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Nucleic Acids Research, 1997, Vol. 25, No. 20 **4093–4097**

# The *spoU* gene of *Escherichia coli*, the fourth gene of the *spoT* operon, is essential for tRNA (Gm18) 2′-*O*-methyltransferase activity

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Received June 10, 1997; Revised and Accepted August 18, 1997

## ABSTRACT

**We have evidence that the open reading frame previously denoted *spoU* is necessary for tRNA (Gm18) 2′-*O*-methyltransferase activity. The *spoU* gene is located in the *gmk–rpoZ–spoT–spoU–recG* operon at 82 minutes on the *Escherichia coli* chromosome. The deduced amino acid sequence of *spoU* shows strong similarities to previously characterized 2′-*O*-methyltransferases. Comparison of the nucleoside modification pattern of hydrolyzed tRNA, 16S rRNA and 23S rRNA from wild-type and *spoU* null mutants showed that the modified nucleoside 2′-*O*-methylguanosine (Gm), present in a subset of *E.coli* tRNAs at residue 18, is completely absent in the *spoU* mutant, suggesting that *spoU* encodes tRNA (Gm18) 2′-*O*-methyltransferase. Nucleoside modification of 16S and 23S rRNA was unaffected in the *spoU* mutant. Insertions in the downstream *recG* gene did not affect RNA modification. Absence of Gm18 in tRNA does not influence growth rate under the tested conditions and does not interfere with activity of the SupF amber suppressor, a suppressor tRNA that normally has the Gm18 modification. We suggest that the *spoU* gene be renamed *trmH* (tRNA methylation).**

## INTRODUCTION

A total of 29 different modified nucleosides have been identified in *Escherichia coli* tRNA (1). It has been estimated that some 45 enzymes are required for their synthesis, since many of the nucleoside modifications are present at several positions in the tRNA and others are complex molecules requiring a number of enzymatic steps for synthesis. About 1% of the genetic information in *E.coli* is devoted to tRNA nucleoside modification, which is 10 times more than that for tRNA. The most common tRNA modification is methylation of either the base or the 2′-hydroxyl of the ribose. So far, only enzymes methylating the base of the nucleoside have been identified in *E.coli*.

One of the earliest tRNA modifying activities studied was methylation of the 2′-hydroxyl of guanosine (Gm) (2). The Gm modification is found at nucleoside 18 in the D loop of 13 out of a total 46 *E.coli* tRNAs (3). The enzyme has been purified from *Thermus thermophilus* (4), but the corresponding gene has not been identified in any organism.

Recent accumulation of DNA sequences from different organisms allows identification of putative 2′-*O*-ribose methyltransferases through their similarity to other known ribose methylases. The *spoU* open reading frame (ORF) has previously been suggested to encode an RNA ribose methylase by sequence similarity (5,6). We here show that the *spoU* gene is essential for tRNA (Gm18) methyltransferase activity. Initial studies suggest that the absence of Gm18 in tRNA has no significant effect on growth at 37 or 42°C or on the efficiency of decoding during translation.

## MATERIAL AND METHODS

### Bacterial strains and growth conditions

The strains used were derivatives of *E.coli* K-12 and are listed in Table 1. Strains were grown at 37°C. LB medium (7) was used as a complex rich medium. Solid medium was obtained by adding 1.5% agar. Medium E (8) supplemented with 1.5% agar, 0.2% glucose and 40 µg/ml of the appropriate amino acids was used as minimal solid medium. Liquid cultures were grown in MOPS minimal medium (9) supplemented with 0.2% glucose or glycerol. When necessary chloramphenicol (12.5 µg/ml), kanamycin (50 µg/ml) or rifampicin (50 µg/ml) was added to the medium. F factor conjugations and P1 transductions were carried out as described (10).

