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Transcription of the tRNA-*tufB* operon of *Escherichia coli*: activation, termination and antitermination

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ABSTRACT

Signals setting the level of transcription of the tRNA-*tufB* operon have been studied by deletion mapping. *TufB* transcription was measured *in vivo* with plasmid-borne tRNA-*tufB*:*galK* operon fusions. Removal of the sequences from -133 to -58 with respect to the transcription start point, results in a 90% decrease of *tufB* transcription. This demonstrates the presence of a region, upstream of the tRNA-*tufB* promoter, that enhances the expression of the operon. DNA fragments bearing this upstream activator region do not display an abnormal electrophoretic mobility, as has been observed for the *rrnB* P1 upstream activator.

Deletions starting in the first tRNA gene and directing towards *tufB* reveal at least two sites that influence *tufB* transcription. One signals transcription termination in the intergenic region between *thrT* and *tufB*. The other may be involved in antitermination. Possible mechanisms underlying antitermination and termination are considered in the light of the nucleotide sequence.

INTRODUCTION

The tRNA-*tufB* operon of *Escherichia coli* is a mixed operon coding for four different tRNA species and the protein EF-TuB (1,2). The regulation of this operon is complex. Transcription has been shown to be under stringent control (3,4). Products of the *rrn* operons repress, either directly or indirectly, the transcription of the tRNA genes (5,6, our unpublished results). The EF-Tu protein acts as an autogenous repressor of *tufB* (7,8). *TufB* is cotranscribed with the four upstream tRNA genes (2,9,10). Termination of transcription within or at the end of the tRNA cluster partially uncouples tRNA synthesis from *tufB* transcription. Processing of the cotranscript occurs in the intergenic region at a site about 72-74 nucleotides upstream of the initiation codon of *tufB* (11).

In order to shed more light on the regulation of the transcription of this interesting operon we have deleted parts of the operon and have studied the consequences for transcription. These studies have revealed the presence of an activator region upstream of the major promoter similar to activators

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found upstream of the tyrT promoter (12) and the rrnB promoter (13). Downstream of the promoter other regulatory sequences are present, involved in termination and probably in antitermination.

MATERIALS AND METHODS

Strains and plasmids

The E. coli strain used for experiments with galK plasmids is AB 2463 (arg, his, leu, pro, thr, thi, lac, gal, ara, xyl, mnt, str^{R}; 14). LBE 12020 (tufB, rpoB, recA56, xyl) is described by van der Meide (7). pTuB1 and pTuB2 are gifts of Dr. Y. Kaziro (15,16) and pCA95 is a gift of Dr. C. van Sluis (17).

Construction of deletions

Deletions were made by treatment of linear plasmid DNA with Bal31 exonuclease, followed by blunting with "Klenow" DNA polymerase and ligating with BamHI linkers (CCGGATCCGG). After nicking with BamHI and an other restriction endonuclease, the deleted DNA fragments were cloned in pDS1 or a derivative thereof. Deletion endpoints have been mapped by sequencing with the method of Maxam and Gilbert (18), starting at the BamHI linker. All constructions were also checked by digestion with AluI, HaeIII or HinfI restriction endonuclease. Recombinant DNA methods were according to Maniatis et al. (19).

Galactokinase activity and plasmid copy number determination

The galactokinase activity of cell extracts was determined according to a slightly modified method of Adams and Hatfield (20) as is described in Van Delft et al. (11). For a comparison of the galK expression of the various transformants, galactokinase activities were corrected for differences in plasmid copy number. Copy numbers were determined according to Adams and Hatfield (20) using pDS1 DNA as a probe.

S1 nuclease fine mapping

RNA isolation and S1 nuclease fine mapping procedures are described in Van Delft et al. (11). The labeling of the 3′ terminus of the AluI site from the AluI-ClaI DNA probe was as follows: pTuB10 was nicked with AluI and the fragment comprising the thrT-tufB intercistronic region was eluted from a polyacrylamide gel. The 3′ ends were labeled with [α-^{32}P]-dCTP and T4 DNA polymerase as described by Maniatis et al. (19), nicked with ClaI and the labeled AluI-ClaI fragment that bears the thrT-tufB intercistronic region was purified on a polyacrylamide gel.

