The EMBO Journal Vol.2 No.6 pp.899–905, 1983

The nucleotide sequence of an *Escherichia coli* operon containing genes for the tRNA(m^1G)methyltransferase, the ribosomal proteins S16 and L19 and a 21-K polypeptide

Anders S. Byström*, Karin J. Hjalmarsson[1], P.Mikael Wikström and Glenn R. Björk

Department of Microbiology, University of Umeå, S-901 87 Umeå, Sweden

Communicated by P. Reichard
Received on 2 March 1983

The nucleotide sequence of a 4.6-kb *Sal*I-*Eco*RI DNA fragment including the *trmD* operon, located at min 56 on the *Escherichia coli* K-12 chromosome, has been determined. The *trmD* operon encodes four polypeptides: ribosomal protein S16 (*rpsP*), 21-K polypeptide (unknown function), tRNA-(m^1G)methyltransferase (*trmD*) and ribosomal protein L19 (*rplS*), in that order. In addition, the 4.6-kb DNA fragment encodes a 48-K and a 16-K polypeptide of unknown functions which are not part of the *trmD* operon. The mol. wt. of tRNA(m^1G)methyltransferase determined from the DNA sequence is 28 424. The probable locations of promoter and terminator of the *trmD* operon are suggested. The translational start of the *trmD* gene was deduced from the known NH_2-terminal amino acid sequence of the purified enzyme. The intercistronic regions in the operon vary from 9 to 40 nucleotides, supporting the earlier conclusion that the four genes are co-transcribed, starting at the major promoter in front of the *rpsP* gene. Since it is known that ribosomal proteins are present at 8000 molecules/genome and the tRNA-(m^1G)methyltransferase at only ~80 molecules/genome in a glucose minimal culture, some powerful regulatory device must exist in this operon to maintain this non-coordinate expression. The codon usage of the two ribosomal protein genes is similar to that of other ribosomal protein genes, i.e., high preference for the most abundant tRNA isoaccepting species. The *trmD* gene has a codon usage typical for a protein made in low amount in accordance with the low number of tRNA-(m^1G)methyltransferase molecules found in the cell.

Key words: nucleotide sequence/regulation/ribosomal proteins/*trmD* operon/tRNA(m^1G)methyltransferase

Introduction

The translational apparatus in *Escherichia coli* is composed of RNAs, such as rRNA and tRNA, and a variety of proteins such as initiation factors, elongation factors and aminoacyl-tRNA ligases. These different macromolecules constitute the major part of the cell content (Maaløe, 1979). Measurements of macromolecular composition have been made in order to understand the mechanisms which govern cell growth. An early observation was that fast growing cells have a higher content of rRNA and tRNA than slow growing cells. Such growth rate-dependent regulation is also true for all ribosomal proteins, elongation factors and many, but not all, aminoacyl-tRNA ligases (Maaløe, 1979; Nierlich, 1978; Neidhardt *et al.*, 1975). Proteins with growth rate-dependent regulation are made in large amounts in the cell although exceptions exist (Pedersen *et al.*, 1978). Ribosomal proteins, as well as other proteins made in large amounts, are also regulated in a way similar to stable RNA when monitored during shift-up and shift-down conditions. Although much is known of the regulatory behaviour of such proteins and many models have been discussed, the underlying mechanism is still unknown (Maaløe, 1979; Nierlich, 1978; Lindahl and Zengel, 1982).

During the biosynthesis of tRNA, several endo- and exonucleases operate together with the tRNA-modifying enzymes before the emergence of the mature tRNA (Mazzara and McClain, 1980). At least 40 different tRNA-modifying enzymes are involved in the maturation process but only a few of their genes have been identified (Björk *et al.*, 1982; Björk, 1983). So far little is known about gene organization and regulation of this group of enzymes. However, the regulation of tRNA(m^5U)methyltransferase is similar to that of stable RNA and ribosomal proteins, although the output of this enzyme is low (Ny and Björk, 1980). Another tRNA biosynthetic enzyme, the tRNA(m^1G)methyltransferase, is regulated in quite a different way. The specific activity of this enzyme increases only slightly with increased growth rate and the regulation of this enzyme under amino acid limitation is different from that of ribosomal proteins and rRNA (Ny *et al.*, 1980). Moreover, the enzyme is present at ~80 molecules/genome in sharp contrast to the ribosomal proteins which are present at 8000 molecules each/genome at the same growth rate (Dennis and Bremer, 1974; Hjalmarsson *et al.*, 1983).