### Analysis of modified nucleosides

tRNA was isolated according to the method of Buck *et al.* (11). Ribosomal 30S subunits (containing 16S rRNA) and 50S subunits (containing 23S and 5S rRNA) were separated on

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10–40% sucrose gradients (12). rRNA was isolated from the sucrose gradient fractions by phenol extraction and ethanol precipitation. The isolated RNA was digested to nucleosides with P1 nuclease (Boehringer) and bacterial alkaline phosphatase (Sigma) (13). Reverse phase HPLC analysis of modified nucleosides was performed on a Waters HPLC system equipped with the Millennium 2000 data analysis program (Millipore). Nucleosides were separated on a Supelcosil LC-18S column and the gradient used was according to Gehrke and Kuo (14). Modified nucleosides were identified by comparison with their known absorbance spectra and retention times.

### β-Galactosidase activity measurements

Strains containing an F′ plasmid with a *lacI–lacZ* fusion with or without nonsense mutations in the *lacI* part were grown in MOPS glucose medium. In each experiment four independent clones were analyzed. Determination of β-galactosidase activity was according to Miller (10), except that cells were lysed by addition of 25 µl chloroform and 50 µl 0.1% SDS per 500 µl sample.

### Sequence analysis

The deduced amino acid sequence of previously characterized 2′-*O*-methyltransferases were used as probes to search for homologous proteins in the GenBank and SwissProt databases. The databases were searched using the BLAST program (15). The alignment of *spoU* homologs was done using the PILEUP, GAP and BESTFIT programs, which are part of the GCG package (Genetics Computer Group, Madison, WI).

## RESULTS

### Amino acid sequence homologies

The deduced amino acid sequences of two genes encoding RNA 2′-*O*-methyltransferases are currently available: *tsr* from *Streptomyces azureus* (SwissProt accession no. P18644) encoding the 23S rRNA (Am1067) 2′-*O*-methyltransferase (16); PET56 from yeast (accession no. 431760) encoding the 23S rRNA (Gm2251) methyltransferase (17). (Numbering of rRNA nucleosides is given according to the *E.coli* equivalent rRNA.) In addition, two orthologs (i.e. the ‘same’ gene from different organisms) of *tsr* have been sequenced. The *tsr* orthologs used here as additional probes have the accession nos P52393 and P52391.

The deduced amino acid sequences of PET56 and *tsr* (and its orthologs) were used to probe the SwissProt database for *E.coli* ORFs that may encode 2′-*O*-methyltransferases. Five *E.coli* ORFs were identified by the search, as were several homologs and orthologs in a variety of organisms (5,6). One of the ORFs identified in this database search was the previously studied but phenotypically uncharacterized *spoU* gene. The *spoU* encoded gene product was identified as a potential 2′-*O*-methyltransferase based on its homology to *tsr* and PET56.

