A secondary promoter for elongation factor Tu synthesis in the str ribosomal protein operon of Escherichia coli

ARTICLE *in* MGG - MOLECULAR AND GENERAL GENETICS · FEBRUARY 1982

DOI: 10.1007/BF00334145 · Source: PubMed

2 AUTHORS:

Janice M Zengel
Carnegie Mellon University
70 PUBLICATIONS **2,622** CITATIONS

SEE PROFILE

Lasse Lindahl
University of Maryland, Baltimore County
85 PUBLICATIONS **3,594** CITATIONS

SEE PROFILE

Mol Gen Genet (1982) 185:487–492

MGG

A Secondary Promoter for Elongation Factor Tu Synthesis in the *str* Ribosomal Protein Operon of *Escherichia coli*

Janice M. Zengel and Lasse Lindahl
Department of Biology, The University of Rochester, Rochester, New York 14627, USA

Summary. The *str* operon of *Escherichia coli* contains genes for ribosomal proteins S12 and S7 and for elongation factors EF-G and EF-Tu (Jaskunas et al. 1975). We have subcloned various segments of DNA from this operon onto multicopy plasmids. We found that cells carrying a recombinant plasmid which lacks the major promoter for the *str* operon but contains the 5′ portion of the EF-Tu gene synthesize a novel protein which we have identified as a truncated EF-Tu molecule. Moreover, cells carrying plasmids with an intact EF-Tu gene synthesize the elongation factor at a 3-to 5-fold higher rate than haploid cells. Thus the EF-Tu gene can be expressed in the absence of the major promoter for the *str* operon. This expression is not due to read-through from plasmid promoters, but it is dependent on the presence of the distal portion of the EF-G gene on the plasmids. These results indicate that there is a secondary promoter for EF-Tu expression, apparently located within the structural gene for elongation factor EF-G.

Introduction

The synthesis of elongation factor EF-Tu in *Escherichia coli* provides an interesting and unusual model for studying regulation of gene expression. The reason is that EF-Tu is encoded by two nearly identical, but unlinked, genes (Jaskunas et al. 1975). One of these two genes (*tufA*) is located at 72 minutes on the chromosome and is the most distal gene in the *str* operon, which also encodes the ribosomal proteins (r-proteins) S12 and S7 as well as the elongation factor EF-G (Jaskunas et al. 1975; see map in Fig. 1). The other gene for EF-Tu (*tufB*) is located at 88 minutes and is at the distal end of an operon which also encodes four tRNA molecules (Lee et al. 1981). The total amount of EF-Tu synthesized from the two genes is regulated coordinately with the synthesis of r-proteins during changes in growth rate and during amino acid limitation (Furano 1975; Furano and Wittel 1976; Blumenthal et al. 1976; Reeh et al. 1976; Pedersen et al. 1978).

It is not clear why *E. coli* maintains two genes for EF-Tu or how their expression is regulated and coordinated. Many r-protein operons are regulated autogenously by one of the r-proteins encoded by the operon (for a review, see Lindahl and Zengel 1982). However, the regulatory protein in the *str* operon, S7, regulates only the synthesis of S7 and EF-G, but not EF-Tu (Dean et al. 1981). We have previously investigated if EF-Tu regulates its own synthesis by measuring EF-Tu synthesis after accumulation of EF-Tu to about three times the normal concentration (Zengel and Lindahl 1982). These experiments failed to reveal any regulatory role of EF-Tu itself. Thus no mechanism for regulation of EF-Tu synthesis has yet emerged. In an effort to gain insight into this mechanism we have analyzed EF-Tu synthesis in strains containing multicopy plasmids carrying various parts of the *str* operon. One unexpected result of these experiments was the observation that the *tufA* carried by such plasmids can be expressed in the absence of the major promoter for the *str* operon. Here we report these experiments, which suggest that there is a secondary promoter for *tufA*, apparently located within the gene for EF-G.

Offprint requests to: Janice Zengel

Materials and Methods

Strains and Bacteriological Techniques. All bacterial strains used in this study were derivatives of the *E. coli* K12 strain LL308 (F′ *pro laci*^{q}*z*$^{\Delta M15}$/*Δ*(*lac-pro*) *recA*; Zengel et al. 1980), transformed with the indicated plasmids. Media and growth conditions have been described previously (Zengel et al. 1980; Zengel and Lindahl 1981).