RESULTS

In order to define the regions of the tRNA-tufB operon involved in setting the level of tufB transcription we have fused this operon to the promoterless galK gene, thus placing the expression of galK under control of tufB transcription (21). Translation stop codons in three reading frames between the junction point and galK prevent translational readthrough from the cloned tufB fragment into galK. The operon fusion was carried by a plasmid that was introduced into a galK defective E. coli strain. Galactokinase activity of cell-free extracts was determined as a measure of galK transcription.

The tRNA-tufB:galK operon fusion was constructed by cloning a 1050 bp BglII-ClaI fragment from the plasmid pTuB10 into the BamHI-ClaI sites of pDSI (Figure 1). The resulting plasmid pDS10 contained the sequences ranging from

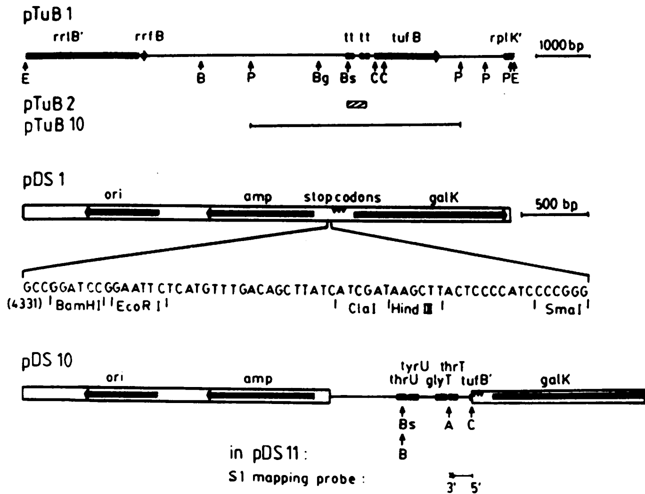

Figure 1. Maps of the EcoRI fragment from pTuB1 containing the tRNA-tufB operon and of the galK plasmids pDS1 and pDS10. pTuB2 is identical to pTuB1 except for the deletion indicated by the shaded box. pTuB10 is made by cloning the indicated PstI fragment of pTuB1 in the PstI site of pBR322. pDS1 is constructed by cutting pCA95 (a derivative of pKO1 (21)) with EcoRI, blunting of the generated "sticky" ends with "Klenow" DNA polymerase and ligating with a synthetic BamHI linker. During this procedure about 30 bp of the original plasmid were deleted. The number 4331 represents the corresponding G-residue of pBR322 (48). The construction of pDS10 is described in the text. In pDS11 the BstEII site of pDS10 is altered into a BamHI site. The S1 nuclease mapping probe is a 179 bp long AluI-ClaI fragment, labeled with ^{32}P at the 3' terminus of the AluI site. Endonuclease sensitive sites are abbreviated as follows: A=AluI; B=BamHI; Bg=BglII; Bst=BstEII; C=ClaI; E=EcoRI; H=HpaI and P=PstI.

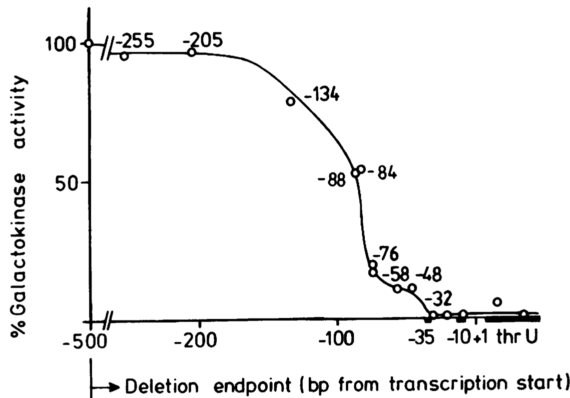

Figure 2. Galactokinase activities of cells transformed with pDS10 derivatives harbouring deletions in the region upstream of the tRNA-tufB operon. All deletions start at the BglII site 500 bp upstream of the transcription initiation point. The endpoints -255 and -205 were estimated by analysis with restriction endonucleases, the others were determined by sequencing. The activity of the pDS10 transformant was set at 100%.

500 bp upstream of the transcription initiation site of the tRNA-tufB operon up to and including the tufB translation initiation codon. Various deletions of the insert were constructed and their effects on galK expression were measured. All deletions harboured a BamHI linker (CCGGATCCGG) at the junction between the undeleted regions. Their sizes were determined by DNA sequencing starting at this linker according to Maxam and Gilbert (18). Galactokinase levels were corrected for copy number variations of the plasmids. Corrections never exceeded a factor 2.