The *trmD* gene encodes the tRNA(m^1G)methyltransferase. It has recently been located on the chromosomal map of *E. coli* and cloned (Byström and Björk, 1982a). Subcloning, deletion mapping and analysis of Tn5 insertions have established that the *trmD* gene is the third promoter-distal gene in an operon composed of four genes (Byström and Björk, 1982b). Expression *in vivo*, using the minicell system, revealed that the promoter-proximal gene is expressed in much larger amounts than *trmD*. The fourth gene, i.e., the gene following *trmD*, is also expressed at a high level compared with *trmD* (Figure 1). This paper describes the entire DNA sequence of this operon and its nearby regions. From published amino acid sequences, the first and the last gene in the *trmD* operon were shown to encode the ribosomal proteins S16 and L19, respectively. Thus, the *trmD* gene, located between the two ribosomal protein genes, is expressed at a 100-fold lower level (Figure 1). A unique regulatory device must exist to maintain this non-coordinate expression.

Results

Strategy for the determination of the nucleotide sequence of the trmD operon

Figure 1 summarizes the gene organization of the 4.6-kb DNA fragment including the *trmD* operon (Byström and Björk, 1982b). The 3.4-kb *Sal*I_1-*Ava*I_1 and the 1.7-kb *Hind*III_2-*Eco*RI_1 fragments were isolated and shotgun cloned into the replicative form of M13mp701 (Figure 1, lane g). The DNA sequences of the different cloned fragments were determined using the dideoxy method (Sanger *et al.*, 1977, 1980).

[1]Present address: National Defence Research Institute, Department 4, S-901 82 Umeå, Sweden.
*To whom reprint requests should be sent.

© IRL Press Limited, Oxford, England.

In this way most of the DNA sequence on both strands was determined for the entire 4.6-kb *Sal*I_1-*Eco*RI_1 DNA fragment. The remaining sequence was determined following isolation of specific DNA fragments (Figure 1, lane h). Some regions were sequenced according to Maxam and Gilbert (Figure 1, lane i). Figure 2 shows the DNA sequence of the 4.6-kb *Sal*I_1-*Eco*RI_1 fragment.

Identification of open reading frames

Using the computer program 'TransQ' of Staden (1979), open reading frames were identified. It has been established that the 4.6-kb *Sal*I_1-*Eco*RI_1 DNA fragment encodes six polypeptides with mol. wts. 48 K, 13 K, 25 K, 31 K, 15 K and 16 K (Byström and Björk, 1982b). Six open reading frames were found in the DNA sequence which agree with the positions of the corresponding genes and sizes of these six proteins (Byström and Björk, 1982b). Four of these polypeptides are part of the *trmD* operon, starting around position 1700 and ending around position 3829 (Byström and Björk, 1982b). The *trmD* gene product, the tRNA(m^1G)methyltransferase, has been purified to homogeneity and the NH_2-terminal sequence has been determined to be H_2N-(Met)-(Trp)-Ile-Gly-Ile-Ile-Ser-Leu-Phe-Pro (Hjalmarsson *et al.*, 1983). This stretch of amino acids corresponds to the 10 codons from position 2617 – 2646 (Figure 2). This open reading frame consists of 255 codons and ends with an ochre codon (UAA at position 3382). The mol. wt. of a polypeptide translated from this open reading frame will be 28 424 compared with the 31 000 obtained with pure enzyme and minicell extracts on SDS-gel electrophoresis (Hjalmarsson *et al.*, 1983; Byström and Björk, 1982b). Table I shows that the amino acid composition of the pure enzyme is in good agreement with the amino acid composition predicted from the nucleotide sequence. The first and fourth proteins in the *trmD* operon have mol. wts. of 13 K and 15 K, respectively, on SDS-gel electrophoresis. These mol. wts. are in agreement with those of ribosomal proteins S16 and L19 (Wittman, 1974). Furthermore, the corresponding genes have been located close to *tyrA* (69% co-transducible to *tyrA*, Isono, 1978), as well as the *trmD* gene (80% co-transducible to *tyrA*; Byström and Björk, 1982a). The amino acid sequences for the first polypeptide (13 K) and the fourth polypeptide (15 K) deduced from the DNA sequence were identical with the amino acid sequences reported for the ribosomal proteins S16 and L19, respectively (Vandekerckhove *et al.*, 1977; Brosius and Arfsten, 1978). Ribosomal protein S16 starts at position 1771 and ends with an ochre codon (UAA) at position 2017. Ribosomal protein L19 starts at position 3426 and ends, also with an ochre codon, at position 3771. The second protein in the operon has a mol. wt. of 25 K on SDS-gel electrophoresis. There exist three possible start code words at positions 2029, 2035 and 2038 and an ochre (UAA) at position 2584. These alternative start positions would give polypeptides of mol. wts. 20 869, 20 639 and 20 508, respectively. At present we cannot determine which of these start codons is used to produce the 25-K polypeptide. Furthermore, there also exists an additional stop codon (UAA) at position 2596 in the same reading frame. Thus, the *trmD* operon is compact, with interspace regions between the ribosomal protein S16, 21-K polypeptide, tRNA(m^1G)methyltransferase and ribosomal protein L19 of only 9 – 18, 30 and 40 nucleotides, respectively (Figure 3A).