Plasmid Construction. The structures of the recombinant plasmids used for this study are summarized in Fig. 1. General conditions for recombinant DNA techniques have been described previously (Lindahl and Zengel 1979; Zengel et al. 1980). The vector pLL140 (Zengel et al. 1980) is a derivative of pBGP120 (Polisky et al. 1976) that contains a unique *Kpn*I site downstream from the widl-type *lac* promoter and partial *lacz* gene. The *Kpn*I site is flanked on the promoter proximal side by about 100 base pairs from the r-protein L3 gene and on the promoter distal side by about 400 base pairs from the C-terminal end of the r-protein L2 gene (Zengel et al. 1980). Genes inserted at the *Kpn*I site on pLL140 are under control of the *lac* operator-promoter. Plasmid pLL145 was constructed by cloning the 3.2 kb *Kpn*I fragment carrying an intact *fus* (EF-G) gene and a partial *tufA* (EF-TuA) gene from the specialized transducing phage *λfus*3 (Jaskunas and Nomura 1977) into the *Kpn*I site on pLL140. Plasmid pLL184 was derived from pLL145 by digesting the latter DNA with *Hin*dIII and religating. The resulting plasmid carries a delection extending from the *Hin*dIII site about 3 kb upstream of the *lac* promoter (Zengel et al. 1980) through the *lac* region to the *Hin*dIII site within the structural gene for EF-G. Plasmid pLL180 was constructed by transferring an *Eco*RI fragment carrying the partial *tufA* gene from pLL145 to pBGP120 (Polisky et al. 1976). pLL101 contains the 4.4 kb *Eco*RI fragment from *λfus*3 carrying the intact *tufA* gene inserted into the *Eco*RI site