Identification of an upstream region required for high tRNA-tufB expression

Many E. coli genes encoding stable RNA species owe a high level of expression to a functional region upstream of the -35 box of their promoter. To search for such an activator region upstream of the tRNA-tufB operon relevant DNA sequences of pDS10 were deleted. To this aim pTuB10, linearized at the BglII site, was treated with Bal31 exonuclease and ligated with BamHI linkers. The plasmid was then nicked with BamHI and ClaI and the fragment bearing the tRNA-tufB region cloned in pDS1. Galactokinase activities of cells transformed with the various derivatives of pDS10 were determined and are presented in Figure 2. This figure shows that the activity of the tRNA-tufB promoter decreases upon removal of upstream sequences, leveling off at about 10% of the original level when the deletion end point reaches positions

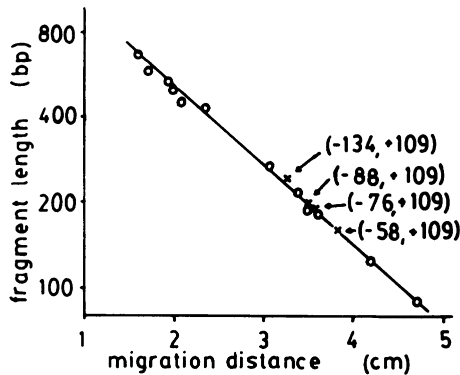

Figure 3. Electrophoretic mobilities of restriction fragments bearing the tRNA-tufB promoter (x) and of fragments of a HaeIII digest of pBR322 (o). The mobility was determined by electrophoresis at 5°C on a 5% polyacrylamide gel (20:1=acrylamide:bisacrylamide) in Tris-Acetate buffer (19).

-76 to -48. Elimination of the -35 box, completely abolishes promoter activity. The main decrease in galactokinase activity is observed upon deleting the sequences from -135 to -58 with a sharp drop between -85 to -76.

That the upstream region required for high levels of tufB transcription harbours a secondary promoter is highly improbable. First, a deletion ranging from -33 to +56 caused an almost complete abolishment of galK expression (results not shown). This deletion would not eliminate a secondary promoter with a -35 box at position -76. Second, no clear homologies are found between the upstream sequence and a consensus promoter. Third, in vitro experiments did not reveal transcripts initiated upstream of +1 (10).

No marked sequence conservations have been noted in the upstream region of different promoters for stable RNA genes. A rather unusual physical conformation, possibly involving kinking or bending of the DNA helix has been proposed, since DNA fragments carrying two different stable RNA promoters (hisR and rrnB P1) display an anomalous electrophoretic mobility on non-denaturing polyacrylamide gels (13,22). No evidence for such aberrant features in helix geometry could be detected in the upstream region of the tRNA-tufB operon (Figure 3). This figure illustrates the electrophoretic mobility at 5°C of various tRNA-tufB promoter fragments together with that of fragments from pBR322. Electrophoreses carried out at 20°C or 40°C gave the same results (not shown). Since the nucleotide sequence upstream of position -157 is unknown, analysis of upstream fragments beyond -134 was not feasible.

If a DNA region with an unusual physical conformation is present, it may be located far more upstream than that reported thus far for the hisR and rrnB P1 promoters. Alternatively, a location close to position -134 on the fragment (-134,+109) may mask its presence during electrophoresis (compare 22).

Sequences signalling termination

In a previous paper we presented evidence for transcription termination within the tRNA-tufB operon (11). In order to study the nature and the location of the terminator(s) in more detail we constructed a number of deletions starting at position +54 downstream of the transcription start site. In this case pDS10 was linearized at the BstEII site (see Figure 1) treated with Bal31 and ligated with BamHI linkers. After nicking with BamHI and ClaI the BamHI-ClaI fragments containing tufB were used to replace the corresponding fragment of pDS11 and galactokinase activity was measured of cells transformed with the deletion derivatives of pDS11.

As can be seen in Figure 4 the various deletions cause rather drastic changes in the expression of galK. The rise in transcription with deletions extending beyond position 409 is in accordance with the results reported in Van Delft et al. (11). There an increase in both the cellular content and the synthesis rate of tufB transcripts was observed with a deletion extending to position 459 (similar to pDS11.1 of Figure 5). This increase is most readily explained by the removal of sequences required for transcription termination. Striking is the low level, close to zero, of the activity resulting from deletions with endpoints ranging from position 245 to 409. These deletions apparently cause virtually complete termination. It seems as if sequences have been removed that normally counteract termination. We come back to them below.

