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Genetic and molecular analysis of the tRNA–*tufB* operon of the myxobacterium *Stigmatella aurantiaca*

Laure Bremaud[1], Christophe Fremaux[1,2], Soumaya Laalami[1] and Yves Cenatiempo[1,*]

[1]Institut de Biologie Moléculaire et d'Ingénierie Génétique–CNRS URA 1172, Université de Poitiers, 40 Avenue du Recteur Pineau, 86022 Poitiers Cedex, France and [2]Texel, groupe Rhône-Poulenc, ZI de Buxières, BP 10, 86220 Dangé-St Romain, France

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ABSTRACT

The *tufB* gene, encoding elongation factor Tu (EF-Tu), from the myxobacterium *Stigmatella aurantiaca* was cloned and sequenced. It is preceded by four tRNA genes, the first ever described in myxobacteria. The tRNA synthesized from these genes and the general organization of the locus seem identical to that of *Escherichia coli*, but differences of potential importance were found in the tRNA sequences and in the intergenic regions. The primary structure of EF-Tu was deduced from the *tufB* DNA sequence. The factor is composed of 396 amino acids, with a predicted molecular mass of 43.4 kDa, which was confirmed by expression of *tufB* in maxicells. Sequence comparisons between *S.aurantiaca* EF-Tu and other bacterial homologues from *E.coli*, *Salmonella typhimurium* and *Thermus thermophilus* displayed extensive homologies (75.9%). Among the variable positions, two Cys residues probably involved in the temperature sensitivity of *E.coli* and *S.typhimurium* EF-Tu are replaced in *T.thermophilus* and *S.aurantiaca* EF-Tu. Since two or even three *tuf* genes have been described in other bacterial species, the presence of multiple *tuf* genes was sought for. Southern and Northern analysis are consistent with two *tuf* genes in the genome of *S.aurantiaca*. Primer extension experiments indicate that the four tRNA genes and *tufB* are organized in a single operon.

INTRODUCTION

Elongation factor Tu (EF-Tu) of *Escherichia coli* was discovered about 30 years ago (1) and since then has been subjected to numerous studies. Its role in protein biosynthesis has been elucidated (2). EF-Tu is involved in the binding of aminoacyl-tRNA to ribosomes. It is a multifunctional protein interacting with a variety of different components, such as guanine nucleotides, ribosomes, aminoacyl-tRNAs and elongation factor Ts (EF-Ts). During the elongation step of protein biosynthesis, EF-Tu, coupled to aminoacyl-tRNA and guanosine triphosphate (GTP), forms a reactive ternary complex. Binding of this ternary complex to the A-site of the ribosome leads to GTP hydrolysis, followed by release of EF-Tu–GDP (3).

Elongation factor EF-Tu is a monomeric protein of ~44 kDa and one of the most abundant proteins in *E.coli* (4). Its amino acid sequence (5) has been established, as well as the tertiary structure of first its GTP binding domain and recently a larger trypsin-treated form lacking residues 45–58 (6, and for a review 7). EF-Tu is present in two forms in various bacterial species. They are encoded by two *tuf* genes, namely *tufA* and *tufB*, in *E.coli* (8,9), in *Salmonella typhimurium* (10) and in *Thermus thermophilus* (11,12). In *E.coli* and *T.thermophilus* the amino acid sequence deduced from *tufA* and *tufB* show that their gene products are almost identical. They differ by a single amino acid at the C-terminus in *E.coli* (5,13) and at four internal positions in *T.thermophilus* (12). In spite of these structural differences, the products of *tufA* and *tufB* were found to exhibit identical physical, chemical and catalytic properties (14).

The *tufA* gene is located at 74 min on the *E.coli* chromosome in the *str* operon and is co-transcribed with the *fusA*, *rpsG* and *rpsL* genes, encoding respectively elongation factor G and ribosomal proteins S7 and S12 (15). *tuf*A is expressed more efficiently (~3.5 times higher) than the other genes of the operon (16). *tufB* is preceded by four tRNA genes in a second operon (the tRNA–*tufB* operon) at a distant location at 90 min on the chromosome (17).