**Table 1.**

| Strain | Genotype | Reference |
|---|---|---|
| CF898 | F⁻, *thi*-1, *pyrE60*, *argE3*, *proA2*, *thr*-1, *leuB6*, *mtl*-1, *xyl*-5, *ara*-14, *galK2*, *rpsL31*, *supE44*, *cdd*, *gyrA* | M.Cashel |
| CF1499 | Like CF898, but *trmH*(*spoU*)::*cat* | M.Cashel |
| CF1500 | as CF1499, separate isolate | M.Cashel |
| CF3324 | Δ*recG163* (Δ*spoV1*::*kan*) | 23 |
| CF3325 | as CF3324, separate isolate | M.Cashel |
| CF4666 | Δ*trmH*::*kan* (Δ*spoU3*::*kan*) | M.Cashel |
| CF4667 | as CF4666, separate isolate | M.Cashel |
| GRB1253 | F′ Δ-14 *lacI*am112, *proA*⁺*B*⁺/UB585 | This study |
| GRB1254 | F′ Δ-14 *lacI*am117, *proA*⁺*B*⁺/UB585 | This study |
| GRB1255 | F′ Δ-14 *lacI*am121, *proA*⁺*B*⁺/UB585 | This study |
| GRB1256 | F′ Δ-14 *lacI*am128, *proA*⁺*B*⁺/UB585 | This study |
| GRB1257 | F′ Δ-14 *lacI*amA24, *proA*⁺*B*⁺/UB585 | This study |
| GRB1258 | F′ Δ-14 *lacI*am122, *proA*⁺*B*⁺/UB585 | This study |
| GRB1260 | F′ Δ-14 *lacI*⁺, *proA*⁺*B*⁺/UB585 | This study |
| GRB1296 | *argE*(am), *ara*, Δ(*lac-proB*), *gyrA*, *rpoB*, *thi*, *tyrT* (*supF*), *metB*, *trmH*(*spoU*)::*cat* | This study |
| GRB1297 | F′ Δ-14 *lacI*am112, *proA*⁺*B*⁺/GRB1296 | This study |
| GRB1298 | F′ Δ-14 *lacI*am117, *proA*⁺*B*⁺/GRB1296 | This study |
| GRB1299 | F′ Δ-14 *lacI*am121, *proA*⁺*B*⁺/GRB1296 | This study |
| GRB1300 | F′ Δ-14 *lacI*am128, *proA*⁺*B*⁺/GRB1296 | This study |
| GRB1301 | F′ Δ-14 *lacI*amA24, *proA*⁺*B*⁺/GRB1296 | This study |
| GRB1302 | F′ Δ-14 *lacI*am122, *proA*⁺*B*⁺/GRB1296 | This study |
| GRB1304 | F′ Δ-14 *lacI*⁺, *proA*⁺*B*⁺/GRB1296 | This study |
| UB585 | *argE*(am), *ara*, Δ(*lac-proB*), *gyrA*, *rpoB*, *thi*, *tyrT* (*supF*), *metB* | L.A.Isaksson |

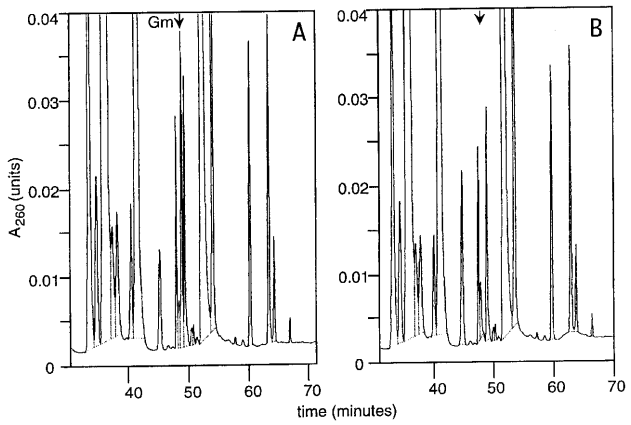


**Figure 1.** HPLC analysis of modified nucleosides in tRNA. (**A**) Strain CF898 *trmH*$^+$. (**B**) Strain CF1499 *trmH*::*cat*. The arrow indicates the retention time of 2′-*O*-ribose methylated guanosine (Gm).

In addition to known and putative 2′-*O*-methyltransferases found in the database search when using SpoU as probe, we also identified the HIV TAR RNA binding protein TRP-185 (18) as having substantial homology at the C-terminal to SpoU (probability of an accidental match $3.3 \times 10^{-7}$).

Another enzyme that utilizes the 2′-hydroxyl of the tRNA ribose backbone as substrate is RIT1 (19). Instead of adding a methyl group, the RIT1 enzyme adds a ribosyl phosphate to the 2′-hydroxyl of A64 of $tRNA^{fmet}$ in yeast. Alignment of RIT1 to SpoU and SpoU homologs identified a shared motif (V,L,I,M)-x-L-$x_7$-N-x-G-x-(V,L,I,M)-x-(KR) (x, any residue), with the only exception for RIT1 being a 13 amino acid insertion between residues 11 and 12. We suggest that the motif shared by 2′-*O*-methyltransferases and RIT1 is involved in recognition of the ribose substrate.

