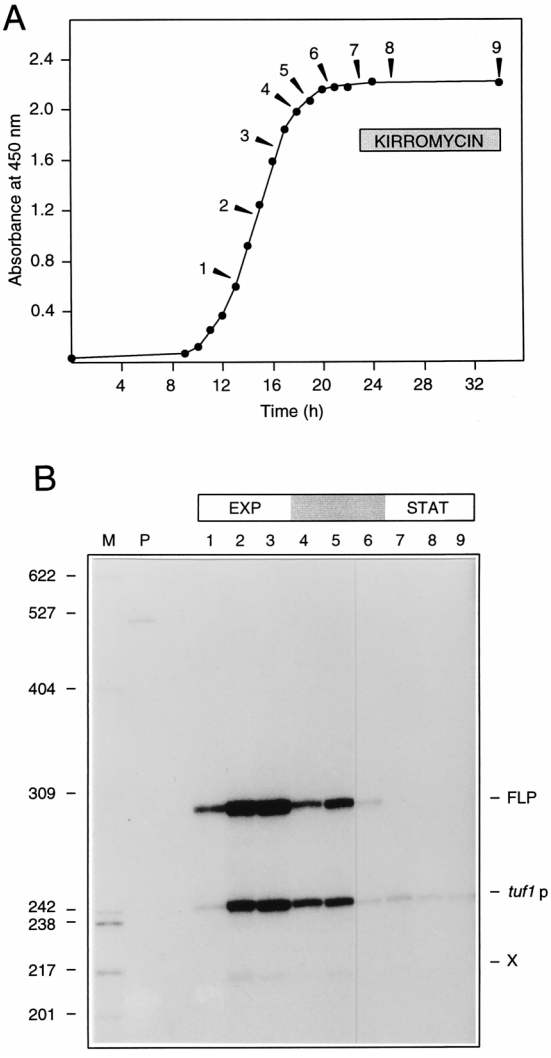

FIG. 5. (A) Growth curve of *S. ramocissimus* B7. Arrowheads indicate the time points at which RNA and kirromycin were isolated as described in Materials and Methods. The shaded box labelled KIRROMYCIN denotes the presence of kirromycin in the filtrate. The doubling time during exponential growth was 2.5 h. (B) S1 nuclease protection analysis of the *tuf1* transcripts in RNA isolated at the time points indicated in panel A (10 μg of RNA per sample). EXP and STAT indicate exponential and stationary growth phases, respectively, and the shaded box indicates the transition phase. FLP, the signal for full-length protection of the *fus-tuf1* region of the probe; *tuf1*p, a transcript initiated at *tuf1*p; X, a minor additional transcript or processed product. In lane P, the location of the 490-nt full-length *tuf1* probe can be seen. Lane M contains end-labelled *Hpa*II-digested pBR322 size markers (sizes are given in nucleotides).

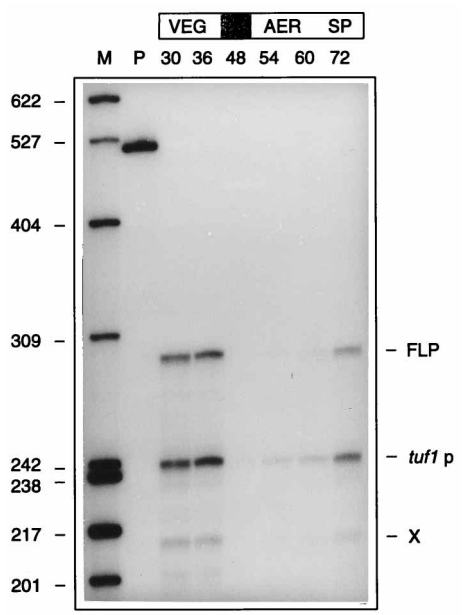

FIG. 6. S1 nuclease protection analysis of the *tuf1* transcripts in RNA isolated from surface-grown cultures at the time points indicated in hours (40 μg of RNA per sample). VEG, AER, and SP indicate the appearance of vegetative mycelium, aerial mycelium, and spores, respectively, and the shaded area corresponds to the transition phase. FLP marks the signal for full-length protection of the *fus-tuf1* region of the probe; *tuf1*p indicates a transcript initiated at *tuf1*p; X, P, and M are as in Fig. 5B.