0026-8925/82/0185/0487/$ 01.20

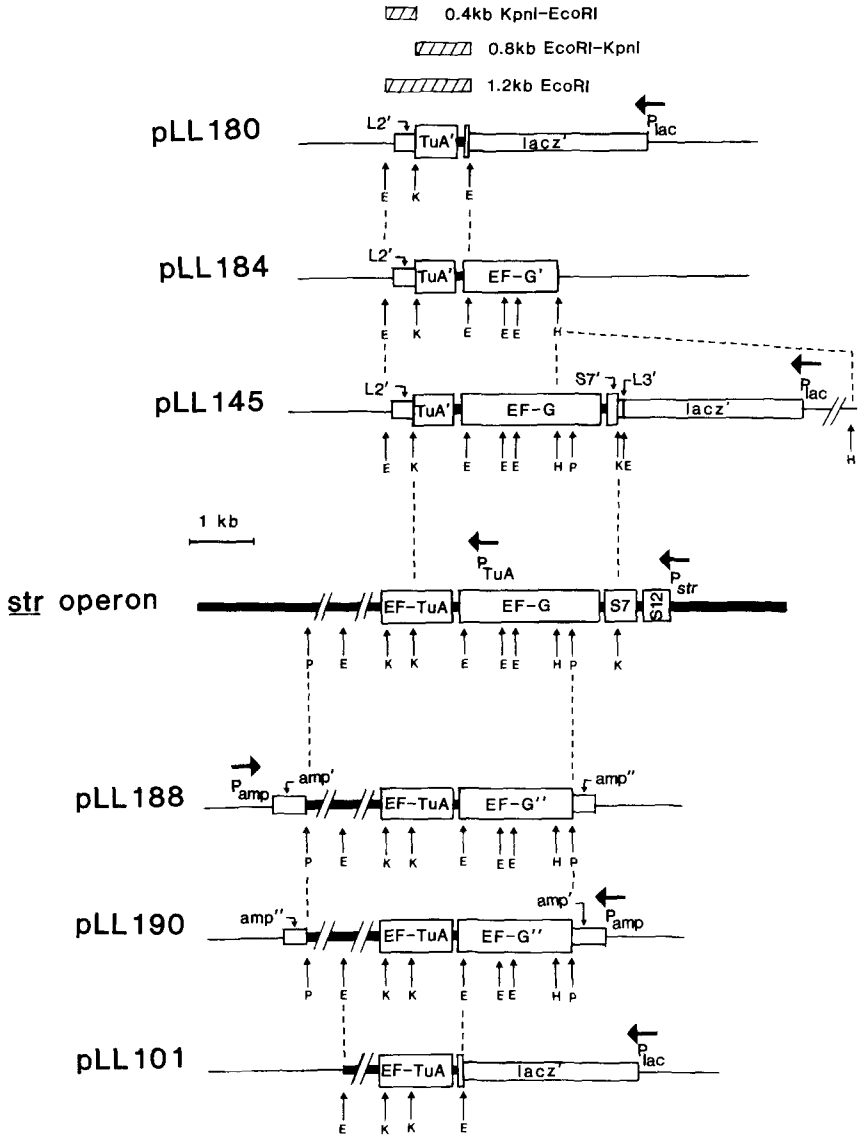

Fig. 1. The *str* operon and fragments of the operon cloned on plasmids. The genetic organization of the *str* operon is shown in the middle of the figure, drawn approximately to scale. The genes in the operon are identified by their products, and indicated by boxes whose lengths are approximately proportional to the expected lengths based on the molecular weights of the corresponding proteins. P_{str} designates the position of the major promoter of the *str* operon (Jaskunas et al. 1975; Post et al. 1978); the arrow above indicates the direction of transcription. The approximate position of the secondary promoter P_{TuA}, described in this report, is also shown. The vertical arrows indicate sites in or near the *str* operon for restriction endonucleases *Eco*RI (E), *Kpn*I (K), *Pst*I (P) and *Hin*dIII (H). The figure also shows the regions of the *str* operon cloned on the indicated plasmids (see Materials and Methods for details). The orientation of vector promoters relative to the cloned DNA is also given, with direction of transcription from these promoters indicated by the horizontal arrows. P_{lac}: *lac* promoter; P_{amp}: promoter for β-lactamase (ampicillin resistance) gene. The *Eco*RI and *Eco*RI-*Kpn*I fragments mentioned in the text are indicated at the top of the figure. Details of the plasmid construction and description of the vectors, including the origin of the complete or partial genes for L2, L3 and β-lactamase (amp) carried by these vectors, are given in Materials and Methods

on pBGP120 (Lindahl and Zengel 1979). Plasmids pLL188 and pLL190 were constructed by transferring the 7 kb *Pst*I fragment carrying the intact *tufA* gene from λ*fus*3 into the *Pst*I site on pBR322, which is located inside the β-lactamase gene carried by this vector (Bolivar et al. 1977). The orientation of the *tufA* gene in pLL188 and pLL190 relative to the promoter for the β-lactamase gene is shown in Fig. 1.

Labeling and Gel Electrophoresis of Proteins. For analysis by one-dimensional gel electrophoresis, a 0.2 ml aliquot of cells growing exponentially in glycerol or glucose minimal medium was labeled for one minute with 5 μCi L-[^{35}S]methionine (Amersham; specific activity 750–1,400 Ci/mmol), chased for two minutes with nonradioactive methionine (50 μg/ml), and mixed with 0.5 ml boiling sample buffer. Total proteins were fractionated by electrophoresis in 12% sodium dodecyl sulfate polyacrylamide gels (Laemmli 1970). For analysis by two-dimensional gel electrophoresis, a 2.5 ml aliquot of exponentially growing cells was labeled for one minute with 30 μCi [^{35}S]methionine, chased for 2 min with nonradioactive methionine (20 μg/ml) and harvested on ice. The cells were washed and then mixed with reference cells which had been labeled for several generations with 160 μCi L-[4,5-^{3}H(N)]-leucine (New England Nuclear, specific activity 42.0 Ci/mmol). Total protein extracts were prepared and fractionated by two-dimensional gel electrophoresis essentially as described by O'Farrell (1975). Procedures for autoradiography and determining radioactivity in protein spots have been described (Lindahl and Zengel 1979). The ^{35}S to ^{3}H ratio in a given protein spot was normalized by dividing by the ^{35}S to ^{3}H ratio in total protein.

Tryptic Peptide Mapping. [^{35}S]methionine-labeled proteins for peptide mapping were purified by two-dimensional gel electrophoresis (O'Farrell 1975), digested with trypsin (Jaskunas et al. 1975), and fractionated on a discontinuous acidic 50% acrylamide gel (West and Bonner 1980).

Results

1. Synthesis of a Novel Protein from DNA Cloned from the str Operon

To analyze the regulation of EF-Tu synthesis, we constructed a variety of plasmids carrying different segments of the *str* operon fused to the *lac* promoter-operator. One such plasmid, pLL101, carries the intact *tufA* (EF-TuA) gene cloned on pBGP120. An-

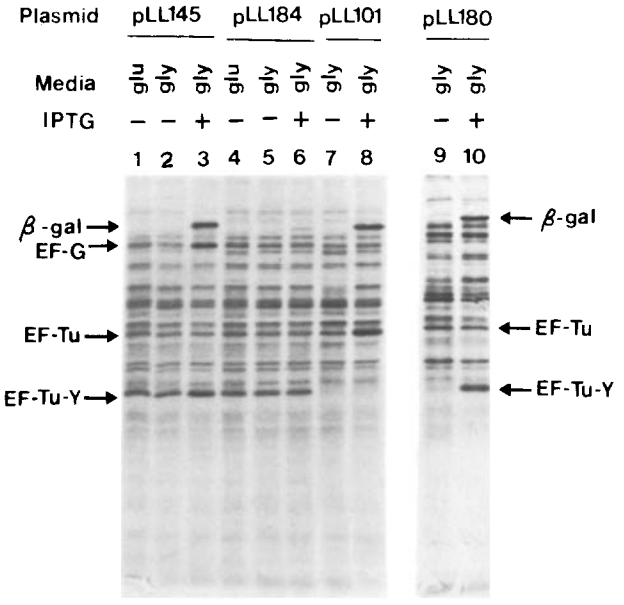

Fig. 2. Synthesis of EF-Tu-Y in cells carrying pLL145, pLL184, or pLL180. Cells containing pLL145 (lanes 1–3), pLL184 (lanes 4–6), pLL101 (lanes 7–8), or pLL180 (lanes 9–10) growing exponentially in minimal glycerol ("gly") or minimal glucose ("glu") media were pulse labeled with [^{35}S]methionine in the absence of IPTG or 10 min after induction with IPTG. The total protein extracts were electrophoresed on a 12% sodium dodecylsulfate-polyacrylamide gel. The figure shows an autoradiogram of the dried gel, with the pertinent protein bands identified. The strong β-galactosidase ("β-gal") band seen in lanes 3, 8, and 10 represents protein synthesized predominantly from the plasmid pLL145 (lane 3), pLL101 (lane 8), or pLL180 (lane 10); a small fraction of the band is due to the *lacz*$^{\Delta M15}$ gene on the F′. The weak band in the same region of the gel in lane 6 is β-galactosidase synthesized from the gene carried by the F′.