The steep rise in transcription caused by deletions beyond position 409 hints at the location of the putative transcription terminator (Figure 4). These deletions eliminate the 3' half of thrT and the subsequent 10 nucleotides, implicating this part of the transcript in termination (compare pDS11.3 and pDS11.1 in Figure 5).

In order to investigate the role of the 5' half of thrU that is fused to the 3' half of thrT in plasmids deleted up to position 409, the deletion of pDS11.3 was extended in the direction of the tRNA-tufB promoter yielding pDS16.2 (Figure 5). First it was verified that the deletion of the 5' half of thrU by itself does not affect transcription (compare pDS14.2). The galactokinase activity of pDS16.2 transformants demonstrated that the

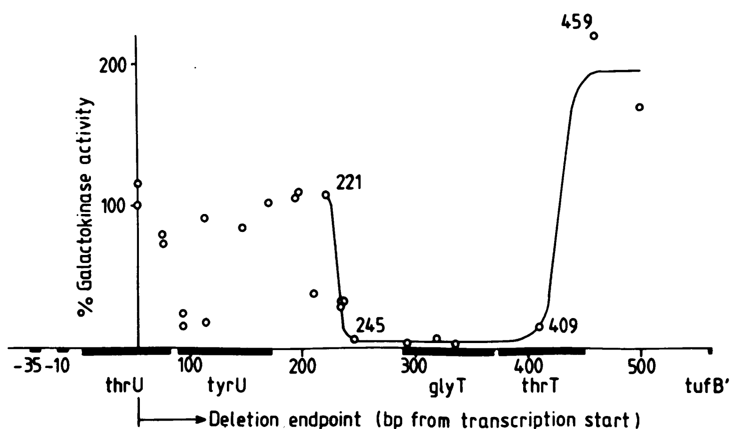

Figure 4. Galactokinase activities of cells transformed with pDS11 derivatives harbouring deletions in the tRNA region of the *tufB* operon. All deletions start at the *BstEII* site, 54 bp downstream of the transcription initiation point. The activity of pDS11 transformants was set at 100%.

deletion had not eliminated the transcription termination signal. We conclude that the 5' half of *thrU* is dispensable for termination.

A deletion described by Miyajima *et al.* (16) seems to be in conflict with the data presented here. This deletion, present on the plasmid pTuB2 (cf. Figure 1), runs from position 33 to 398 and was obtained by homologous recombination between *thrU* and *thrT*, resulting in a replacement of the four tRNA genes by a single *thrU*:*thrT* fusion gene. According to Miyajima *et al.* this deletion had no effect on EF-TuB synthesis in minicells and cell-free extracts and did not alter the synthesis of β-galactosidase in lysogens carrying a λ transducing phage with a *tufB*-*lacZ* fusion. Since this deletion is almost identical to that of pDS11.3, the result of Miyajima *et al.* is in clear contrast to the 90% drop in transcription caused by the deletion in the latter plasmid (Figure 5). To study this discrepancy we transferred the deletion of pTuB2 to a plasmid harbouring the tRNA-*tufB*:*galK* operon fusion. The *BglII*-*ClaI* fragment of pTuB2 was cloned in the *BamHI*-*ClaI* sites of pDS1, generating the recombinant pDS20. Galactokinase activity of pDS20 transformants is 15% of that of the control cells (pDS10 transformants; Figure 5) indicating that the deletion leaves the transcription termination site intact in accordance with the deletion of pDS11.3. Since Miyajima *et al.* examined a fragment corresponding to the entire *EcoRI* fragment of pTuB1 (compare Figure 1) and this fragment contains a much larger region upstream