In this report we present molecular cloning and characterization of the *tufB* locus of the myxobacterium *Stigmatella aurantiaca*. Myxobacteria are of special interest among prokaryotes because under starvation they undergo a developmental cycle leading to the formation of multicellular structures termed fruiting bodies (18). Our starting hypothesis was that protein factors of the translational machinery could be involved directly or indirectly in this developmental process. Therefore, we designed a PCR-based strategy to selectively amplify a segment of any gene encoding a G protein (19). Instead of using heterologous probes that could reveal either irrelevant genes or several G protein-encoding genes, this approach increases the probability of cloning a gene of interest. We first amplify and then identify two DNA segments of *S.aurantiaca* encoding the G domain of initiation factors IF2 and EF-Tu respectively (19). These fragments were used as homologous probes to clone *infB*, the gene encoding IF2 (Bremaud, manuscript in preparation) and *tufB*.

* To whom correspondence should be addressed

Table 1. Bacterial strains and plasmids

Strains and plasmids	Characteristics	Source or reference
Escherichia coli		
DH5α	*supE44*, Δlac_{U169}(ϕ80*lacZ*ΔM15) *hsdR17*, *recA1*, *endA1*, *gyrA96*, *thi-1*, *relA1*	Bethesda Research Laboratory
Y1090	*supF*, *hsdR*, *araD139*, Δ*lon*, Δlac_{U169}, *rpsL*, *proA*$^+$, *strA*, *trpC22*::*Tn*10, pMC9	(21)
CSR603	*F*$^-$, *recA1*, *uvrA6*, *phr-1*, *leuB6*, *proA2*, *argE3*, *thi-1*, *ara-14*, *lacY1*, *galK2*, *xyl5*, *mtl-1*, *rpsl31*, *tsx-33*, *supE44*	(27)
LBYC1	DH5α (pLBYC1)	This study
LBYC2	DH5α (pLBYC2)	This study
LBYC3-1 and 3-2	CSR603 (pLBYC2)	This study
LBYC4	CSR603 (pUC18)	This study
Plasmids		
pBluescript II SK+	Amp^R, *lacZ*, cloning vector, 2.9 kb	Stratagene
pUC18	Amp^R, *lacZ*, cloning vector, 2.9 kb	Pharmacia
pLBYC1	pBluescript II SK+::2.0 kb *Eco*RI–*Xho*I fragment	This study
pLBYC2	pUC18::2.0 kb *Eco*RI–*Xho*I fragment	This study

MATERIALS AND METHODS

Bacterial strains, plasmids and media

Bacterial strains and plasmids used in this study are listed in Table 1.

S.aurantiaca strain DW4 (ATCC 33878) was grown to late exponential phase in 1% Bactocasitone (Difco) with 8 mM $MgSO_4$ at 30°C and harvested at ~4×10^8 cells/ml. *E.coli* strains were grown in or plated on Luria–Bertani medium at 37 or 42°C. DH5α transformants were grown in the presence of 100 μg/ml ampicillin. Isopropyl-β-D-thiogalactopyranoside (IPTG) and 5 bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) were used at concentration of 50 μg/ml each. Y1090 strain was grown in the presence of 0.4% maltose.

DNA and RNA manipulations

Genomic DNA of *S.aurantiaca* was extracted as described previously (20). Plasmid DNA and mini and maxi preparations of λgt11 were performed as already described (21). Plasmid and genomic DNA were digested with restriction enzymes purchased from Gibco-BRL. Restriction fragments were separated on agarose gels and isolated using the Prep-A-Gene Kit (BioRad Laboratories, Richmond, CA). Recombinant DNA was obtained with T4 DNA Ligase (Gibco-BRL). *E.coli* competent cells were prepared and transformed according to Hanahan (22).

Total RNA was extracted from *S.aurantiaca* by a method derived from Ausubel *et al.* (23). All aqueous solutions used in these manipulations were treated with 0.1% diethyl pyrocarbonate (DEPC) and autoclaved. A RNA sample was resuspended in DNase buffer for an RNase-free DNase digestion (23). After digestion, the enzyme was extracted with 1 vol phenol/chloroform (v/v), 1 vol chloroform and RNA was precipitated and resuspended in 10 μl water. RNA concentration was estimated spectrophotometrically and 10 μg were electrophoresed on formamide–formaldehyde denaturing 1.2% agarose gel.