other plasmid, pLL145, carries the intact *fus* (EF-G) gene and the 5′ portion of *tufA* on the vector pLL140 (Fig. 1; Zengel and Lindahl 1982). Neither plasmid carries the natural promoter for the *str* operon. When we analyzed protein synthesis in cells carrying pLL101 we found, as expected, that the expression of the cloned EF-Tu gene was dependent on induction of transcription at the upstream *lac* promoter (Fig. 2, lanes 7 and 8). Similarly, synthesis of EF-G from the plasmid pLL145 was dependent on the presence of *lac* inducer (Fig. 2, lanes 2 and 3). In addition, cells containing pLL145 synthesized a large amount of a protein with a molecular weight (estimated from its electrophoretic mobility) of approximately 30,000 (Fig. 2, lanes 1–3). This protein (called "EF-Tu-Y") was synthesized in the absence of *lac* inducer (Fig. 2, lanes 1 and 2) but addition of IPTG led to an increased rate of synthesis (Fig. 2, lane 3). Although EF-Tu-Y comprised a significant fraction of the total protein in the cells carrying pLL145, little protein of comparable molecular weight was synthesized with or without IPTG in cells carrying the *tufA* plasmid pLL101 (Fig. 2, lanes 7 and 8) or the vector plasmid pLL140 (data not shown).

These results indicated that the new protein EF-Tu-Y is encoded, at least in part, by the DNA inserted into pLL140 to construct pLL145, and that the gene for EF-Tu-Y lies in the same ("correct") orientation relative to the *lac* promoter as the cloned *fus* and partial *tufA* genes. Furthermore, the constitutive synthesis of EF-Tu-Y from pLL145 suggests that the transcription of the gene for this protein can initiate at a promoter other than P_{lac}. Since this novel protein was not an expected

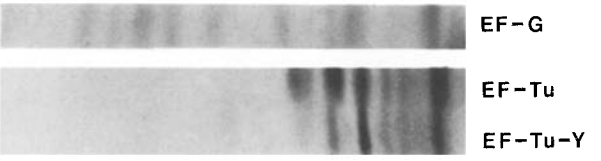

Fig. 3. Comparison of the tryptic fingerprints of EF-Tu and EF-Tu-Y. EF-Tu and EF-Tu-Y labeled in vivo with [^{35}S]methionine were purified from cells carrying pLL145 (see Materials and Methods for details). The tryptic peptides were separated by gel electrophoresis. An autoradiogram of the dried gel is shown. For comparison, the tryptic fingerprint of EF-G is included

product of the cloned DNA derived from the *str* operon, we wanted to determine the location of the structural gene and promoter for EF-Tu-Y.

2. Identification of the Structural Gene for EF-Tu-Y

To identify the region of DNA encoding EF-Tu-Y, we subcloned *Eco*RI fragments from pLL145 into the vector pBGP120 (Fig. 1). Cells containing one of these subclones, pLL180 (Fig. 1), synthesized EF-Tu-Y in the presence of IPTG (Fig. 2, lane 10; see below for discussion of inducible versus constitutive EF-Tu-Y synthesis). The plasmid pLL180 contains the 1.2 kb *Eco*RI fragment from pLL145. This *Eco*RI fragment consists of a 0.8 kb *Eco*RI-*Kpn*I fragment originating from the *str* operon and a 0.4 kb *Kpn*I-*Eco*RI fragment originating from the vector pLL140 (see top of Fig. 1). From DNA sequencing studies (Yokota et al. 1980) we know that the 0.8 kb *Eco*RI-*Kpn*I fragment contains approximately 70 base pairs from the distal end of the *fus* gene and the proximal 677 base pairs from the *tufA* gene. The 0.4 kb *Kpn*I-*Eco*RI fragment contains the distal portion of the gene for r-protein L2 (Zengel et al. 1980). Of these various partial genes present on the 1.2 kb *Eco*RI fragment in pLL180, only the 677 base pair portion of *tufA* has the coding capacity for a protein the size of EF-Tu-Y. We conclude, therefore, that EF-Tu-Y is encoded, at least in part, by the partial *tufA* gene present on pLL145 and pLL180.