Codon usage

Table II shows the codon usage of the genes in the *trmD* operon. The *rpsP*(S16) and *rplS*(L19) genes show similar codon usage as genes for other ribosomal proteins and other proteins made in large amounts in the cell (Nomura *et al.*, 1980). Thus, these genes avoid certain codons such as AUA(Ile), CGA/G(Arg), AGA/G(Arg), UCA/G(Ser), ACA/G(Thr) and GGA/G(Gly) (Ikemura, 1981). These codons, except UCA/G and ACG, are identical with the modulator codons suggested by Grosjean and Fiers (1982). Furthermore, these codons are read by tRNA species found in small amounts in the cell. In contrast, the *trmD* gene uses these codons more frequently in correlation with the low amount of this enzyme produced in the cell. Results obtained using minicell systems suggest that the expression of the 21-K

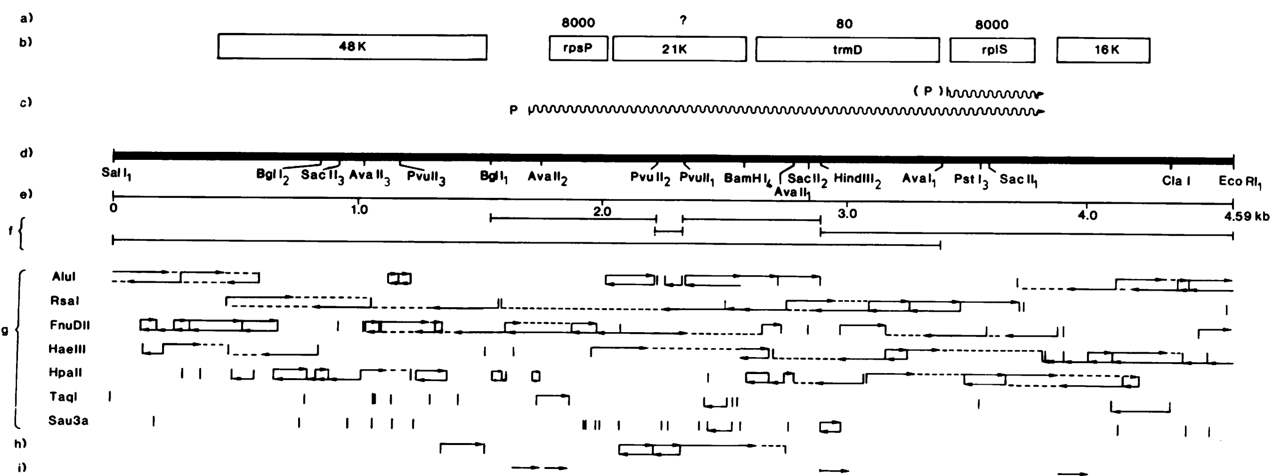

Fig. 1. Restriction map and sequencing strategy of the 4.6-kb *Sal*I-*Eco*RI fragment containing the *trmD* operon of *E. coli* K-12. **(a)** Number of molecules of each gene product, encoded by the *trmD* operon, at a specific growth rate of k = 1.0 (Dennis and Bremer, 1974; Hjalmarsson *et al.*, 1983). **(b)** Genes within the 4.6-kb *Sal*I-*Eco*RI fragment. **(c)** Promoters of the *trmD* operon. P indicates the major promoter, and (P) indicates a suggested weak internal promoter (Byström and Björk, 1982b). **(d)** Restricton map of the 4.6-kb *Sal*I-*Eco*RI fragment. **(e)** Scale in kb of the 4.6-kb chromosomal DNA fragment of pBY03 containing the *trmD* operon. **(f)** DNA fragments isolated from pBY03 used in **g**, **h** and **i**. **(g)** Fragments in **f** were shotgun cloned into vector M13mp701, with respective enzyme. When using restriction enzymes giving blunt ends, T4 kinase phosphorylated *Bam*HI or *Eco*RI linkers were ligated onto the fragments before ligating them into M13mp701. Vertical lines indicate restriction sites within the sequenced area. Horizontal continued lines indicate sequenced DNA and dashed lines represent the rest of the cloned DNA. **(h)** Specific fragments cloned into vector M13mp701. **(i)** Regions sequenced by the method of Maxam and Gilbert (1977, 1980).