Screening of a *S.aurantiaca* λgt11 library

The λgt11 library was constructed in Pr. Schairer's Laboratory (Heidelberg, Germany). *S.aurantiaca* genomic DNA was partially digested with *Hpa*II and fragments ranging from 2 to 6 kb were selected. They were then appended with *Hpa*II–*Eco*RI adaptors and finally cloned into the *Eco*RI site of λgt11. Amplified DNA products (19) were labelled with [α-^{32}P]dCTP (ICN Biomedicals) by random priming (24) and used to screen 15 000 clones previously plated (21).

Southern hybridization

Chromosomal and plasmid DNA were digested to completion with appropriate restriction endonucleases and analysed by agarose gel electrophoresis. The DNA was transferred from the gels onto Hybond-N+ filters (Amersham), essentially according to the method of Southern (25). The filters were pre-hybridized for 1 h at 65°C and then hybridized overnight at 65°C with a probe. Hybridization conditions are detailed in Results. After washing, the filters were autoradiographed with X-ray films. Amplified DNA products (19) or restriction fragments used as probes were labelled by random priming as above (24).

Northern hybridization

RNA was transferred from the denaturing gel onto Hybond-N+ filters by capillarity. Filters were hybridized overnight at 42°C in the presence of 50% formamide.

DNA sequencing and computer analysis

DNA sequencing was performed by the dideoxy chain termination method (26) using an automated sequencer (ALF, Pharmacia). Sequencing reactions were carried out on fragments cloned into the pBluescript II SK+ vector using fluorescent universal and reverse primers and T7 DNA polymerase (Autoread Sequencing Kit, Pharmacia).

Sequence analysis was performed with the programs of the Genetics Computer Group (GCG, Madison, WI) sequence analysis software package.

Maxicells analysis

Expression of plasmid-encoded proteins was analysed as described previously (27). Cell extracts were submitted to

SDS–PAGE electrophoresis (10% polyacrylamide). Labelled proteins were detected by autoradiography of the dried gel.

Primer extension

Primer extension was performed to map the transcription initiation start of the tRNA–*tufB* operon of *S.aurantiaca* using the primer extension oligonucleotide 5′-ACTGCCAGATACTTC-CG-3′. The reverse transcripts obtained as previously described (28) were analysed by electrophoresis through a 5% polyacrylamide sequencing gel. A sequencing reaction of pLBYC1 performed with the primer extension oligonucleotide was used to provide the size markers.

RESULTS

Cloning and identification of the *tufB* locus

A PCR fragment specific to a part of the G domain of *S.aurantiaca* EF-Tu (19) was used as a specific probe to screen a λgt11 library of *S.aurantiaca* DW4 (kindly provided by H. U. Schairer, Heidelberg, Germany). One clone carrying a 6.0 kb *Eco*RI insert was subsequently isolated. The presence of an internal *Eco*RI site led to two *Eco*RI fragments of 3.1 and 2.9 kb respectively. By using the same PCR probe in a Southern blot analysis, the G domain of the *tuf* gene was found to be borne on a 2.0 kb *Eco*RI–*Xho*I subfragment arising from the above 3.1 kb segment. Restriction fragments from pLBYC1 (Table 1) were subcloned in the pBluescript II SK+ vector and sequenced. Figure 1 shows the restriction map and the genetic organization of the 2.0 kb *Eco*RI–*Xho*I fragment. The overall nucleotide sequence of the latter appears in the EMBL data bank under accession no. X82820. This DNA segment is organized as an operon starting by a promoter (see below and Discussion) and ending by a putative terminator with a stable hairpin structure from position 1924–1951 (calculated $\Delta G = -25$ kcal). An open reading frame (ORF) of 1191 bp (396 amino acids), encoding a putative protein of 43.4 kDa, presumably EF-Tu, was found. This open reading frame is preceded by four tRNA genes clearly identified by computer analysis using the PC gene software tRNA SEARCH. According to their anti-codon sequences and by comparison with *E.coli*, these tRNA genes were named *thrU*, *tyrU*, *glyT* and *thrT* respectively (Fig. 2). The average G+C content in this 2.0 kb fragment was 62.3%. Moreover, the percentage of the third base of the codon being G or C was 91.4% along this gene, which is an essential characteristic of myxobacterial genes. These results identify the myxobacterial origin of these genes.