The truncated *tufA* gene encodes the first 225 amino acids of EF-Tu (Arai et al. 1980; Jones et al. 1980). This portion of the EF-Tu molecule has a predicted molecular weight of 24,600 Daltons, which is only slightly smaller than the apparent molecular weight of EF-Tu-Y (30,000). This difference is probably due to the fact that the partial *tufA* gene does not include a termination codon and therefore an unknown portion of the C-terminal end of EF-Tu-Y must be derived from the partial L2 gene originating from the vector pLL140. In fact, if the reading frames of the partial genes for EF-Tu and L2 are aligned properly, EF-Tu-Y could be a fusion protein consisting of the N-terminal part of EF-Tu and the C-terminal part of L2. In any event, at least two thirds of EF-Tu-Y must be derived from *tufA*.

The genetic evidence that EF-Tu-Y is a partial EF-Tu molecule is supported by chemical studies. We analyzed the tryptic peptide patterns of ^{35}S-methionine labeled EF-Tu and EF-Tu-Y from cells carrying pLL145 (Fig. 3). The tryptic digestions apparently were incomplete, since the pattern for EF-Tu contains more than the five methionine-containing tryptic peptides predicted from the amino acid sequence (Arai et al. 1980; Jones et al. 1980). Nevertheless, it is clear that the EF-Tu-Y digest contains several methionine-containing tryptic peptides which are also found in the digest of the complete EF-Tu protein. Furthermore, EF-Tu-Y can be precipitated by EF-Tu specific

antisera (data not shown). On the basis of the genetic and biochemical evidence we therefore conclude that EF-Tu-Y is the N-terminal fragment of EF-Tu, possibly fused to the C-terminal end of r-protein L2.

3. *Identification of a Secondary Promoter in the str Operon*

We next considered the question of the identity of the promoter responsible for the constitutive synthesis of EF-Tu-Y from pLL145. The observation that the synthesis of EF-Tu-Y proceeds in the absence of *lac* inducer suggests that the partial *tufA* gene can be expressed from a promoter other than P_{lac}. Consistent with this conclusion is the observation that pLL145-containing cells grown in glucose medium (and therefore presumably catabolite repressed) still synthesize EF-Tu-Y at about the same rate as cells grown in glycerol medium (Fig. 2, lanes 1 and 2). Moreover, since the expression of the EF-G gene on pLL145 is dependent on induction of P_{lac}, the promoter responsible for the constitutive expression of EF-Tu-Y is almost certainly downstream from the beginning of the *fus* gene. To conclusively demonstrate that the constitutive EF-Tu-Y synthesis is due to a promoter other than P_{lac} we deleted a *Hin*dIII fragment from pLL145 (Fig. 1). The resulting plasmid (pLL184) is missing the *lac* promoter, the *lacz'* gene and part of the EF-G gene (Fig. 1). However, cells carrying this deletion plasmid continue to synthesize EF-Tu-Y at a rate comparable to that found in cells containing pLL145 (Fig. 2, lanes 4, 5, and 6). These experiments demonstrate that *tufA* can be expressed from a promoter, which we will call P_{TuA}, which must lie downstream from the *Hin*dIII site in the *fus* gene (Fig. 1).

EF-Tu-Y synthesis from the subclone pLL180 (described above) occurs only in the presence of IPTG (Fig. 2, lanes 9 and 10). Expression of the partial *tufA* gene on this plasmid is dependent therefore on transcription from P_{lac}, suggesting that P_{TuA} is *not* present on the 0.8 kb *Eco*RI-*Kpn*I fragment that harbors the 5′ portion of *tufA* (Fig. 1). Consistent with this conclusion is the observation that the synthesis of EF-Tu from the *tufA* plasmid pLL101 (described above) is also dependent on IPTG induction (Fig. 2, lanes 7 and 8). The DNA cloned on pLL101 terminates at the same upstream *Eco*RI site as the DNA cloned on pLL180 (see Fig. 1). These results indicate that at least part of P_{TuA} maps upstream from this *Eco*RI site. As mentioned above, this *Eco*RI site lies about 70 base pairs upstream from the 3′ end of the *fus* gene (Yokota et al 1980). Therefore, P_{TuA} apparently maps within the structural gene for EF-G between the *Hin*dIII site and the most distal *Eco*RI site.

4. *Gene Dosage Effects of Plasmids Carrying tufA and the Secondary Promoter*

The mapping of a promoter for the partial *tufA* gene within the *fus* gene on pLL145 implies that the intact *str* operon has a secondary promoter for EF-Tu synthesis. We would predict, therefore, that a strain carrying a multicopy plasmid with the intact *tufA* gene and P_{TuA} would constitutively oversynthesize the complete EF-TuA molecule. To test this prediction, we cloned a 7 kb *Pst*I fragment carrying all of *tufA* and a portion of the *fus* gene into the *Pst*I site in the β-lactamase gene of pBR322 (Bolivar et al. 1977; Sutcliffe 1978). We examined two such plasmids (pLL188 and pLL190; see Fig. 1) carrying the 7 kb DNA fragment in opposite orientations. We found that, in cells carrying either of these plasmids the rate of EF-Tu synthesis is three-to five-fold higher than in control cells (Fig. 4, Table 1).