## The *spoU* gene is necessary for tRNA(Gm18)methyltransferase activity

The *spoU* gene is the only gene in the *spoT* operon to which no function has been assigned. The *spoT* operon has been extensively studied by several laboratories and a number of genetic constructions involving genes of the *spoT* operon have been published. To determine the function of the *spoU* gene, we used strains where the *spoU* gene had been inactivated by deletion and marked by the insertion of either a kanamycin resistance gene cassette or a chloramphenicol resistance gene cassette (20; Table 1). The modification pattern of tRNA and of 16S and 23S rRNA isolated from wild-type and *spoU* mutant cells was analyzed by separation of nucleosides by reverse phase HPLC. When the modified nucleosides of tRNA were analyzed, 2′-*O*-methylguanosine (Gm) was missing in all *spoU* mutant strains (Fig. 1 and Table 2). No differences in modification pattern could be found when 16S or 23S rRNA was analyzed (data not shown).

Since both the kanamycin and chloramphenicol resistance cassette insertions in *spoU* might also inhibit expression of the downstream *recG* gene, we examined strains deleted for the *recG* gene for the presence of Gm in the tRNA. The *recG*::*kan* mutant strains all had normal amounts of Gm18 in their tRNA (Table 2). Thus we conclude that the *spoU* gene product is likely to be the tRNA (Gm18) methyltransferase. We suggest that the *spoU* gene be denoted *trmH* (tRNA methylation), in accordance with the nomenclature for tRNA methyltransferases (21).

## The absence of Gm18 does not influence growth rate or activity of the SupF amber suppressor tRNA

No difference in growth rate between wild-type and *trmH* mutant strains was observed, either in rich medium (LB) or in minimal MOPS/glucose medium at 37 or 42°C. This is in agreement with previous findings (20) and indicates that the Gm18 deficiency causes no major disturbance of cell function under these growth conditions.

One of the tRNA species in *E.coli* which has the modified nucleoside Gm in position 18 is $tRNA^{Tyr}$. Since the amber (UAG) suppressor tRNA SupF, which is a mutated $tRNA^{Tyr}$, also has Gm18, we used SupF to determine the influence of the Gm modification on translation efficiency of this tRNA. The suppressor activity of the SupF tRNA was measured using a set of F′ factors harboring an in-frame *lacI′–′lacZ* fusion with or without amber nonsense codons in the *lacI* part (22). Since the influence of the absence or presence of a certain modification on read-through of nonsense codons can differ considerably depending in which codon context the nonsense codon is found, we used six different F′ episomes that have their UAG stop codon situated in different parts of the *lacI* gene. These F′ episomes were introduced into strains containing the SupF amber suppressor tRNA and either the wild-type *trmH* gene (UB585) or the *trmH*::*cat* deletion mutation (GRB1296). The β-galactosidase activity resulting from suppressor activity of SupF was determined and expressed as a percentage of the β-galactosidase activity found in the same strain harboring a *lacI′–′lacZ* fusion with no amber stop codon (Table 3). At most the efficiency of SupF containing or lacking Gm18 differed by 30%. We do not believe that this consitutes a significant difference, as the values obtained would routinely vary by 10–20% between experiments. Therefore, we conclude that no significant difference in read-through activity of Gm18-containing and Gm18-deficient suppressor tRNA can be detected with this experimental system.

**Table 2.** Amount of Gm or $m^1G$ in tRNA (mol/mol Ψ)

| Strain | Gm18 | $m^1G37$ |
|---|---|---|
| CF898 *spoU*$^+$ | 0.48 | 0.37 |
| CF1499 *trmH*(*spoU*)::*cat* | nd[a] | 0.36 |
| CF1500 *trmH*(*spoU*)::*cat* | nd | 0.30 |
| CF3324 *recG*::*kan* | 0.46 | 0.34 |
| CF3325 *recG*::*kan* | 0.48 | 0.35 |
| CF4666 *trmH*(*spoU*)::*kan* | nd | 0.36 |
| CF4667 *trmH*(*spoU*)::*kan* | nd | 0.36 |

[a]Not detectable. The detection limit was <0.001 mol Gm/mol Ψ.