The amino acid sequence predicted from the ORF resembles that of EF-Tu from other sources. A comparison of the deduced amino acid sequence with that of *E.coli* (accession no. X57091), *S.typhimurium* (X55117) and *T.thermophilus* (X61957) revealed a 76% homology. The amino acids, especially at the active site in the G domain, are highly conserved, with even several long stretches of identical residues. Whereas two Gram-negative organisms (*E.coli* and *S.typhimurium*) bear almost identical EF-Tu, differing at only one internal position and at the C-terminus, it should be stressed that the situation is quite different in *S.aurantiaca*, another Gram-negative bacterium. At 33 positions the amino acid found in *S.aurantiaca* is identical to that of *T.thermophilus* EF-Tu and consequently differs from that of the two other Gram-negative organisms.

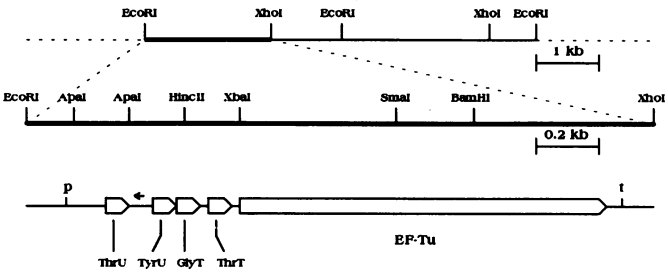

Figure 1. Physical map of the *S.aurantiaca* tRNA–*tufB* operon. The fragments that were successively cloned are shown in the upper part of the figure. The insert carried by the λgt11 bacteriophage is bordered by two *Eco*RI sites that were introduced during library construction. The 2.0 kb *Eco*RI–*Xho*I fragment sequenced is represented by a heavy bar. The open boxes show the genes identified throughout the fragment and indicate the direction of transcription. 'P' and 't' indicate putative promoter and terminator respectively. A *Sma*I–*Bam*HI restriction fragment was used in Southern experiments, whereas *Bam*HI–*Xho*I and *Eco*RI–*Hin*cII fragments were used in Northern experiments. The probe used in primer extension is represented by the leftward horizontal arrow.

Heterologous expression of the *tufB* gene

A 2.0 kb *Eco*RI–*Xho*I fragment was inserted into plasmid pUC18 (digested with *Eco*RI and *Sal*I). The resulting plasmid pLBYC2 was used to transform bacterial strain CSR603 for maxicell analysis of the plasmid-encoded EF-Tu.

Two LBYC3 recombinant clones were tested (Fig. 3, lanes 2 and 3), as well as LBYC4 (CSR603 transformed with the control plasmid pUC18) (Fig. 3, lane 1).

In addition to the β-lactamase protein detected at the bottom of the autoradiogram in lanes 1–3, a protein of ~43 kDa, putatively EF-Tu, is only produced by the clones containing pLBYC2.

Detection of a second *tuf* gene in *S.aurantiaca*

Southern analysis was used to test *S.aurantiaca* for the presenee of two *tuf* genes. A 257 bp *Sma*I–*Bam*HI fragment from the *tufB* gene of *S.aurantiaca* was used as a probe (see Fig. 1). Under high stringency conditions (0.1× SSC, 1% SDS, 20 min at 65°C) the probe hybridized to two distinct fragments of *S.aurantiaca* genomic DNA digested with restriction endonucleases *Apa*I, *Nco*I, *Sal*I and *Sno*I (Fig. 4). These enzymes did not cut within the *tufB* gene of *S.aurantiaca* and therefore our results indicate that there are at least two *tuf* genes in the *S.aurantiaca* genome. Similar results were obtained with genomic DNA of another myxobacterial species, *Myxococcus xanthus* (data not shown). The second *tuf* gene has not been cloned yet.