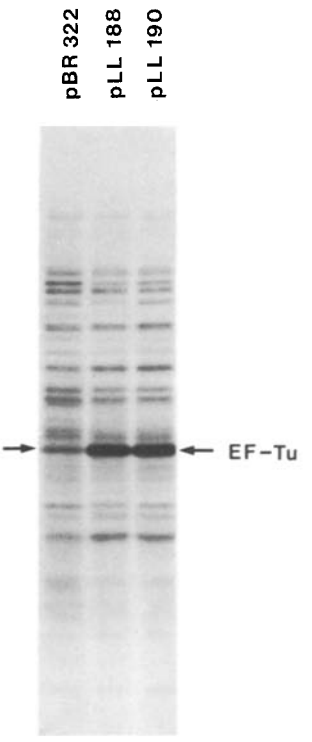

Fig. 4. Synthesis of EF-Tu in strains carrying multiple copies of the *tufA* gene. Cells containing pLL188 or pLL190 (see Fig. 1) or the vector pBR322 were grown exponentially in glucose minimal medium, labeled for 1 min with [^{35}S]methionine and electrophoresed as described in the legend to Fig. 2 and in Materials and Methods. The figure shows an autoradiogram of the gel

Table 1. Stimulation of EF-Tu synthesis in cells carrying the 7 kb *Pst*I fragment cloned on pBR322

Protein	Relative synthesis rate in cells carrying plasmid			
	pLL188		pLL190	
	Glucose	Glycerol	Glucose	Glycerol
EF-G	0.89	0.86	0.89	1.09
EF-Tu	*4.39*	*5.53*	*4.34*	*3.17*
EF-Ts	0.81	0.73	0.90	0.95
α	0.70	0.75	0.64	0.82

Cells containing recombinant plasmid pLL188 or pLL190 or the vector pBR322 were grown exponentially in minimal glycerol or minimal glucose media. The cultures were pulse-labeled for 1 min with [^{35}S]methionine. The total protein extracts were fractionated by two-dimensional gel electrophoresis (O'Farrell 1975) and the radioactivity in the indicated proteins was determined. The relative synthesis rates were calculated by dividing the normalized $^{35}S/^{3}H$ ratio for a given protein in the pLL188 or pLL190 strain by the normalized ratio for the same protein in the pBR322 control. For further details, see Materials and Methods

The β-lactamase DNA on pBR322 is transcribed predominantly from the β-lactamase promoter (P_{amp} in Fig. 1), although it apparently is also transcribed weakly from the opposite direction (Chang et al. 1978; Stuber and Bujard 1981). If the expression of the *tufA* gene on pLL188 and pLL190 were due to transcription initiated at promoters on the vector DNA (such as the β-lactamase promoter), we would therefore expect that

the expression should depend strongly on the orientation of the DNA insert relative to the vector DNA. This was not observed; rather, we found that the *tufA* gene is expressed with similar efficiency in both orientations. These experiments therefore suggest that the EF-Tu synthesis from pLL188 and pLL190 is due to a promoter carried by the cloned *Pst*I fragment. This result is consistent with our conclusion that there is a secondary promoter for *tufA* within the structural gene for EF-G.

Discussion

E. coli EF-Tu is encoded by two almost identical unlinked structural genes (Jaskunas et al. 1975; Yokota et al. 1980; An and Friesen 1980). One of these genes, *tufA*, has been mapped at the promoter distal end of the *str* operon, which also encodes EF-G and r-proteins S7 and S12 (Jaskunas et al. 1975). Several laboratories have shown that 50 to 70% of the total EF-Tu is synthesized from the *tufA* gene (Pedersen et al. 1976; van der Meide et al. 1980). Given the seven-to fourteen-fold excess of EF-Tu to ribosomes and EF-G (Furano 1975; Pedersen et al. 1978), these results imply that EF-Tu is synthesized from *tufA* at greater than three times the synthesis rates of the other three proteins from the *str* operon. In this paper we report that there is a secondary promoter for *tufA* apparently located within the structural gene for EF-G. Initiation of transcription at this secondary promoter, P_{TuA}, may in part account for the relative increase in *tufA* gene expression compared to the expression of the upstream genes of the *str* operon. Another possible function of P_{TuA} is to allow the cell to differentially regulate EF-Tu synthesis from *tufA* without perturbing the synthesis of EF-G and r-proteins S7 and S12. In this regard, it is interesting to note that the ratio between EF-TuA and EF-G decreases with increasing growth rate (Reeh and Petersen 1977).