Fig. 2. DNA sequence of the *trmD* operon and nearby regions from *E. coli* K-12. Only one strand with the polarity of the mRNA is given, except for the region encoding the 16-K polypeptide which is transcribed in the opposite direction. The amino acid sequences of the coded proteins are shown. The *trmD* operon consists of: the *rpsP*(S16) gene, which begins at position 1771; the 21-K (unknown function) gene, which begins at position 2029–2038; the *trmD* [tRNA(m[1]G)methyltransferase] gene, which begins at position 2617; and the *rplS* (L19) gene, which begins at position 3426. The first 10 amino acids of the tRNA(m[1]G)methyltransferase were confirmed by determination of the NH_2-terminal amino acid sequence of the purified enzyme (Hjalmarsson *et al.*, 1982). Two presumptive Pribnow sequences are boxed in front of the *rpsP* gene. Regions of dyad symmetry able to form hairpin structures with a ΔG value less than -8.4 kcal are indicated by underlining. A dot indicates the center of symmetry. Probably, the dyad symmetry, with a $\Delta G = -20.0$ kcal, following the *rplS* gene is the terminator of the *trmD* operon.

polypeptide is intermediate between the ribosomal proteins and the tRNA(m¹G)methyltransferase (Byström and Björk, 1982b). Table II shows that the codon usage of the gene for the 21-K polypeptide is somewhere between that of the two ribosomal protein genes and the *trmD* gene. Thus, the UCA/G(Ser), ACA/G(Thr) and GGA/G(Gly), which are completely avoided by the ribosomal protein genes but used rather frequently by the *trmD* gene, are used to some extent by the 21-K protein gene. The frequent use of CUC(Leu) by the 21-K protein gene is also in sharp contrast to the usage by ribosomal protein and *trmD* genes.

Discussion

In this paper the entire nucleotide sequence of a 4.6-kb *Sal*I$_1$-*Eco*RI$_1$ DNA fragment including the *trmD* operon is presented (Figure 2). The restriction enzyme pattern (*Sal*I, *Hind*III, *Eco*RI) of this sequenced DNA fragment was shown by the Southern blot technique to be identical to that of the *E. coli* chromosome (unpublished results). The *trmD* gene is the structural gene for the tRNA(m¹G)methyltransferase. Surprisingly, the promoter proximal gene of this operon was found to be *rpsP*, which encodes for ribosomal protein S16. The last gene of the operon is *rplS*, which encodes for ribosomal protein L19. This provides the first example where a gene for a tRNA biosynthetic enzyme is part of an operon. The two surrounding genes, encoding the ribosomal proteins, are expressed at a high level, each resulting in 8000 molecules/genome in glucose minimal medium. The third promoter-distal gene, *trmD*, is expressed at a very low level resulting in only ~80 molecules of tRNA(m¹G)methyltransferase/genome (Figure 1). Furthermore, the regulation of these proteins, both as a function of growth rate and as a response to the level of ppGpp in the cell, is quite different (Ny *et al.*, 1980).

Two presumptive promoters were found in front of the *rpsP* gene. The two possible −10 regions are indicated in Figure 3b (Rosenberg and Court, 1979). The sequence of one of these promoters (−10 from position 1690 to 1696) is identical to the −10 region of the str-promoter and the −35 region of the α-promoter (Nomura *et al.*, 1980). In position 1730−1731, located ~39 nucleotides in front of the start of the *rpsP* gene, there is a region of dyad symmetry. This hairpin structure is followed by a stretch of four Ts (Figure 3B). Similar features have been found in other ribosomal operons and in the str-operon such a hairpin structure was shown to be a terminator *in vitro* (Post *et al.*, 1978). The *in vivo* significance of this hairpin structure in the *trmD* operon is yet to be established.

Transposon Tn5 insertions in the *rpsP* or the 21-K gene abolished the synthesis of the *trmD* gene product, the

Table I. Comparison of the amino acid composition of the tRNA(m¹G)methyltransferase obtained from the protein hydrolysate and the DNA sequence. Results are shown as residues per molecule

Residue	Number of residues	
	Protein hydrolysate[a]	DNA sequence
Methionine	7.1	8
Tryptophan	n.d.	5
Isoleucine	13.7	15
Glycine	24	24
Serine	14.6	14
Leucine	27.9	28
Phenylalanine	6.1	6
Proline	11.5	13
Glutamic acid		22
Glx	33	
Glutamine		11
Aspartic acid		14
Asx	18.6	
Asparagine		4
Arginine	18.3	19
Alanine	20.9	21
Threonine	11.9	12
Tyrosine	6.9	6
Valine	13.3	14
Lysine	7.9	8
Histidine	8.9	9
Cysteine	2.1	2

