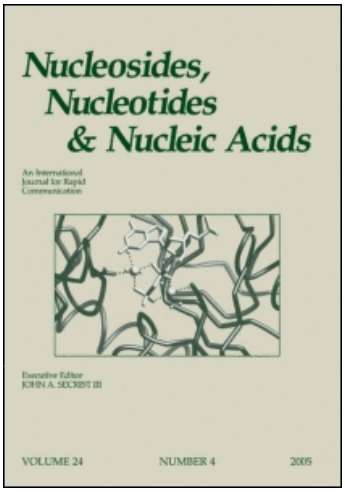

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Ascidian Mitochondrial tRNA^{Met} Possessing Unique Structural Characteristics

Akiko Kondow[a]; Shin-ichi Yokobori[b]; Takuya Ueda[a]; Kimitsuna Watanabe[a]
[a] Department of Chemistry and Biotechnology, Graduate School of Engineering, University of Tokyo, Tokyo, Japan [b] Department of Molecular Biology, School of Life Science, Tokyo University of Pharmacy and Life Science, Tokyo, Japan

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ASCIDIAN MITOCHONDRIAL tRNA^{Met} POSSESSING UNIQUE STRUCTURAL CHARACTERISTICS *

Akiko Kondow[1], Shin-ichi Yokobori[2], Takuya Ueda[1] and Kimitsuna Watanabe[1],* *
[1]Department of Chemistry and Biotechnology, Graduate School of Engineering, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan, [2]Department of Molecular Biology, School of Life Science, Tokyo University of Pharmacy and Life Science, Horinouchi, Hachioji, Tokyo 192-03, Japan

ABSTRACT: Methionine tRNA was purified from muscle mitochondria of the ascidian *Halocynthia roretzi* and its RNA sequence was determined. Analysis of the nucleotide sequence revealed that unlike most metazoan mitochondrial tRNAs^{Met}, which have a highly conserved cytidine (C) or C-derivative at the wobble position, the *H. roretzi* mitochondrial tRNA^{Met} possesses 5-carboxymethylaminomethyluridine (cmnm^{5}U) at the first position of the anticodon. This is the first report of a single mitochondrial tRNA^{Met} species having uridine (U) or a U-derivative at the wobble position.

INTRODUCTION

It has been so far elucidated that most metazoan mitochondria use various non-universal codons (1), which are recognized by tRNA anticodons whose first position is often modified (2). In the case of the methionine codon, not only AUG but also AUA is known to specify methionine in the mitochondria of most metazoa except for cnidarias, echinoderms and platyhelminths (1). The tRNAs^{Met} responsible for decoding this non-universal AUA codon have been sequenced for some metazoan mitochondria, and 5-formylcytidine (f^{5}C) was found at the first position of both bovine and nematode (*Ascaris suum*) mitochondrial (mt) tRNAs^{Met} (3-5). This modified nucleoside is thus considered to be responsible for decoding the AUA as well as the AUG codon, which has recently been verified experimentally (Takemoto *et al.*, submitted).

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*This paper is dedicated to the memory of the late Prof. Tsujiaki Hata of the Tokyo Institute of Technology.

**To whom correspondence should be addressed, Tel+FAX: 81-3-5800-6950; E-mail: kw@kwl.t.u-tokyo.ac.jp

Yokobori *et al.* (6) analyzed the mt genome of an ascidian, *Halocynthia roretzi*, and found that the non-universal codon AUA is used to specify methionine in this organella. It has also been observed that the mt $tRNA^{Met}$ gene, which is present in the genome as a single gene, has the anticodon TAT instead of the usual CAT (Yokobori *et al.*, in preparation). These findings indicate that the $tRNA^{Met}$ transcribed from this single gene is responsible for decoding the AUA as well as the AUG codon.

With the aim of verifying this at the RNA level, we purified mt $tRNA^{Met}$ from *H. roretzi* muscle to determine its nucleotide sequence, and found that the tRNA possessed several unique features: a modified nucleoside, 5-carboxymethyl-aminomethyluridine ($cmnm^5U$) at the wobble position, a truncated T arm, and a lack of the three G-C pairs at the bottom of the anticodon stem commonly present in the initiator $tRNAs^{Met}$ so far studied. Since none of these features has ever been observed in initiator $tRNAs^{Met}$ of other organisms and mitochondria (7), they appear to be specific to the ascidian $tRNA^{Met}$.