The strength of P_{TuA} can be estimated from the synthesis rates of EF-Tu, or EF-Tu fragment, in several of our strains. For example, cells carrying a plasmid with the 7 kb *Pst*I fragment inserted in either orientation of pBR322 synthesize about four-fold more EF-Tu than haploid cells (Table 1). If we assume that the plasmid is present at twenty copies per genome (Hershfield et al. 1974), that synthesis of EF-Tu from the two chromosomal genes is still proceeding normally, and that one-half of the chromosome-derived EF-Tu is synthesized from *tufA*, then the twenty *tufA* genes carried by the plasmid and expressed from the secondary promoter are contributing 6/8ths of the EF-Tu being synthesized in these cells. Thus, the cloned secondary promoter is roughly 30% (6/20) as active as P_{str}. A similar value for the strength of P_{TuA} was estimated from the amount of the incomplete EF-Tu (EF-Tu-Y) synthesized from cells containing pLL145. These estimates do not necessarily reflect the relative rates of transcription from P_{str} and P_{TuA} because the efficiencies of translation from the two messenger RNA molecules may differ. Nevertheless, these calculations suggest that the secondary promoter could make a significant contribution to the overall synthesis rate of EF-Tu.

Studies of UV-irradiated cells infected with λ transducing phages carrying mutations in the *str* operon have suggested that the synthesis of EF-TuA is dependent on transcription from the major *str* promoter (Jaskunas et al. 1975; Post et al. 1978). It is not clear why the secondary promoter activity was not observed in these experiments. Perhaps the secondary promoter is active only under certain (unknown) physiological conditions. If so, removing the promoter from the intact *str* operon can apparently also trigger its activation. However, it is important to note that the high level of secondary promoter activity that we observe is not the result of fusing P_{TuA} to a foreign gene or of changing DNA sequences immediately upstream from the secondary promoter. This suggests that the promoter is entirely within the DNA from the *str* operon and is not simply an artifact created by fortuitous combinations of bases at the joints between DNA inserts and vector DNA.

We do not understand how the synthesis of EF-Tu is regulated. Ribosomal protein S7 regulates its own and EF-G synthesis but not the synthesis of EF-Tu (Dean et al. 1981). One possibility is that EF-TuA regulates its own synthesis. However, we saw no effect on EF-Tu-Y synthesis under conditions where EF-Tu was oversynthesized two-fold (Zengel and Lindahl 1982). Moreover, in cells carrying a plasmid containing an intact *tufA* gene and secondary promoter, the synthesis of EF-TuA is increased three-to five-fold. These experiments indicate that excess accumulation of EF-Tu does not affect the expression of *tufA* from P_{TuA}. In other studies we have shown that excess accumulation of EF-Tu also has no significant effect on the expression of the *tuf* genes from their primary promoters (Zengel and Lindahl 1982). Thus, our studies have failed to reveal any evidence for autogenous regulation of EF-Tu synthesis. On the other hand, Young and Furano (1981) report that cells can compensate for a decrease in the level of functional EF-Tu from *tufB* (EF-TuB) by increasing the level of EF-TuA synthesis. Their hybridization studies indicate that *tufB* inactivation results in an increased level of mRNA transcribed from DNA immediately upstream from the *tufA* gene. One interpretation of these results is that the cell responds to a decrease in the level of EF-TuB by increasing the transcription of the entire *str* operon (Young and Furano 1981). However, another possibility is that EF-TuA synthesis in this *tufB* mutant is increased by initiating additional transcripts at the secondary promoter. Experiments are in progress to determine the molecular basis for regulation of *tufA* and *tufB* expression and to characterize the role of the secondary promoter in this regulation.

Acknowledgements. We are grateful to R. H. Archer for technical assistance and to N. Brot for antisera. This work was supported by a grant from the National Institute for Allergy and Infectious Diseases.

References

An G, Friesen JD (1980) The nucleotide sequence of *tufB* and four nearby tRNA structural genes of *Escherichia coli*. Gene 12:33–39

Arai K, Clark BFC, Duffy L, Jones MD, Kaziro Y, Laursen RA, L'Italien JL, Miller DL, Nagarkatti S, Nakamura S, Nielsen KM, Petersen TE, Takahashi K, Wade M (1980) Primary structure of elongation factor Tu from *Escherichia coli*. Proc Natl Acad Sci USA 77:1326–1330

Blumenthal RM, Lemaux PG, Neidhardt FC, Dennis PP (1976) The effects of the *relA* gene on the synthesis of aminoacyl-tRNA synthetases and other transcription and translation proteins in *Escherichia coli* B. Mol Gen Genet 149:291–296

Bolivar F, Rodriguez RL, Green PJ, Betlach MC, Heyneker HL, Boyer HW, Crosa JH, Falkow S (1977) Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene 2:95–113

Chang ACYC, Nunberg JH, Kaufman RJ, Erlich HA, Schimke RT, Cohen SN (1978) Phenotypic expression in *E. coli* of a DNA sequence coding for mouse dihydrofolate reductase. Nature 275:617–624