[a]Data are from Hjalmarsson *et al.* (1983). The number of residues per tRNA(m¹G)methyltransferase molecule in the protein hydrolysate was calculated by multiplying the molar % of its amino acid composition with the total number of residues obtained from the DNA sequence (trp residues subtracted). Aspartic acid and glutamic acid were not distinguished from their respective amino derivatives. Tryptophan was degraded during the analysis.

```
A    INTERCISTRONIC REGIONS IN THE TRMD OPERON

     RPSP - 21K
     2002
     Val Asn Lys Ala Ala •••                                 Met Ser Lys Gln Leu Thr Ala Gln
     GTA AAC AAA GCA GCT TAA TCT GTC ACG GTG GTC ATG ATG AGC AAA CAA CTC ACC GCG CAA
                             ——— 18 ———

     21K - TRMD
     2572
     Asp Pro Gly Phe •••             •••                         Met Trp Ile Gly Ile
     GAT CCT GGT TTT TAA ACC ACC GGA TAA ACG GTA AAA GAC GGC GCT ATG TGG ATT GGC ATA
                          ——— 30 ———

     TRMD - RPLS
     3376
     Met Ala •••                                                   Met Ser Asn
     ATG GCG TAA GCC CCC GAG ATA TCA GTT TAC CCA GGA TAA GAG ATT AAA T ATG AGC AAC
                      ——— 40 ———

B    THE PROMOTER REGION OF THE TRMD OPERON

                                                                    C T
                                                                   C   C
                                                                  T     G
                                                                  T     A
                                                                   G   T
                                                                    C G
                                                                    C G
                                                                    T G
                                                                    C G
                                                                    A T
                                                                    G C
                                                                    G C
                                                                    C G
                                                                    C G
     1660                1680                 1700                  A T T
     ACTGTTGATTGCAATTCCCGCAAAAATGAG[TAAAAT]TTTCGGGCTTT[TAATAT]GA    C G   TTTTATTCACACAAGAGGATGTTATGGTA
                                                                              1760           MetVal

C    THE TERMINATOR OF THE TRMD OPERON

                                                 A T
                                                A   T
                                                 C G
                                                 C G
                                                 G C
                                                 G T
                                                 T G
                                                 C G
                                                 G C
                                                 G C
     LeuAsn•••      3780                         G C          3840
     CTTAACTAAGATTTCGCTTAAGCGACATCCTGTTAAGAA        TTTTTTATCTGTTTGCTGGTGTTTTA
```

Fig. 3. The DNA sequence of: **(A)** The intercistronic regions in the *trmD* operon. The distances in nucleotides are given below the sequences. One of the three possible translational initiator codons for the 21-K polypeptide is indicated (for further explanation see the text). **(B)** The potential promoter region preceding the *rspP* (S16) gene. **(C)** The potential transcriptional terminator downstream of the *rplS* (L19) gene.

tRNA(m^1G)methyltransferase. Furthermore, deletion analysis as well as analysis of different subclones established that the promoter of the *trmD* gene was to the left of *rpsP* (Figure 1, Byström and Björk, 1982b). However, in all TrmD$^-$ plasmid derivatives there was a small production of the *rplS* gene product, the ribosomal protein L19, suggesting an internal promoter somewhere upstream of *rplS* but to the right of the *Hind*III$_2$ site in the *trmD* gene. The latter was inferred from the observation that a *Hind*III$_2$-*Eco*RI$_1$ subclone synthesized a small amount of the *rplS* product, ribosomal protein L19 (Byström and Björk, 1982b). However, the DNA sequence in this region does not reveal any apparent promoter structure. This further strengthens our suggestion that the *rplS* is part of the *trmD* operon, but does not explain the small synthesis of L19 in the absence of the major promoter.

The potential transcriptional terminator of the *trmD* operon has its point of dyad symmetry at position 3814–3815, with a ΔG value of -20.0 kcal (Figure 3C). This dyad symmetry is followed by a stretch of six Ts, which is more than the minimal length of four Ts proposed to be important for terminators (Christie *et al.*, 1981). Other dyad symmetries with ΔG values less than -8.4 kcal are shown in Figure 2. The significance of these and the postulated promoters and terminators have to be analysed more extensively.

Genes for ribosomal proteins have been shown to be part of operons containing only ribosomal protein genes as well as part of composite operons. The latter operons contain genes encoding non-ribosomal proteins involved in translation, like EF-Tu and EF-G, or transcription, like subunits of the RNA polymerase. Some of these operons express the genes differently (An *et al.*, 1982; Young and Furano, 1981; Fiil *et al.*, 1980). The expression of the cistrons in the *trmD* operon is sharply dis-coordinate. So far no ribosomal protein operon has been found with a 100-fold different level of expression of its constituent cistrons. In the *trmD* operon two UAA stop codons are spaced by three codons following the 21-K gene, where a translational polarity mechanism may well be operating (Figure 3A). Furthermore, among the ribosomal protein operons, only the str- and α-operons show a higher expression of the last genes, compared with the proximal genes. Thus, in the str-operon the expression of EF-Tu from the *tufA* gene is 3- to 5-fold higher compared with the production of EF-G from the preceding *fusA* gene. Two explanations of this phenomenon have been suggested: (i) a more efficient translation of the *tufA* mRNA (Young and Furano, 1981); (ii) a weak internal promoter located within the 3′ terminus of the *fusA* gene (An *et al.*, 1982; Zengel and Lindahl, 1982). Similar mechanisms may be operating in the *trmD* operon.