tufB transcription analysis

A small number of genes from myxobacteria have so far been characterized and, therefore, little is known about promoter sequences in these organisms. Northern hybridization experiments were performed in order to determine whether or not *tufB* is co-transcribed with the four tRNAs. Total RNA was extracted from *S.aurantiaca* in late exponential phase. A first probe, corresponding to a 573 bp *Bam*HI–*Xho*I fragment (the C-terminal part of the *tufB* gene), hybridized to two transcripts of ~1.7 and 1.3 kb (Fig. 5A). A second probe, prepared from a 480 bp *Eco*RI–*Hin*cII fragment overlapping the *tufB* upstream region, hybridized to a single transcript of ~1.7 kb and to the tRNAs (Fig. 5B).

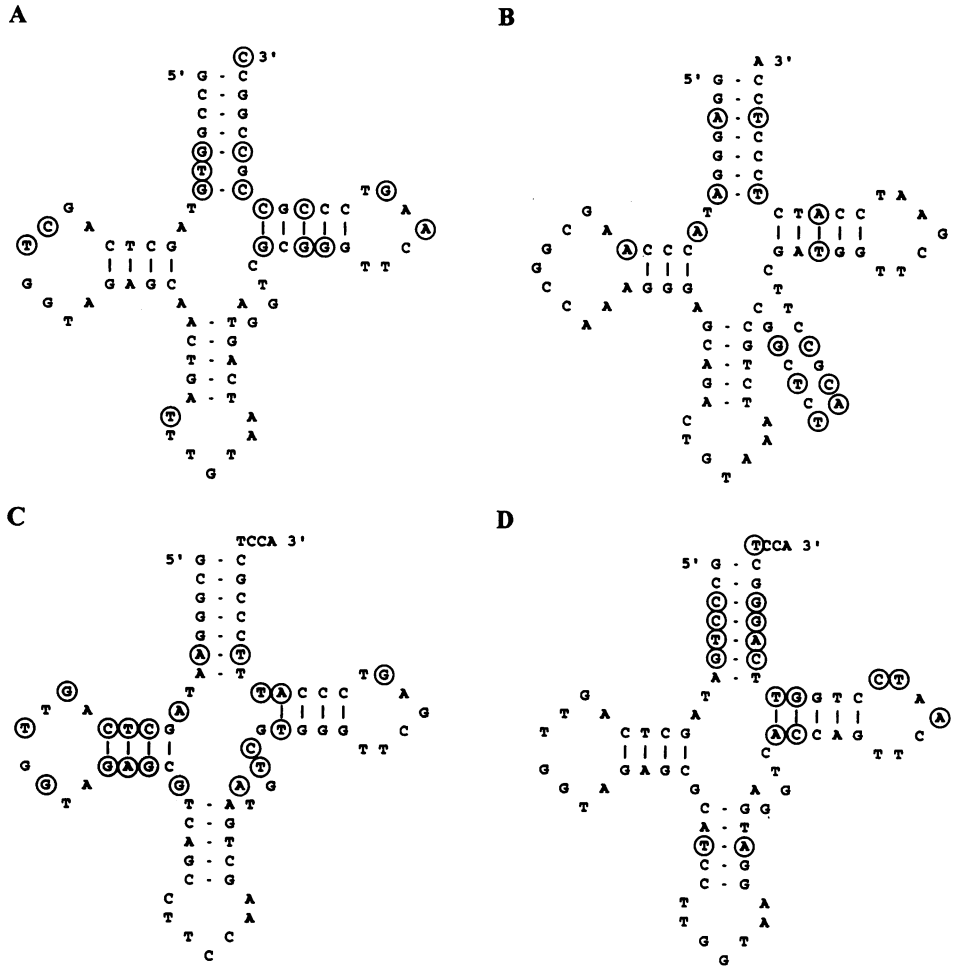

Figure 2. Schematic representation of the four tRNA encoded upstream of the *tufB* gene in *S.aurantiaca*: (**A**) $tRNA_4^{Thr}$, (**B**) $tRNA_2^{Tyr}$, (**C**) $tRNA_2^{Gly}$ and (**D**) $tRNA_3^{Thr}$. The tRNA were named in accordance with those characterized in the *E.coli* tRNA–*tufB* operon. The differences in nucleotides observed between *S.aurantiaca* tRNAs and their counterparts in the tRNA–*tufB* operon in *E.coli* are circled.