Dean D, Yates JL, Nomura M (1981) Identification of ribosomal protein S7 as a repressor of translation within the *str* operon of *E. coli*. Cell 24:413–419

Furano AV (1975) Content of elongation factor Tu in *Escherichia coli*. Proc Natl Acad Sci USA 72:4780–4784

Furano AV, Wittel FP (1976) Synthesis of elongation factors Tu and G are under stringent control in *Escherichia coli*. J Biol Chem 251:898–901

Hershfield V, Boyer HW, Yanofsky C, Lovett MA, Helinski DR (1974) Plasmid ColE1 as a molecular vehicle for cloning and amplification of DNA. Proc Natl Acad Sci USA 71:3455–3459

Jaskunas SR, Lindahl L, Nomura M, Burgess RR (1975) Identification of two copies of the gene for the elongation factor EF-Tu in *E. coli*. Nature 257:458–462

Jaskunas SR, Nomura M (1977) Organization of ribosomal protein genes of *Escherichia coli* as analyzed by polar insertion mutations. J Biol Chem 252:7337–7343

Jones MD, Pedersen TE, Nielsen KM, Magnusson S, Sottrup-Jensen L, Gausing K, Clark BFC (1980) The complete amino acid sequence of elongation factor Tu in *Escherichia coli*. Eur J Biochem 108: 507–526

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lee JS, An G, Friesen JD, Fiil NP (1981) Location of the *tufB* promoter of *E. coli*: Co-transcription of *tufB* with four transfer RNA genes. Cell 25:251–258

Lindahl L, Zengel JM (1979) Operon-specific regulation of ribosomal protein synthesis in *Escherichia coli*. Proc Natl Acad Sci USA 76:6542–6546

Lindahl L, Zengel JM (1982) Expression of ribosomal genes in bacteria. Adv Genet 21:53–121

O'Farrell PH (1975) High resolution two-dimensional gel electrophoressis of proteins. J Biol Chem 250:4007–4021

Pedersen S, Bloch PL, Reeh S, Neidhardt FC (1978) Patterns of protein synthesis in *Escherichia coli*. Catalog of amount of 140 individual proteins at different growth rates. Cell 14:179–190

Pedersen S, Blumenthal RM, Reeh S, Parker J, Lemaux P, Laursen RA, Nagarkatti S, Friesen JD (1976) A mutant of *Escherichia coli* with an altered elongation factor Tu. Proc Natl Acad Sci USA 73:1698–1701

Polisky B, Bishop RJ, Gelfand DH (1976) A plasmid cloning vehicle allowing regulated expression of eukaryotic DNA in bacteria. Proc Natl Acad Sci USA 73:3900–3904

Post LE, Arfsten AE, Nomura M (1978) Isolation and characterization of a promoter mutant in the *str* ribosomal protein operon in *E. coli*. Cell 15:231–236

Reeh S, Pedersen S (1977) Regulation of elongation factor synthesis in vivo. FEBS Federation of European Biochemical Societies, vol 43, Gene Expression (Clark BFC et al. eds), pp 89–98. Pergamon Press, New York

Reeh S, Pedersen S, Friesen JD (1976) Biosynthetic regulation of individual proteins in *relA*$^+$ and *relA* strains of *Escherichia coli* during amino acid starvation. Mol Gen Genet 149:279–289

Stuber D, Bujard H (1981) Organization of transcriptional signals in plasmids pBR322 and pACYC184. Proc Natl Acad Sci USA 78:167–171

Sutcliffe JG (1978) Nucleotide sequence of the ampicillin resistance gene of *Escherichia coli* plasmid pBR322. Proc Natl Acad Sci USA 75:3737–3741

Van der Meide PH, Borman TH, Van Kimmenade AMA, Van de Putte P, Bosch L (1980) Elongation factor Tu isolated from *Escherichia coli* mutants altered in *tufA* and *tufB*. Proc Natl Acad Sci USA 77:3922–3926

West MHP, Bonner WM (1980) Histone 2A, a heteromorphous family of eight protein species. Biochemistry 19:3238–3245

Yokota T, Sugisaki H, Takanami M, Kaziro Y (1980) The nucleotide sequence of the cloned *tufA* gene of *Escherichia coli*. Gene 12:25–31

Young FS, Furano AV (1981) Regulation of the synthesis of *E. coli* elongation factor Tu. Cell 24:695–706

Zengel JM, Lindahl L (1981) High efficiency, temperature-sensitive suppression of amber mutations in *Escherichia coli*. J Bacteriol 145:459–465

Zengel JM, Lindahl L (1982) Studies on the oversynthesis of elongation factors G and Tu in *Escherichia coli*. J Bacteriol 149:793–797

Zengel JM, Mueckl D, Lindahl L (1980) Protein L4 of the *E. coli* ribosome regulates an eleven gene r-protein operon. Cell 21:523–535

Communicated by K. Isono

Received December 21, 1981