Although the relative rate of synthesis of the ribosomal proteins increases with increasing growth rate, the relative rate of synthesis of the corresponding mRNAs are more or less invariant with growth rate (Miura *et al.*, 1981; Lindahl and Zengel, 1982). The specific activity of the tRNA(m^1G)-methyltransferase is invariant with growth rate (Ny *et al.*, 1980). Therefore, the regulatory behaviour of the tRNA-(m^1G)methyltransferase might be a reflection of the relative rate of synthesis of the mRNA of the *trmD* operon. If so, the ribosomal proteins S16 and L19 must be subjected to autoregulation at the translational level. Such a regulatory mechanism has been proposed for the regulation of other ribosomal proteins (Fallon *et al.*, 1979).

The efficiency of reading of any particular codon may vary over at least a 10-fold range (Bossi and Roth, 1980). This might exert a significant influence on the rate of protein synthesis in certain regions of the mRNA. It has been suggested that translational rates may be set by the use of codons whose tRNAs are present in low levels in the cell and that these regions may be larger than one codon (Ames and Hartman, 1963; Stent, 1964). Thus, depending on its environment, a

Table II. Codon usage of: (a) the *rpsP* (S16) gene; (b) the *rplS* (L19) gene; (c) the 21-K (unknown function) gene, AUG at position 2038 used as start codon; and (d) the *trmD* (tRNA(m^1G)methyltransferase) gene

	U	a,	b,	c,	d,	C	a,	b,	c,	d,	A	a,	b,	c,	d,	G	a,	b,	c,	d,	
U	Phe	0	0	5	3	Ser	1	3	3	0	Tyr	0	0	1	2	Cys	0	0	1	0	U
		4	4	1	3		0	3	1	3		1	2	4	4		0	0	1	2	C
	Leu	1	0	0	4		0	0	2	2	term	1	1	1	1	term	0	0	0	0	A
		0	0	1	5		0	0	2	3		0	0	0	0	Trp	1	1	6	5	G
C	Leu	0	2	1	3	Pro	0	1	3	5	His	0	0	0	6	Arg	4	12	4	5	U
		0	0	7	1		0	0	2	1		2	2	2	3		6	1	0	6	C
		0	0	0	0		1	0	0	3	Gln	0	2	3	7		0	0	0	0	A
		3	4	7	15		1	2	1	4		2	4	10	4		0	0	0	5	G
A	Ile	2	5	4	9	Thr	3	3	3	2	Asn	1	0	3	2	Ser	0	0	0	2	U
		4	2	7	3		0	1	4	4		3	4	2	2		2	2	3	4	C
	Met	0	0	0	3		0	0	0	0	Lys	4	8	7	6	Arg	0	0	1	2	A
		1	2	7	8		0	0	1	6		1	3	6	2		0	0	0	1	G
G	Val	5	7	5	4	Ala	6	5	0	2	Asp	3	1	11	8	Gly	2	5	6	5	U
		1	2	6	1		0	0	1	4		1	2	7	6		4	3	3	10	C
		2	3	4	2		4	2	2	10	Glu	4	4	9	16		0	0	2	5	A
		0	3	2	7		1	0	5	5		1	5	1	6		0	0	3	4	G

The numbers represent the use of codons in the respective genes, obtained from the DNA sequence in Figure 2. In all four cases stop codon UAA was used.

specific codon may or may not have a regulatory effect on the efficiency of translation. The codon usage of *trmD* is typical for a gene whose product is made in small amounts (Table II). One possible regulatory device of the *trmD* gene may be the availability of a certain subset of minor tRNA species. The *trmD* gene shows a positive response to gene dosage, indicating no deficiency in a putative limiting cell component like a subset of minor tRNA species (Byström and Björk, 1982a). However, modulator codons could still influence the rate of translation (Grosjean and Fiers, 1982). Furthermore, other elements, such as the dyad symmetries found in the *trmD* gene, might also play a pivotal role in determining the efficiency of translation.