These data led us to conclude that the transcript of ~1.7 kb corresponds to the *tufB* gene co-transcribed with the four tRNA genes. The RNA fragment of ~1.3 kb may correspond to *tuf* mRNA without the tRNAs. This length is in good agreement with the size of the ORF encoding EF-Tu, but could as well match a *tufA* transcript.

Characterization of the tRNA–*tufB* operon

The 5′-end of the tRNA–*tufB* operon transcript was identified by primer extension analysis. Total RNA from *S.aurantiaca* was submitted to primer extension using an internal oligonucleotide primer complementary to the sequence from nucleotides 357 to 373 (upstream of *tufB* between the tRNA genes, *thrU* and *tyrU*, see Fig. 1). Transcription initiation sites were identified by alignment with a sequence ladder generated on pLBYC2 using the same oligonucleotide. Two reverse transcripts were generated (Fig. 6), defining two transcriptional starts, at a T (nucleotide 231) and at a C (nucleotide 237) respectively. The two transcription initiation sites are located 10 and 15 bp upstream of the first tRNA gene (*thrU*), i.e. 463 and 457 bp respectively upstream of the *tufB* initiation codon.

DISCUSSION

One of the two EF-Tu structural genes from the myxobacterium *S.aurantiaca* has been cloned and sequenced. The analysis of a 2.0 kb fragment revealed, upstream of the EF-Tu gene, the presence of four tRNA genes. This organization appears to be similar to that of the tRNA–*tufB* operon of *E.coli* (17) and we propose, on the basis of the anticodon sequences, the same nomenclature as in *E.coli*: *thrU* for $tRNA_4^{Thr}$, *tyrU* for $tRNA_2^{Tyr}$, *glyT* for $tRNA_2^{Gly}$ and *thrT* for $tRNA_3^{Thr}$. However, such a nomenclature needs to be confirmed in *S.aurantiaca* and will depend upon the number of other tRNA isoacceptor species, which remain to be discovered.

Within the tRNA, the nature of the bases normally found at highly conserved positions (e.g. nucleotide 8 being a T) was as expected. However, primary structures of the four tRNA differ from that of their *E.coli* counterparts in several instances. Whereas, as in *E.coli*, the 3′-ends of $tRNA_2^{Gly}$ and $tRNA_3^{Thr}$ are CCA, this is not the case for $tRNA_4^{Thr}$ and $tRNA_2^{Tyr}$. The latter tRNA sequences have a 3′-CCA terminus, but this is probably not their amino acceptor end, because, in both cases, it is partly paired with the 5′-end. Therefore, the CCA end is likely to be added after processing of the pre-tRNA, a situation usually encountered in

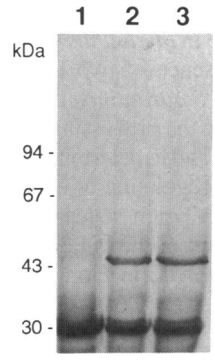

Figure 3. Maxicell analysis of *S.aurantiaca* EF-Tu synthesis from plasmid pLBYC2. Plasmid-encoded proteins were labelled with [^{35}S]methionine and analysed by SDS–PAGE (10% polyacrylamide). The figure shows an autoradiogram of the dried gel. Lane 1, LBYC4; lane 2, LBYC3-1; lane 3, LBYC3-2.