**Table 3.** Comparison of the efficiency of suppresion by amber suppressor tRNA SupF at different UAG codons of *lacI*

| *lacI′–′lacZ* fusion | β-Galactosidase activity (%) | | |
|---|---|---|---|
| | UB585 (*trmH*$^+$) | GRB1296 (*trmH*::*cat*) | GRB1296/UB585 |
| F′ Δ-14 *lacI*am112 | 15 | 18 | 1.20 |
| F′ Δ-14 *lacI*am117 | 16 | 18 | 1.13 |
| F′ Δ-14 *lacI*am121 | 53 | 36 | 0.68 |
| F′ Δ-14 *lacI*am128 | 50 | 48 | 0.96 |
| F′ Δ-14 *lacI*amA24 | 43 | 45 | 1.05 |
| F′ Δ-14 *lacI*am122 | 40 | 55 | 1.36 |
| F′ Δ-14 *lacI*$^+$ | 100 | 100 | 1 |

## DISCUSSION

We have analyzed the modified nucleosides present in hydrolyzed tRNA and rRNA from wild-type *E.coli* and *spoU* null mutants. The *spoU* null mutants were found to be devoid of detectable Gm in their tRNA (Fig. 1). No difference in the presence of modified nucleosides in 16S or 23S rRNA of the isogenic *spoU* pair could be detected. Only residue 18 in *E.coli* tRNA has been shown to contain a Gm nucleoside modification (3). The absence of Gm18 from hydrolyzed tRNA isolated from *spoU* null mutants together with the pronounced amino acid sequence similarities of the *spoU* encoded protein with two RNA 2′-*O*-methyltransferases strongly supports the notion that *spoU* encodes tRNA (Gm18) methyltransferase, although purification of the protein and establishment of an *in vitro* assay for the enzyme would be necessary to provide final proof. We therefore rename the *spoU* gene *trmH* (tRNA methylation), in accordance with the nomenclature for tRNA methyltransferases (21).

The *spoT* operon is located at 82 minutes on the *E.coli* chromosome. It encodes five ORFs in the following order: *gmk* (guanylate kinase); *rpoZ* (the ω subunit of the RNA polymerase); *spoT* (ppGpp 3′-pyrophosphatase); *spoU* (ORF of unknown function); *recG* (junction-specific DNA helicase) (23–25). Interestingly, three of the genes encoded in the *spoT* operon are intrinsically linked to guanosine metabolism. The gene product of the *trmH* gene methylates the ribose moiety of G18 in tRNA, the *gmk* gene product phosphorylates (d)GMP to (d)GDP and the *spoT* gene product dephosphorylates ppGpp to GDP, all enzymes acting on guanosine derivatives. The remaining two proteins encoded by the operon, RpoZ and RecG, are both involved in other aspects of nucleic acid metabolism. The gene order within the *spoT* operon is conserved in *Haemophilus influenzae*, with the exception of the precise deletion of *trmH* (26). We have analyzed the modified nucleosides of tRNA from *H.influenzae* by HPLC chromatography and, as expected, hydrolyzed tRNA from this organism was found to lack the Gm modification (data not shown).

The enzymatic activity of tRNA (Gm18) methyltransferase has been shown in *E.coli* (2) and the tRNA (Gm18) methyltransferase protein has been purified from *T.thermophilus* (4). It has been suggested that the recognition element for the enzyme consists of the D loop of the tRNA and the presence of G18 and G19 (27), although there clearly has to be another determinant, or anti-determinant, in the recognition element, since all *E.coli* tRNA carries these features, whereas only 13 tRNA species (out of 45 sequenced) retain the modified nucleoside Gm18. In yeast rRNA 55 and in mammalian rRNA over 100 2′-*O*-ribose methylations can be found. The recognition of the sites to be methylated is provided by a large number of small nucleolar RNAs (snoRNAs) which are bound by the methylase and each are complementary to the rRNA sequences flanking a site to be methylated (28–30). It is not known if similar RNA-based recognition can be found in bacteria.