One can speculate why the *trmD* gene is part of an operon containing ribosomal proteins, since apparently a very elaborate regulatory mechanism must exist which governs the differential synthesis of these proteins. The ribosomal proteins in the operon are involved directly in translation. The tRNA(m^1G)methyltransferase is indirectly involved in translation since it methylates 12% of the tRNAs in *E. coli*, among them $tRNA_1^{Leu}$ and $tRNA_3^{Leu}$. It seems that in order to ensure a proper regulation of the tRNA(m^1G)methyltransferase it is advantageous to have the *trmD* gene as part of an operon whose products may all be involved in translation. With the help of the known DNA sequence and the knowledge of the gene organization it will be possible to construct proper mutants *in vitro*, which might be helpful in the elucidation of the regulatory mechanism behind the synthesis of the tRNA(m^1G)methyltransferse and the function of m^1G in the tRNA.

Materials and methods

Materials

Isotopes [α-^{32}P]dCTP and [γ-^{32}P]ATP were purchased from Radiochemical Centre, Amersham. DNA polymerase I (Klenow subfragment) and T4 polynucleotide kinase were purchased from Boehringer Mannheim and New England Biolabs, respectively. Restriction enzymes and T4 DNA ligase were obtained from Boehringer Mannheim or New England Biolabs. *Bam*HI (CCGGATCCGG) and *Eco*RI (GGAATTCC) linkers were obtained from Collaborative Research. The dideoxynucleotide triphosphates were from PL Biochemicals. The deoxynucleotide triphosphates, isopropyl β-D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) were obtained from Sigma. Polyethylene glycol 6000 was obtained from BDH Chemicals Ltd., Poole, England. The synthetic oligonucleotide primer CGACGGCCAGTG used in dideoxy DNA sequencing was a generous gift from KabiGen AB. X-ray film used was Cronex 4 from DuPont.

Preparation of DNA and DNA sequencing

Plasmid pBY03 was the source of DNA and was prepared as described elsewhere (Byström and Björk, 1982a). Plasmid pBY03 was subjected to restriction endonuclease cleavage and different chromosomal DNA fragments were purified by separating the digested mixture on a 5% polyacrylamide gel. The cleaved fragments were electroeluted essentially as described by McDonell *et al.* (1977). The DNA fragments were then shotgun cloned into the replicative form of vector M13mp701 using different restriction enzymes with four-base recognition sequences. The vector M13mp701 is a derivative of M13mp7 (Bentley, personal communication; Messing *et al.*, 1981). When restriction enzymes giving blunt end fragments were used, T4 polynucleotide kinase-phosphorylated *Bam*HI or *Eco*RI linkers were ligated onto the fragments before ligating them into the replicative form of M13mp701. The fragments generated by restriction enzymes *Taq*I, *Hpa*II and *Sau*3a were ligated directly into the replicative form of M13mp701 using the *Acc*I and the *Bam*HI enzyme restriction sites, respectively. Ligation was performed as described by the manufacturer. Competent cells of strain JM101 were transformed with the ligation mixture using, essentially, the method of Mandel and Higa (1970). Preparation of single-stranded M13mp701 templates, T-lane screening and DNA sequencing by the dideoxy method were according to Sanger *et al.* (1977, 1980). When needed, additional DNA sequencing was made according to Maxam and Gilbert (1977, 1980).

Computer analysis

The computer programs of Staden (1979), modified by P. Gustafsson and P. Hagblom, Umeå, and the program by Harr *et al.*, 1982 were used to analyse accumulated DNA sequences. All analyses were made on a Cyber 170/730 Dual computer.

Acknowledgements

We are greatly indebted to KabiGen AB for the generous gift of the synthetic oligonucleotide primer for the M13 DNA sequencing system. Skilful technical assistance by Åsa Andersson and critical reading of the manuscript by Drs. Petter Gustafsson, Ivor Tittawella, Berndt-Eric Uhlin, Professor Staffan Normark, all at Umeå, and Dr. Leif Isaksson, Uppsala, are gratefully acknowledged. This work was supported by the Swedish Cancer Society (Project No. 680), and by the Swedish Board for Technical Development.