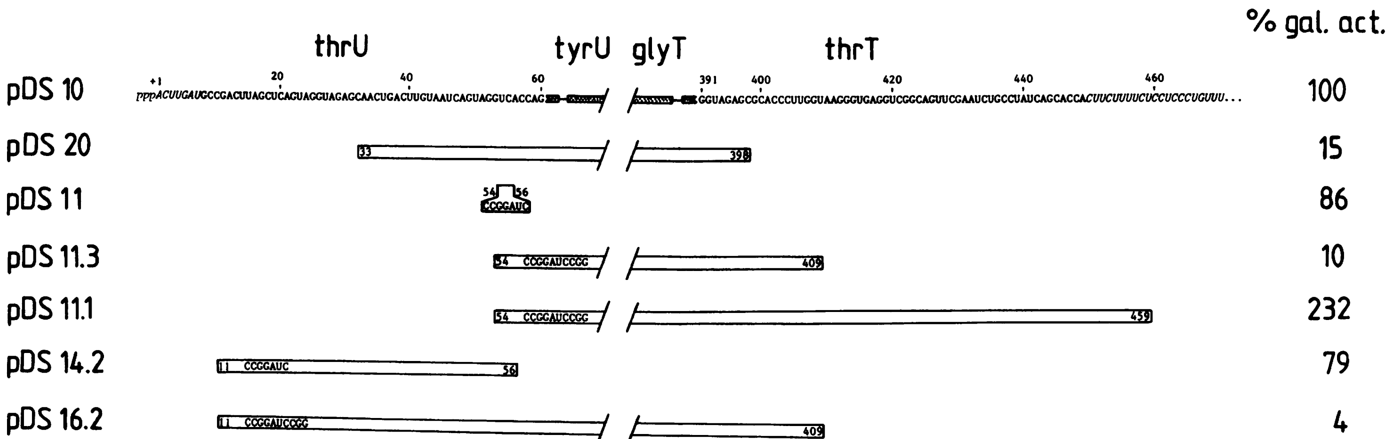

Figure 5. Galactokinase activities of cells transformed with pDS10 derivatives harbouring deletions in the tRNA region of the _tufB_ operon. The activity of pDS10 was set at 100%. Boxes represent the deleted sequences, with end points numbered. The sequence in the boxes is the inserted _BamHI_ linker. The linker of pDS11 and pDS14.2 was found to be shortened. Intergenic and flanking sequences are in italics.

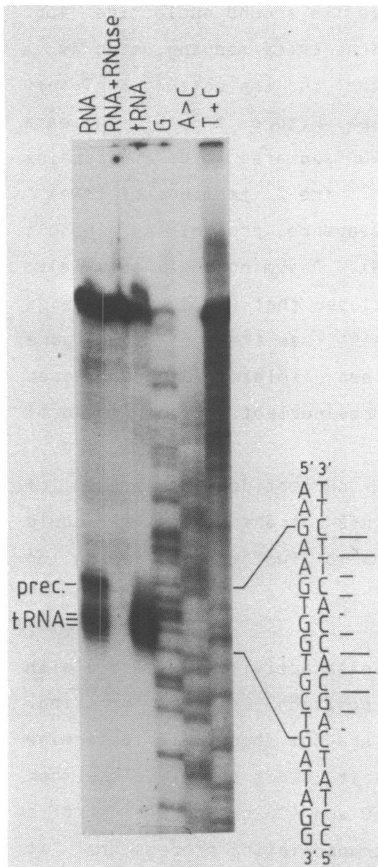

Figure 6. S1 nuclease fine mapping of the 3' ends of thrT transcripts in the thrT-tufB intercistronic region. The DNA probe used is shown in Figure 1. RNA was isolated from the strain LBE 12020. tRNA was obtained from Boehringer Mannheim. G, A>C and T+C indicate three different sequencing reactions according to (18). The ends of S1 nuclease resistant hybrids are indicated by bars.

of the tRNA-tufB operon, we also made constructs bearing this region. The EcoRI-ClaI fragments of pTuB1 and pTuB2 were cloned in pDS1 generating pDS100 and pDS200, respectively. The cellular amount of galactokinase encoded by pDS200 is 11% of that by pDS100, in accordance with the results for pDS10, pDS20 and pDS11.3. This, however, contradicts the data of Miyajima et al. (16), as they observed no differences in EF-TuB synthesis derived from pTuB1 and pTuB2. Whether the discrepancy is due to differences in analysis methods (tufB transcription versus EF-TuB synthesis), genetic background of host strains or DNA constructs (our constructs harboured 2 nucleotides of the tufB sequence, while those of Miyajima et al. 39 or more) is not clear.