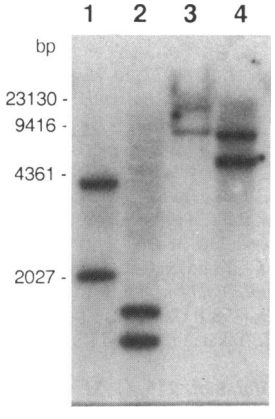

Figure 4. Genomic Southern hybridization of the *S.aurantiaca tuf* genes. *S.aurantiaca* genomic DNA (10 μg) was digested with restriction endonucleases. The fragments generated were separated by electrophoresis on a 0.8% agarose gel and hybridized with the radiolabelled *Bam*HI–*Sma*I *tufB* internal fragment. Lane 1, *Apa*I; lane 2, *Nco*I; lane 3, *Sal*I; lane 4, *Sno*I.

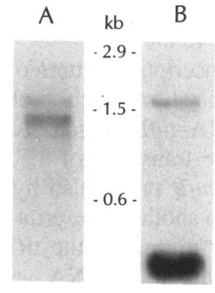

Figure 5. Northern blot analysis of tRNA–*tufB* mRNA. Two different probes, specific for the *tufB* gene of *S.aurantiaca* (**A**) or the *tufB* upstream region (**B**), were used as described in Materials and Methods. RNA sizes were estimated by comparison with a set of molecular weight standards (Boehringer-Mannheim Biochemica).

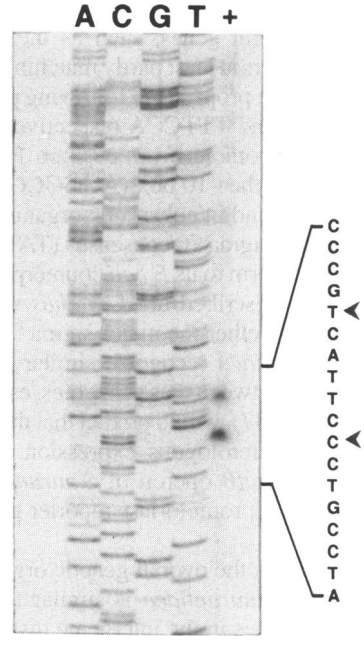

Figure 6. Determination of the *tufB* transcription initiation start by primer extension. A sequence ladder generated on pLBYC2 with the primer extension oligonucleotide (see Materials and Methods) was used as molecular weight marker. Putative transcriptional starts are shown by arrows.

Gram-positive bacteria and in eukaryotic organisms. Individual comparisons between the tRNA sequences from *E.coli* and *S.aurantiaca* show good conservation in the anticodon regions, but reveal strong variations within the variable loop ($tRNA_2^{Tyr}$, $tRNA_2^{Gly}$), the TψC loop ($tRNA_3^{Thr}$, $tRNA_4^{Thr}$), the DHU stem–loop ($tRNA_2^{Gly}$) and the amino acid stem ($tRNA_3^{Thr}$, $tRNA_4^{Thr}$). Of particular interest is the nature of the discriminator base found in the latter tRNA isoacceptor species. This base, the first before the CCA 3′-end, is an A in *E.coli* and an A or U in any other $tRNA^{Thr}$ so far identified (for a review see 29), with only a few exceptions in mitochondrial $tRNA^{Thr}$ (anticodon UGU) of fish, such as *Acipenser transmontanus* (accession no. X13485) or *Cyprinus carpio* (X61010). In this case the discriminator base is a C, as in *S.aurantiaca* $tRNA_3^{Thr}$, which bears the same anticodon. Again, this myxobacterium displays some features that are not found throughout the bacterial world and which should be of great interest, at least from an evolutionary point of view.

When the *tufB* deduced amino acid sequence of *S.aurantiaca* was compared with that of other bacterial species, no major differences were observed. However, at several positions *S.aurantiaca* EF-Tu is similar to that from *T.thermophilus*. For instance, *E.coli* and *S.typhimurium* EF-Tu bear three Cys residues, but in *T.thermophilus* and *S.aurantiaca* EF-Tu two out of these three Cys residues are replaced by Val and/or Ile residues. The conserved Cys, denoted Cys81 in *E.coli* (30), is part of a consensus element in the GTP binding domain of EF-Tu. It is conserved in all bacterial EF-Tu except that of *Micrococcus luteus*. Since it has been reported that the content of Cys residues in thermophilic bacteria is lower than that of mesophilic ones (31), it would be interesting to see whether *S.aurantiaca* EF-Tu

can be classified among the thermostable factors and if this provides an advantage to the cell.