glucose with pregerminated spores. The 490-bp *Pvu*II/*Mlu*I fragment from pUSRT1-1 (described above) was used as a probe. As shown in Fig. 5B, the *tuf1*p and *rpsL*p transcripts were readily detected in RNA isolated from fast-growing cultures and after 16 h contributed almost equally to the total *tuf1* mRNA concentration. Both transcripts reached maximal levels during early exponential growth and decreased markedly when the growth rate declined. Twenty-three hours after inoculation (corresponding to early stationary phase), transcription from *rpsL*p was barely detectable, and stationary-phase transcription of *tuf1*, during kirromycin production, occurred mainly from *tuf1*p, although at a low level. Degradation products of the *tuf1* transcripts were observed throughout growth (data not shown), presumably reflecting their relatively short half-lives.

Transcription of *tuf1* in surface-grown cultures is growth phase dependent. To study *tuf1* transcription during the life cycle of *S. ramocissimus* B7 on solid media, S1 nuclease mapping experiments were performed with RNA isolated from various growth phases, using again the 490-bp *Pvu*II/*Mlu*I fragment from pUSRT1-1 as a probe (Fig. 6). High levels of *tuf1* transcripts were observed during the formation of vegetative hyphae, with transcription initiating from the same start sites as found for the liquid culture (Fig. 5B). Transcript levels of *tuf1*p mRNA were even higher than those of the *str* operon promoter *rpsL*p. A dramatic drop in *tuf1* transcription occurred in the transition phase from vegetative growth to aerial mycelium development, coinciding with the production of kirromycin. At the onset of morphological differentiation, *tuf1* transcript levels increased again, mainly due to the activity of *tuf1*p. Transcripts corresponding to *rpsL*p also reappeared during the later stages of development.

DISCUSSION

In this report, we show that *tuf1* of *S. ramocissimus* is transcribed from a promoter within the *fus-tuf1* intergenic region, in addition to the *str* operon promoter *rpsL*p. S1 nuclease protection experiments revealed a transcript with a 5′ end within the *fus-tuf1* intergenic region at nt −127 relative to the *tuf1* translation start site. The XylE$^+$ phenotype of pISRT1*xylE*-2, revealing promoter activity within 180 bp of the *tuf1* translation start site, and in vitro runoff transcription analysis with *S. coelicolor* M145 RNA polymerase indicated that the potential promoter start site found by S1 mapping does indeed serve to initiate transcription in vivo and is not the result of posttranscriptional mRNA processing. An additional

band which might reflect a weak second promoter in the *fus-tuf1* intergenic region was observed in the S1 nuclease mapping experiments. Compared to the other two *tuf1* transcripts, this mRNA is sparsely present, and the transcript might be a degradation or processing product of the *tuf1*p transcript.

The *tuf1*p transcript, which persists at a relatively high level in both exponential and stationary phases, has a start site preceded by a −10 region (TAGGCT) that is similar to the consensus sequence (TAGRRT, where R = G or A) deduced by Strohl (31) for streptomycete promoters that are probably transcribed by the major RNA polymerase holoenzyme containing σ^{hrdB} (6) but lacks a recognizable −35 region. Perhaps the inverted repeat (Fig. 4A) extending from −163 to −144 binds a transcriptional activator that would obviate the need for a conventional −35 sequence. In addition to this 8-nt inverted repeat, a direct repeat of 17 nt is located at nt −178 to −161 (3′ end of the *fus* gene) and +4 to +21 (5′ end of the *tuf1* gene) (Fig. 4A). Further analysis of the *tuf1*p region revealed the sequence GTAAC 19 bp upstream of the TAGGCT −10 sequence. This region shows a perfect match with the consensus for class H ($GNAACN_{19/20}T$) potential promoter core sequences (5) that might be of the σ^E class (formerly σ^{28}) (22). The presence of two different promoter elements suggests that *tuf1*p transcripts might be the result of both σ^{hrdB}- and σ^E-containing RNA polymerase holoenzymes. Wright and Bibb (41) noticed a strong preference for UGA over UAG, and particularly over UAA, as a translational stop codon in streptomycetes. It is therefore of interest that the frequently translated *S. ramocissimus fus* gene terminates with the unusual UAA codon, thereby creating the GTAAC potential promoter core sequence of *tuf1*p.