The 3' end of thrT transcripts

The proposal put forward above, that the sequence from position 410 to 459 bears a signal required for transcription termination, implies that the 3'

end of thrT transcripts terminated there should lie around nucleotide 450. Total cellular RNA was therefore analysed by S1 nuclease mapping using as a probe a 3′ end-labeled DNA fragment comprising thrT and the entire thrT-tufB intergenic region (an AluI-ClaI fragment, see Figure 1). S1 nuclease resistant hybrids obtained with total cellular RNA appeared to be 6 basepairs longer than those obtained with tRNA (Figure 6). The 3′ terminus of $tRNA^{Thr}_{3}$ mapped here, did not contain the expected CCA sequence, probably as a result of "end-nibbling" of the RNA-DNA hybrid (23,24). Assuming that this also occurred to the thrT transcript, it may be concluded that its 3′ end extends about six nucleotides beyond the CCA end of $tRNA^{Thr}_{3}$ (see the arrow in Figure 7). A precursor of $tRNA^{Gly}_{2}$ and $tRNA^{Thr}_{3}$ has been isolated and sequenced previously, the 3′ end of which was located three nucleotides downstream of $tRNA^{Thr}_{3}$ (25).

ThrT transcripts with a 3′ end at about 40 nucleotides downstream of $tRNA^{Thr}_{3}$, that resulted from processing of tRNA-tufB cotranscripts (11), could not be mapped (Figure 6). Whether this is due to further processing or degradation cannot be decided.

Sequences signalling antitermination

As briefly mentioned above, the low galactokinase activities recorded with plasmids harbouring deletions at and beyond position 245 are striking. Downstream regions so remote from the promoter are not thought to determine the extent of transcription initiation (26,27). It is not conceivable either that removal of sequences between positions 250 and 400 can exert such a dramatic effect on the elongation rate of the transcription process that it drops to virtually zero. Sequences involved in a so-called antitermination mechanism have been reported, however. The steep decline in transcription upon removal of nucleotides from position 222 up to 245 may well be due to the elimination of an antitermination signal normally preventing transcription termination at the downstream terminator. If so, this signal plays a very important role in transcription regulation. Its elimination results in a complete shut off of tufB expression. The data also indicate that the termination of transcription at the end of the tRNA cluster is a very efficient event.

Intriguing are the large variations in transcription caused by deletions ending at positions up to 221. As discussed below the present data do not lend themselves to a clear interpretation in the absence of additional information.

DISCUSSION

The deletion mappings reported in this paper demonstrate that regions both upstream and downstream of the tRNA-tufB promoter determine the level of tufB transcription. A region upstream of this promoter enhances tufB transcription approximately 10-fold. Several regions between the promoter and tufB influence the transcription of tufB by altering the readthrough by RNA polymerase. Evidence is presented that these regions bear elements that are required for termination and antitermination of transcription.

The upstream activator is located in the region from positions -133 to -58 with respect to the transcription start point of the tRNA-tufB cotranscript (Figure 2). Deletions of increasing length cause a gradual decrease of tufB transcription, similar to what is observed with the upstream activators of the tyrT (28), the rrnB P1 (13) and the trp promoters (29). Deletion of sequences upstream of the phage lambda P_L promoter (30) and the spoVG promoter from Bacillus subtilis (31) also resulted in lowered transcription.