We analysed the transcription pattern of the locus by performing sequential hybridizations with probes covering different segments of the tRNA–*tufB* region. A tRNA-specific probe hybridized to a single transcript of 1.7 kb in addition to the tRNAs, whereas the *tufB* probe also hybridized to the 1.7 kb transcript, as well as to another transcript of 1.3 kb. Thus the 1.7 kb transcript is likely to encode the tRNA–*tufB* operon. This result indicates that the four tRNA and the *tufB* genes are co-transcribed. Only limited data are so far available on myxobacterial promoters and primer extension experiments were necessary to precisely map the transcriptional start of the 1.7 kb transcript. Two major reverse transcripts differing by only five bases were generated. To identify promoter sequences, we took into account the consensuses AANGCT (–10) and TTGCNN (–35), determined for another myxobacterium, *M.xanthus* (32). Although this proposal comes from a limited number of examples, it is still believed that it represents the major *M.xanthus* promoter (33) and several genes, such as the *lon* gene (34), bearing putative promoters at least partly matching this consensus were found. We therefore propose the following putative –10 and –35 boxes, AGGGCG and TTTCCA respectively, separated by 18 bases (delimiting a promoter from position 197 to 227 in the sequence). Surprisingly, the –10 box (AGGGCG) resembles the GC box (GGGCGG) found in eukaryotic organisms, but not the –10 region of the *E.coli* sigma 70 consensus (TATAAT). The –35 box seems to better conform to its *E.coli* counterpart (TTGACA), a situation previously described in *M.xanthus* vegA (32). This raises the question of whether the major sigma factors of *E.coli*, *M.xanthus* and *S.aurantiaca* recognize similar sequences. The high degree of identity between these enzymes, especially in their C-terminus regions (35–37), would predict that they act similarly. To verify this point, heterologous expression of the putative promoter of the tRNA–*tufB* operon of *S.aurantiaca* should be performed upstream of a promotorless reporter gene in *E.coli* or *M.xanthus*.

As already pointed out, the overall genetic organization of the tRNA–*tufB* operon of *S.aurantiaca* is similar to that of *E.coli* (17), except for differences in the intergenic distances. In *E.coli* the full-length transcript is first processed to release *tufB* and two pairs of unprocessed tRNA, the final processing leading to the four tRNAs. Our sequence data concerning the intergenic distances would favour a different processing mechanism, since the first tRNA gene is separated from the cluster of the three other tRNA genes, which ends close (20 bp) to the beginning of *tufB*. As mentioned above, in *S.aurantiaca* a 1.3 kb transcript has been visualized using a *tufB*-specific probe. However, because of the high level of identities expected between the two *tuf* genes (*A* and *B*), we cannot assess whether this RNA results from maturation of the tRNA–*tufB* transcript or transcription of *tufA*.

It has been previously demonstrated in *M.xanthus* that translational initiation factor 3 (IF3) is involved in the differentiation process that takes place in myxobacteria under unfavourable conditions (38) and that in another sporulating bacterium, *Bacillus subtilis*, elongation factor G (EF-G) also plays a crucial role (39). We think that this work provides some tools for studying the involvement of translational elongation factor EF-Tu in this special process. The other *tuf* gene (*tufA*) now needs to be cloned and the relative importance of both genes has to be analysed, although such a study could be impaired by the lack of genetic tools in *S.aurantiaca*. However, it would be easier to try to knock out a *tuf* gene in *M.xanthus* (40), where we have already demonstrated the presence of two EF-Tu-encoding genes. The tRNA–*tufB* operon of *S.aurantiaca* deserves *per se* complementary studies to show up the processing intermediates arising from the tRNA gene cluster and to determine the role of the 5′-proximal region of the locus in regulation of expression of the five genes. The occurrence of A+T-rich sequences, an unusual feature in myxobacteria, upstream of the putative promoter may represent UP elements (41) involved in modulation of expression of the tRNA–*tufB* operon.

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