The *tuf1*p and *rpsL*p transcripts were present abundantly during exponential growth in liquid culture. Transcription from both promoters declined markedly upon entry into stationary phase, showing a growth phase dependence similar to that of the *S. coelicolor tuf1* gene (38) and rRNA operon *rrnA* (37). However, transcripts derived from *tuf1*p persisted at least until 34 h after inoculation, corresponding to late stationary phase, albeit at a low level. Analysis of *tuf1* transcription in surface-grown cultures revealed the same transcription start sites as found for the liquid culture experiments. A transitory cessation of *tuf1* transcription was recorded in *S. ramocissimus* at the end of vegetative mycelium formation. The synthesis of *tuf1* transcripts increased again during the formation of aerial hyphae, transcription being mainly due to the activity of *tuf1*p, indicating de novo synthesis of EF-Tu1 during differentiation. These results point to an obligatory presence of EF-Tu1 in aerial mycelium and are consistent with the observation made by Granozzi et al. (16) of a second round of protein and RNA synthesis on solid medium that coincided with the development of aerial hyphae in *S. coelicolor*. Elevated levels of *tuf1*p and *rpsL*p transcripts during the later stages of differentiation may indicate the storage of *tuf1* mRNA or EF-Tu1 in spores or might simply reflect a second round of spore germination on top of old mycelium.

As for many antibiotics, kirromycin production in *S. ramocissimus* was first detected during early stationary phase in liquid medium and correlated temporally with the onset of morphological differentiation in surface-grown cultures. Transcription of *tuf1*, presumably resulting in synthesis of the kirromycin-sensitive EF-Tu1, occurred during kirromycin production in both liquid and surface-grown cultures. These results support the assumption made by Van der Meide (34) and Vijgenboom et al. (40) that kirromycin resistance in *S. ramocissimus* does not reflect alteration of the target protein EF-Tu1, as is the case for some other producers of kirromycin-like antibiotics (1, 14).

The presence of an additional promoter in the *fus-tuf1* spacer region of the *str* operon seems a general feature of streptomycetes, as judged by the conservation of promoter sequences. All −35 sequences of the predicted *tuf1* promoters are part of a direct or inverted repeat, although *S. ramocissimus* seems to be unique in having its class H promoter core sequence around the −35 position with respect to the transcription start site of *tuf1*p.

In contrast to the strong promoter activity of the *S. ramocissimus fus-tuf1* intergenic region, the reported additional promoters for *E. coli tufA*, both located within the *fus* gene, seem to be minor transcription start sites. Although no quantitative studies were done on transcripts derived from the *E. coli* chromosome, on the basis of plasmid studies, Zengel and Lindahl (46) suggested that these promoters were up to 30% as active as the *str* operon promoter, contributing 10 to 15% to the total *tuf* mRNA production in *E. coli*. Here we show that *tuf1*p of *S. ramocissimus* is approximately as active as the operon promoter, and thus the two transcripts contribute more or less equally to the total *tuf1* mRNA concentration. An extra promoter for *tuf1* may allow *S. ramocissimus* to differentially regulate EF-Tu synthesis from *tuf1* without affecting the synthesis of EF-G, S7, and S12, permitting relative increase in *tuf1* gene expression compared to the expression of upstream genes of the *str* operon.

The observation that the duplication of *tuf* is widespread among gram-negative genera suggests that for these bacteria, *tuf* gene duplication fulfills an important role. No functional differences between the nearly identical gene products of *E. coli tufA* and *tufB* could be detected (25, 39), and both genes in *Salmonella typhimurium* were shown to be individually dispensable for growth (18). However, cells with one disrupted *tuf* gene have a decreased growth rate, suggesting that two genes are required to attain high EF-Tu concentrations during rapid growth. Since gram-positive organisms contain only a single copy of the regular *tuf* gene, we propose that the additional strong *tuf1*p promoter in the *str* operon of streptomycetes functions to provide adequate levels of EF-Tu at high growth rates. Whether this is also the case for other gram-positive eubacteria and archaea remains to be determined.

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