MATERIALS AND METHODS

Chemicals and enzymes [5'-^{32}P] pCp (111 TBq/mmol), [γ-^{32}P] ATP (111 TBq/mmol) and L-[^{35}S] methionine (>37TBq/mmol) were purchased from Amersham. RNase T1 and RNase U2 were obtained from Sigma, RNase PhyM from Pharmacia, and RNase CL3 from Boehringer. T4 RNA ligase and T4 polynucleotide kinase were from Toyobo. *Escherichia coli* alkaline phosphatase was purchased from Takara Shuzo, Streptavidin-agarose from Gibco BRL, and Nuclease P1 from Yamasa. A solid- phase DNA probe was obtained from Sci-Media.

Purification of mitochondrial $tRNA^{Met}$ from ascidian muscle Total mitochondrial tRNA was prepared as described previously (8) from ascidian muscle, which was kindly provided by Drs. M. Hoshi and M. Matsumoto of Tokyo Institute of Technology. Northern hybridization was performed as described (9). The sequence of the probe was 5'-AAGTAATAAACATTCGTTATTAGAA-3', which is complementary to the 25 nucleotides of the 3'-terminal of the mt $tRNA^{Met}$ gene (6). It was covalently attached to biotin at the 3'-end. Mitochondrial $tRNA^{Met}$ was fished out by the preparative solid-phase DNA probe method (10) using the same probe as that used for Northern hybridization. The $tRNA^{Met}$ sample to be employed for sequencing was further purified by successive gel electrophoresis.

Sequencing of $tRNA^{Met}$ The nucleotide sequence of $tRNA^{Met}$ was determined mainly by the method of Donis-Keller (11). Modified nucleotides were located and identified according to the method of Kuchino *et al.* (12). Mitochondrial $tRNA^{Met}$ partially

hydrolyzed by incubation at 90°C for 1 min was labeled with [γ-^{32}P] ATP at the 5'-ends of 3'-CCA terminal-containing fragments and the resulting [^{32}P]-labeled oligonucleotides were separated by polyacrylamide gel electrophoresis. They were eluted from the gel and digested with nuclease P1 to produce [^{32}P]-labeled mononucleotides, which were detected by two-dimensional thin-layer chromatography (TLC) on Funacel SF cellulose plates (Funakoshi Pharmaceutical Co.).

Preparation of mitochondrial S100 fraction Mitochondria were prepared from ascidian liver tissue of fresh ascidians by differential centrifugation as reported (13) except that the digitonin treatment was omitted. A supernatant fraction (S100) was prepared from ascidian mitochondria as described for the preparation of S30 (14) except that the homogenate was subjected to centrifugation at 100,000 x g for 2 h. S100 was stored at -80°C until use.

***In vitro* aminoacylation** Aminoacylation was performed at 30°C in a reaction mixture (15 μL) containing 100 mM Tris-HCl (pH 8.0), 15 mM $MgCl_2$, 2 mM ATP, 20 mM KCl, 0.2 mM Spermin, 50 μM [^{35}S]Met (500 MBq/mmol), 0.9 μM relevant tRNA, and 105 μg/ml proteins from the mitochondrial extract. To stop the reaction, the same volume of cold buffer consisting of 9 M urea, 100 mM NaoAc (pH 5.0), 0.02% xylene cyanol, and 0.02% bromophenol blue was added to the reaction mixture, and it was subjected to electrophoresis on 6.5% acid polyacrylamide gel in the presence of 7 M urea and 100 mM NaOAc (pH 5.0). Radiolabeled aminoacyl-tRNA was detected by an imaging analyzer (BAS-1000; Fuji Photo) (15).

RESULTS

Purification of mt $tRNA^{Met}$ Northern hybridization was performed to confirm the expression of the putative $tRNA^{Met}$ which was predicted from the $tRNA^{Met}$ gene of the *H. roretzi* mt genome (Yokobori *et al.*, in preparation). As shown in FIG. 1, a single band with approximately 60-70 nucleotides was detected in the region corresponding to the tRNA.

Mitochondrial $tRNA^{Met}$ was fished out by the preparative hybridization method using a solid-phase DNA probe and further purified by successive gel electrophoresis; 0.16 μg of ascidian (*H. roretzi*) mt $tRNA^{Met}$ was finally obtained from 100 g of ascidian (*H. roretzi*) muscle.