There is no marked sequence conservation in these upstream regions though

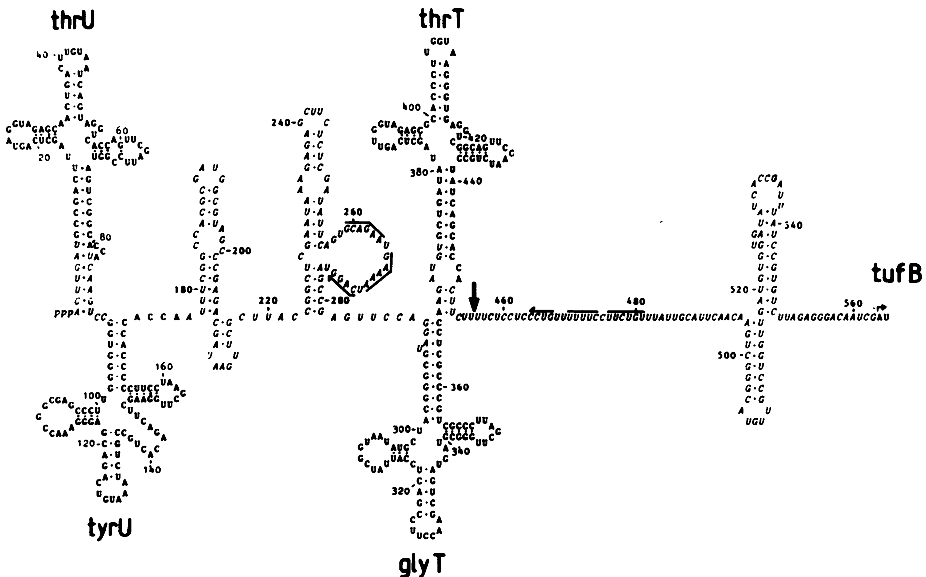

Figure 7. Hypothetical secondary structure of the tRNA region from the tRNA-tufB cotranscript. The structure is based on cloverleaf-like structures of the four tRNAs and analysis with the aid of a computer program (Abrahams et al., in preparation). An interaction between the overlined sequences is discussed in the text. The bold arrow indicates the 3′ end of thrT transcripts mapped in Figure 6.

they all bear A/T-rich sequences. An abnormal DNA conformation has been reported for the upstream activating region of the *rrnB* P1 promoter of *E. coli* (13) and that of the *hisR* promoter of *Salmonella typhimurium* (22). DNA fragments bearing these regions display aberrant electrophoretic mobilities on nondenaturing gels. A similar analysis did not reveal a clear deviation in helix geometry in the case of the tRNA-*tufB* promoter (Figure 3). Whether this region lacks these features on the chromosome or that the technique presently employed failed to reveal them, cannot be decided.

The molecular mechanism of the transcription activation remains to be established. Evidence has been presented that the upstream region of the *tyrT* promoter of *E. coli* contains additional binding sites for RNA polymerase (32). Travers (33) has proposed that binding of RNA polymerase upstream of the promoter may enhance transcription initiation in the case of all stable RNA promoters. Binding of a regulatory protein has been proposed for the *spoVG* gene of *Bacillus subtilis* (31). The existence of A/T-rich boxes in all activator regions described above may allow an easy local unwinding of the two DNA strands (30,34) or cause an abnormal DNA conformation (35) that favours efficient transcription.

Knowledge of the secondary and tertiary structure of the tRNA-*tufB* cotranscript may contribute significantly to our insight into the transcription termination and antitermination events. Since no structural data of this type are available as yet we can only refer to a hypothetical structure based (Figure 7). Taking into account the results of the present investigation two models of transcription termination will be considered here. One is a Rho-independent termination at a site a few nucleotides beyond *thrT*, the other is a Rho-dependent termination in the intergenic region about 50 nucleotides downstream of *thrT*.

Many bacterial terminators have a series of uridine residues at their 3' end that is preceded by a GC-rich region of dyad symmetry. Such terminators are Rho-independent and are generally quite efficient (36). The extended aminoacyl stem of the *thrT* portion of the cotranscript from the tRNA-*tufB* operon provides a double-stranded RNA helix followed by a series of uridines and thus qualifies as a Rho-independent terminator (Figure 7 and (1)). This helix is interrupted by an internal loop but its stability may be increased by coaxial stacking on the aminoacyl stem of the *glyT* portion of the cotranscript, thus forming an extended double-stranded RNA helix comprising 22 base pairs. We do not know whether this helix is formed prior to the termination event. Our data demonstrate, however, that formation of this

helix is not a prerequisite for termination since deletions up to position 409 do not abolish termination. It may be noted that the sequence 410-459 can form a double helix, though with a few mismatches, and thus may be sufficient for signalling termination.

Alternatively transcription may terminate in the intergenic region between thrT and tufB in a Rho-dependent fashion. Previously, Hudson et al. (2) observed the conserved sequence CAACAA when comparing this region with others containing a Rho-dependent terminator. Whether this conserved sequence functions in vivo is unknown; in vitro it has been demonstrated to be insufficient for Rho-dependent termination (37). Rho-dependent termination sites are poorly characterized so far. An unstructured RNA region low in G content upstream of the end points seems to be one of the prerequisites (36). The thrT-tufB intergenic region 453-496 possesses little secondary structure potential and a low G content (7%). The preceding 22 nucleotides are also low in G content. The results of the present study can also be accounted for by a Rho-dependent termination at a site in the intergenic region. The deletion extending up to position 459 removes a part of the unstructured region and almost half of the sequence with a low G content.