We show that absence of the modified nucleoside Gm18 does not in any major way influence activity of the amber suppressor tRNA SupF under the given experimental conditions (Table 3). This suggests that the function of Gm18 in the decoding process is only minor, if any. We were not able to detect any difference in growth rate when comparing the isogenic *spoU* pair in rich or poor medium or at different temperatures (37 or 42°C). It is still possible that Gm plays an important role in recognition by tRNA synthetases or in stabilizing tRNA structure, as has been suggested (4,31). Generally, 2′-*O*-methylation of the ribose stabilizes the C3′-endo form of the ribose, thereby causing conformational rigidity, which is likely to stabilize the interaction between the Gm nucleoside in the D loop and other parts of the tRNA (32,33).

The G in position 18 of tRNA makes a hydrogen bond with residue Ψ55 in the TΨC loop and that hydrogen bond constitutes an important part of the connections between the D and TΨC loops that stabilize the L form tertiary structure of the tRNA molecule (34,35). 2′-*O*-Methylation of the ribose of G18 may contribute to the rigidity of the nucleoside and thus stabilize the Gm18-Ψ55 base pair and the interaction between the D and TΨC loop in tRNA molecules, where that interaction is less stable.

Identification of the gene for tRNA (Gm18) 2′-*O*-methyltransferase and the availability of null mutations in this gene opens the possibility of further studies concerning the structural elements of tRNA necessary for modification and for more detailed analysis of the function of this modification.

## ACKNOWLEDGEMENTS

We are indebted to Glenn Björk for helpful scientific discussions. Michael Cashel and Leif Isaksson are gratefully acknowledged for providing plasmids and strains and Kerstin Jacobsson for technical assistance. This work was supported by grants to Glenn Björk from the Swedish Cancer Society (Project 680) and the Swedish Natural Science Research Council (B-BU 2930). C.G. was funded by a post-doctoral fellowship from the Swedish Natural Research Council.