Nucleotide sequence and secondary structure The nucleotide sequence of the $tRNA^{Met}$ thus obtained was determined by the methods of Donis-Keller (11) and Kuchino et al (12). The sequence (FIG. 2) is identical to that predicted from the $tRNA^{Met}$ gene (Yokobori *et al.*, in preparation), indicating that $tRNA^{Met}$ is actually expressed in

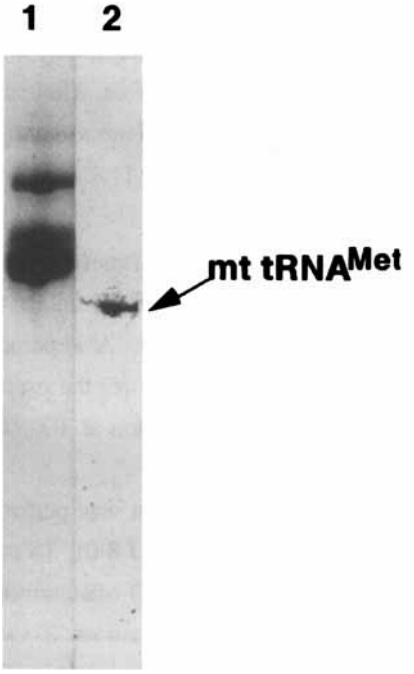

FIG. 1. Detection of ascidian mt tRNA^{Met}. *Lane 1*: Ascidian total RNA was stained with 0.02% (w/v) toluidine blue. *Lane 2*: Northern hybridization using a 5'-^{32}P DNA probe (see text). The composition of the gel was 10% polyacrylamide and 7 M urea.

ascidian mitochondria. An examination of the secondary structure deduced from the nucleotide sequence reveals several characteristic features. First, a modified uridine, 5-carboxymethylaminomethyluridine (cmnm^{5}U), occurs at the first position of the anticodon together with two pseudouridines (Ψ's) at positions 38 and 64; these are clarified further below [the base numbering conforms to the proposed rule (7)]. Second, the T arm is slightly truncated, with only three base pairs in the stem region. This feature is also found in several other tRNA genes of ascidian mitochondria (Yokobori *et al.*, in preparation). Third, there is an absence of three contiguous G-C base pairs at the bottom of the anticodon stem, although all other initiator tRNAs so far elucidated have these G-C pairs (7), which are known to play a crititcal role in the functioning of initiator tRNAs (16).

As shown in FIG. 3, two kinds of modified nucleotides, pcmnm^{5}U and pΨ, were

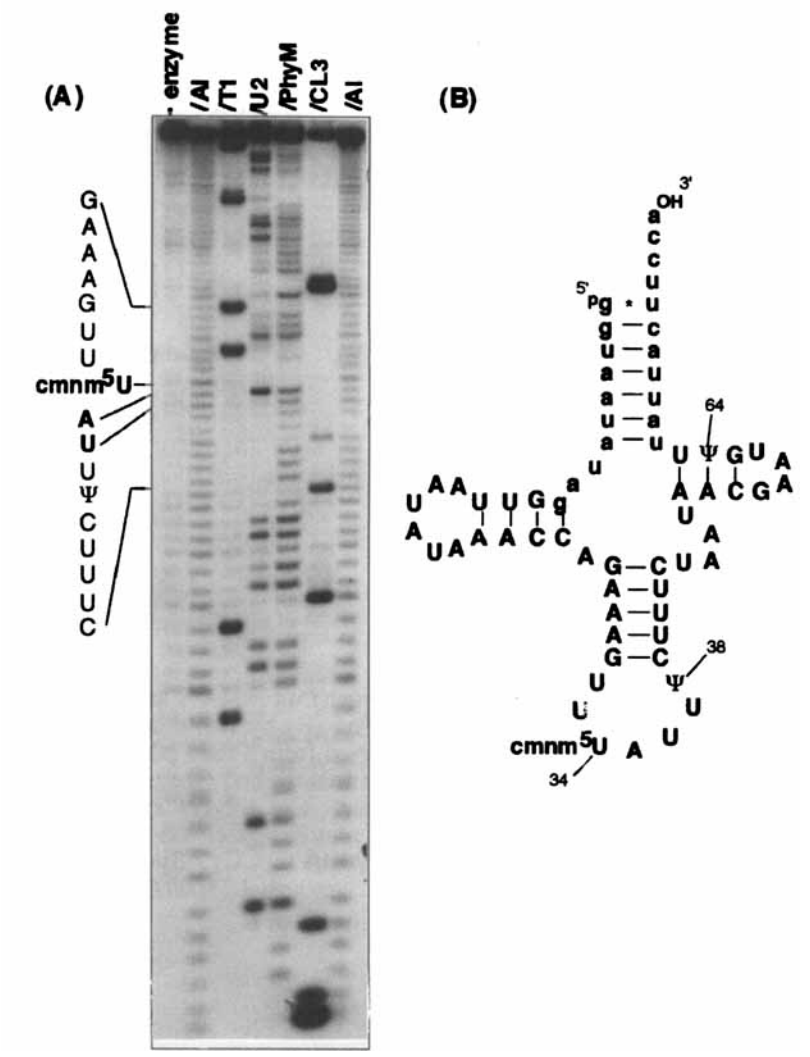

FIG. 2. Nucleotide sequence analysis and cloverleaf structure of *H. roretzi* mt tRNA^{Met}. (A) Nucleotide sequence analysis of 3'-end ^{32}P-labeled tRNA^{Met} by Donis-Keller's method (11). The three anticodon residues are indicated by bold letters. (B) Nucleotide sequence and deduced secondary structure of tRNA^{Met}. Nucleotides shown by lower-case letters are those analyzed only by Donis-Keller's method. The base numbering conforms to the proposed base-numbering rule (7).