References

An,G., Lee,J.S. and Friesen,J.D. (1982) *J. Bacteriol.*, **149**, 548-553.
Ames,B.N. and Hartman,P.E. (1963) *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 349-356.
Björk,G.R., Byström,A.S., Hagervall,T.G., Hjalmarsson,K.J., Kjellin-Stråby,K. and Lindström,P.H.R. (1982) in Usdin,E., Borchardt,R.T. and Creveling,C.R. (eds.), *Biochemistry of S-Adenosylmethionine and Related Compounds*, McMillan Press Ltd., London/Basingstoke, pp. 371-378.
Björk,G.R. (1983) in Apirion,D. (ed.), *Processing of RNA*, CRC Press Inc., USA, in press.
Bossi,L. and Roth,J.R. (1980) *Nature*, **286**, 123-127.
Brosius,J. and Arfsten,U. (1978) *Biochemistry (Wash.)*, **17**, 508-516.
Byström,A.S. and Björk,G.R. (1982a) *Mol. Gen. Genet.*, **188**, 440-446.
Byström,A.S. and Björk,G.R. (1982b) *Mol. Gen. Genet.*, **188**, 447-454.
Christie,G.E., Farnham,P.J. and Platt,T. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 4180-4184.
Dennis,P.P. and Bremer,H. (1974) *J. Bacteriol.*, **119**, 270-281.
Fallon,A.M., Sue Jinks,C., Strycharz,G.D. and Nomura,M. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3411-3415.
Fiil,N.P., Friesen,J.D. and Dennis,P.P. (1980) in Osawa,S., Ozeki,H., Uchida,H. and Yura,T. (eds.), *Genetics and Evolution of RNA Polymerase, tRNA, and Ribosomes*, University of Tokyo Press, and Elsevier/North-Holland Biomedical Press, pp. 33-50.
Grosjean,H. and Fiers,W. (1982) *Gene*, **18**, 199-209.
Harr,R., Hagblom,P. and Gustafsson,P. (1982) *Nucleic Acids Res.*, **10**, 365-374.
Hjalmarsson,K.J., Byström,A.S. and Björk,G.R. (1983) *J. Biol. Chem.*, **258**, 1343-1351.
Ikemura,T. (1981) *J. Mol. Biol.*, **151**, 389-409.
Isono,K. (1978) *Mol. Gen. Genet.*, **165**, 265-268.
Lindahl,L. and Zengel,J.M. (1982) *Adv. Genet.*, **21**, 53-121.
Maaløe,O. (1979) in Goldberger,R.F. (ed.), *Biological Regulation and Development*, Vol. **1**, Plenum Publishing Corporation, pp. 487-542.
Mandel,M. and Higa,A. (1970) *J. Mol. Biol.*, **53**, 159-162.
Maxam,A.M. and Gilbert,W. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 560-564.
Maxam,A.M. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499-560.
Mazzara,G.P. and McClain,W.H. (1980) in Söll,D., Abelson,J.N. and Schimmel,P.R. (eds.), *Transfer RNA: Biological Aspects*, Cold Spring Harbor Laboratory Press, NY, pp. 3-27.
McDonell,M.W., Simon,M.N. and Studier,F.W. (1977) *J. Mol. Biol.*, **110**, 119-146.
Messing,J., Crea,R. and Seeburg,P.H. (1981) *Nucleic Acids Res.*, **9**, 309-321.
Miura,A., Heilig Kreuger,J., Itoh,S., de Boer,H.A. and Nomura,M. (1981) *Cell*, **25**, 773-782.
Neidhardt,F.C., Parker,J. and McKeever,W.G. (1975) *Annu. Rev. Microbiol.*, **29**, 215-250.
Nierlich,D.P. (1978) *Annu. Rev. Microbiol.*, **32**, 393-432.
Nomura,M., Post,L.E. and Jinks,C.S. (1980) in Osawa,S., Ozeki,H., Uchida,H. and Yura,T. (eds.), *Genetics and Evolution of RNA Polymerase, tRNA, and Ribosomes*, University of Tokyo Press, and Elsevier/North-Holland Biomedical Press, pp. 315-328.
Ny,T. and Björk,G.R. (1980) *J. Bacteriol.*, **141**, 67-73.
Ny,T., Thomale,J., Hjalmarsson,K., Nass,G. and Björk,G.R. (1980) *Biochim. Biophys. Acta*, **607**, 227-284.
Pedersen,S., Bloch,P.L., Reeh,S. and Neidhardt,F.C. (1978) *Cell*, **14**, 179-190.
Post,L.E., Arfsten,A.E., Reusser,F. and Nomura,M. (1978) *Cell*, **15**, 215-229.
Rosenberg,M. and Court,D. (1979) *Annu. Rev. Genet.*, **13**, 19-53.

Sanger,F., Coulson,A.R., Barell,B.G., Smith,A.J.H. and Roe,B.A. (1980) *J. Mol. Biol.,* **143**, 161-178.
Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA,* **74**, 5463-5467.
Staden,R. (1979) *Nucleic Acids Res.,* **6**, 2601-2610.
Stent,G.A. (1964) *Science (Wash.),* **144**, 816-820.
Vanderkerckhove,J., Rombauts,W. and Wittmann-Liebold,B. (1977) *Hoppe Seyler's Z. Physiol. Chem.,* **358**, 989-1002.
Wittman,H.G. (1974) in Nomura,M., Tissiéres,A. and Lengyel,P. (eds.), *Ribosomes*, Cold Spring Harbor Laboratory Press, NY, pp. 93-114.
Young,F.S. and Furano,A.V. (1981) *Cell,* **24**, 695-706.
Zengel,J.M. and Lindahl,L. (1982) *Mol. Gen. Genet.,* **185**, 487-492.