## REFERENCES


1 Björk,G.R. (1996) In Neidhardt,F.C., Curtiss,R., Ingraham,J.L., Lin,E.C.C., Low,K.B., Magasanik,B., Riley,M., Schaechter,M. and Umbarger,H.E. (eds), *Escherichia coli and Salmonella typhimurium. Cellular and Molecular Biology*, 2nd Edn. American Society for Microbiology, Washington, DC, pp. 816–886.
2 Gefter,M.L. (1969) *Biochem. Biophys. Res. Commun*., **36**, 435–441.
3 Sprinzl, M., Steegborn, C., Hubel, F. and Steinberg, S. (1996) *Nucleic Acids Res*., **24**, 68–72.
4 Kumagai, I., Watanabe, K. and Oshima, T. (1982) *J. Biol. Chem*., **257**, 7388–7395.
5 Koonin,E.V. and Rudd,K.E. (1993) *Nucleic Acid Res*., **21**, 5519.
6 Gustafsson,C., Reid,R., Greene,P.J. and Santi,D.V. (1996) *Nucleic Acids Res*., **24**, 3756–3762
7 Bertani,G. (1951) *J. Bacteriol*., **62**, 293–300.
8 Vogel,H.J. and Bonner,D.M. (1956) *J. Biol. Chem*., **218**, 97–106.
9 Neidhardt,F.C., Block,P.L. and Smith,D.F. (1974) *J. Bacteriol*., **119**, 736–747.
10 Miller,J.H. (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
11 Buck,M., Connick,M. and Ames,B. (1983) *Anal. Biochem*., **129**, 1–13.
12 Stern,S., Moazed,N. and Noller,H.F. (1988) *Methods Enzymol*., **164**, 481–489.
13 Gehrke,C.W., Kuo,K.C., McCune,R.A., Gerhardt,K.O. and Agris,P.F. (1982) *J. Chromatogr*., **230**, 297–308.
14 Gehrke,C.W. and Kuo,K.C. (1990) In Gehrke,C.W. and Kuo,K.C.T. (eds), *Chromatography and Modification of Nucleosides*, Part A: *Analytical Methods for Major Modified Nucleosides*. Elsevier, Amsterdam, The Netherlands, pp. 3–72.
15 Altschul,S.F., Gish,W., Miller,W., Myers,E.W. and Lipman,D.J. (1990) *J. Mol. Biol*., **215**, 403–410.
16 Bibb,M.J., Bibb,M.J., Ward,J.M. and Cohen,S.N. (1985) *Mol. Gen. Genet*., **199**, 26–36.
17 Sirum-Connolly,K. and Mason,T.L.. (1993) *Science*, **262**, 1886–1889.
18 Wu-Baer,F., Lane,W.S. and Gaynor,R.B. (1995) *EMBO J*., **14**, 5995–6009.
19 Åström,S.U. and Byström,A.S. (1994) *Cell*, **79**, 535–546
20 Xiao,H., Kalman,M., Ikehara,K., Zemel,S., Glaser,G. and Cashel,M. (1991) *J. Biol. Chem*., **266**, 5980–5990.
21 Bachmann,B.J. (1990) *Microbiol. Rev*., **54**, 130–197.
22 Miller,J.H. and Albertini,M. (1983) *J. Mol. Biol*., **164**, 59–71.
23 Kalman,M., Murphy,H. and Cashel,M. (1992) *Gene*, **110**, 95–99.
24 Gentry,D., Bengra,C., Ikehara,K. and Cashel,M. (1993) *J. Biol. Chem*., **268**, 14316–14321.
25 Lloyd,R.G. and Sharples,G.J. (1991) *J. Bacteriol*., **173**, 6837–6843.
26 Fleischmann,R.D. *et al*. (1995) *Science*, **269**, 469–512.
27 Matsumoto,T., Ohta,T., Kumagai,I., Oshima,T., Murao,K., Hasegawa,T., Ishikura,H. and Watanabe,K. (1987) *J. Biochem*., **101**, 191–2298.
28 Kiss-Laszlo,Z., Henry,Y., Bachellerie,J.P., Caizengues-Ferrier,M. and Kiss,T. (1996) *Cell*, **85**, 1077–1088.
29 Nicoloso,M., Qu,L.H., Michot,B. and Bachellerie,J.P. (1996) *J. Mol. Biol*., **260**, 178–195.
30 Tycowski,K.T., Smith,C.M., Shu,M.D. and Steitz,J.A. (1996) *Proc. Natl. Acad. Sci USA*, **93**, 14480–14485.
31 Agris,P.F., Koh,H. and Söll,D. (1978) *Arch. Biochem. Biophys*., **154**, 277–282.
32 Kawai,G., Ue,H., Yasuda,M., Sakamoto,K., Hashizume,T., McCloskey,J.A., Miyazawa,T. and Yokoyama,S. (1991) *Nucleic Acids Symp. Ser*., **25**, 49–50.
33 Kawai,G., Yamamoto,Y., Kamimura,T., Masegi,T., Sekine,M., Hata,T., Iimori,T., Watanabe,T., Miyazawa,T. and Yokoyama,S. (1992) *Biochemistry*, **31**, 1040–1046.
34 Ladner,J.E., Jack,A., Robertus,J.D., Brown,R.S., Rhodes,D., Clark,B.F.C. and Klug,A. (1975) *Proc. Natl. Acad. Sci. USA*, **72**, 4414–4418.
35 Quigley,G.J., Wang,A.H.J., Seeman,N.C., Suddath,F.L., Rich,A., Sussman,J.L. and Kim,S.H. (1975) *Proc. Natl. Acad. Sci. USA*, **72**, 4866–4870.