At the present time we cannot define exactly the site at which termination takes place nor the mechanism employed. Further studies with mutants of E. coli may shed new light on this question. Mapping of the 3′ end of the cellular thrT transcripts revealed a terminus about six nucleotides downstream of thrT (Figure 6). Whether this 3′ end is generated by transcription termination or by trimming of larger thrT transcripts unto the double-stranded RNA helix, cannot be decided, however.

Of great interest are the large fluctuations in tufB transcription observed with deletions ending at positions up to 459. Deletions with endpoints from position 245 up to 409 cause a complete shut off of tufB transcription. So far no deletions ending between 245 and 409 have been found that permit readthrough of tufB. Unless further studies will bring such deletions to light this means that the shut off of tufB transcription is due to termination of transcription downstream of this region. It also means that extending the deletions from 221 to 245 eliminates a region required for the prevention of transcription termination at the downstream site. Such regions have been found in the rrn operon (38,39). It has been shown that they are involved in an antitermination mechanism that ensures that the RNA polymerase elongates through the long untranslated operon and prevents premature termination of transcription at cryptic termination sites. So far no

antitermination signals have been reported in tRNA gene clusters. The antitermination system of the rrn operon of E. coli has many characteristics in common with that of the lambdoid phages (40). In both cases a highly conserved sequence, designated boxA, is an element of the antitermination signals. A search for boxA-like sequences in the tRNA gene region of the tRNA-tufB operon did not yield positive results. Possibly different mechanisms underlie antitermination in the rrn and the tRNA-tufB operon. Of great interest is whether or not transcription antitermination is dependent on host proteins. Mutations in five E. coli genes (nusA-E) affect antitermination of lambda transcription. The products of two of these genes, nusA and nusB, have been implicated in the antitermination of rRNA (42, Sigmund and Morgan quoted in 42). It will be of interest to see whether Nus proteins influence the galactokinase activities studied here with the deletion derivatives of the tRNA-tufB:galK fusion plasmids.

A different type of antitermination has been described for operons involved in the biosynthesis of some amino acids. The leader RNA of the transcript of these polygenic clusters can form mutually exclusive RNA secondary structures, one of which functions as a terminator (36,43). Since our data suggest that the intergenic region between tyrU and glyT contains sequences required for antitermination (Figure 4), interaction between this region and the thrT-tufB intergenic region may be looked for. A potential interaction of this type comprising 15 base pairs with 2 interruptions is indicated in Figure 7 by overlining the two sequences involved. Although these sequences are separated by almost 200 nucleotides, folding of the transcript can bring them in close proximity of each other. Base pairing of the two intergenic regions may prevent Rho-dependent termination by the uptake of the unstructured RNA region in a double-stranded helix. If so, the deletion up to position 245, that causes an increase of termination, should disrupt this proposed interaction between the two intergenic regions. This is conceivable since the deletion probably has a strong effect on the conformation of that part of the transcript. Antitermination through interaction of the two intergenic regions obviously can only affect the Rho-dependent but not the Rho-independent transcription termination discussed above. The merit of a Rho-dependent termination therefore is that a model for the structure of this part of the transcript can be proposed, that may underlie the mechanisms for both termination and antitermination.

Intriguing are the consequences of deleting various parts of thrU, tyrU and the tyrU-glyT intergenic region. Galactokinase activity declines and rises a number of times when the deletion, started at 54, is extended

progressively up to position 245. Whether this reflects the presence of additional transcription signals remains to be investigated. Deletions in this highly structured region may lead to refolding of the RNA chain and to alterations of transcript processing. Consequences for the termination-antitermination mechanism are hard to evaluate therefore on the basis of deletion mapping only.

The effects observed with the different deletions could also be attributed to differences in transcript stability or alterations in transcript processing and consequently to the translation efficiency of galK mRNA. We do not believe this is the case, since it is not true for pTuB11.1. Previously (11) we have shown that the deletion in pTuB11.1 resulted in an increased tufB RNA synthesis, and that processing of the deleted tRNA-tufB cotranscript to monocistronic tufB RNA still occured.

Finally, the question rises to which extent the various regions of the tRNA-tufB operon involved in activation, termination and antitermination of transcription, determine the regulation of the expression of this operon. As yet no evidence for such a role has been reported in the case of the activators upstream of the tyrT and rrnB P1 promoters (12,13,44). Regulation of transcriptional readthrough of a terminator has been described for a number of operons, though never for an operon encoding both structural and informational RNA (43,45,46,47).

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