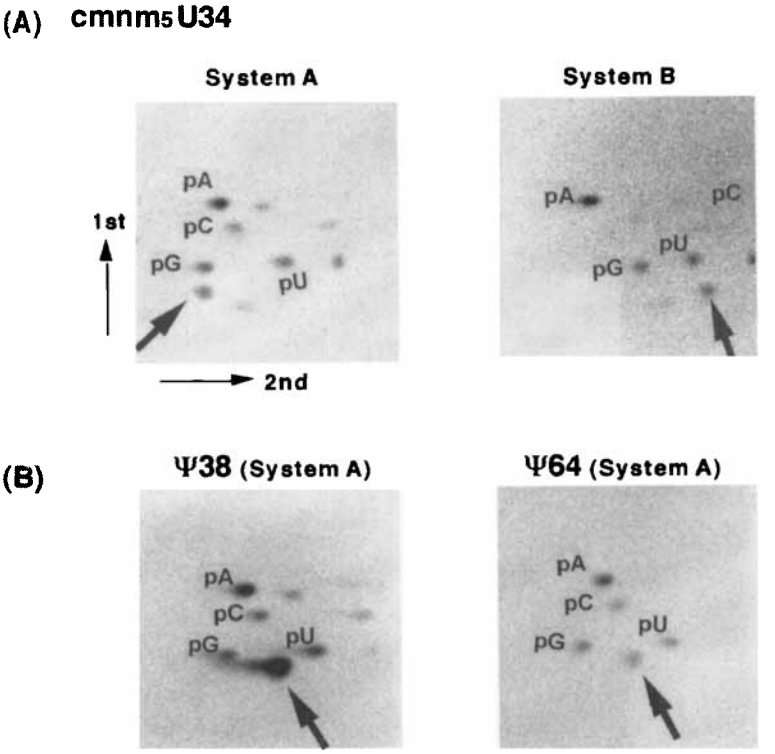

FIG. 3: Analysis of modified nucleotides in *H. roretzi* mt $tRNA^{Met}$ at the anticodon first position (position 34) (A) and positions 38 and 64 (B) by two-dimensional thin-layer chromatography (TLC). The nucleotides were derived from 5'-end labeled nucleotides of partially hydrolyzed tRNA fragments using Kuchino's method (see text). Arrows show the positions of the modified nucleotides defined. The solvent systems were: first dimension, isobutyric acid/ concentrated aqueous ammonia/water (66/1/33, v/v/v); second dimension, 2-propanol/concentrated HCl/water (70/15/15, v/v/v, System A) or 0.1 M sodium phosphate (pH 6.3) /ammonium sulfate/ 1-propanol (100/60/2, v/w/v, System B).

detected. Their locations were clarified by two-dimensional thin-layer chromatography using two different solvent systems. The first position of the anticodon was found to be $cmnm^5U$ [FIG. 2 (A)]. In addition, the nucleoside 3'-adjacent to the anticodon is an unmodified U. This also seems to be exceptional, since purine or its derivatives are found in almost all tRNA species (7).

***In vitro* aminoacylation activity of $tRNA^{Met}$** To verify that the $tRNA^{Met}$ species thus characterized is actually the mt $tRNA^{Met}$ in *H. roretzi*, the methionine acceptor activity of the $tRNA^{Met}$ was examined using the homologous mt S100 fraction. As

described in Materials and Methods, the $tRNA^{Met}$ reacted with [^{35}S]-labeled methionine in the presence of mt S100 was subjected to acid polyacrylamide gel electrophoresis. A single band was detected on the gel by an imaging analyzer, the location of which corresponded to that of tRNAs with chain lengths of about 70 nucleotides (data not shown) as detected by staining, clearly demonstrating that the $tRNA^{Met}$ characterized in this work is the $tRNA^{Met}$ present in *H. roretzi* mitochondria.

DISCUSSION

Unique modification at the anticodon first position of ascidian (H. roretzi) mt $tRNA^{Met}$ It is well known that in mt genetic systems, a modified U at the anticodon first position of tRNA can base-pair only with A or G in the third position of the corresponding codon, and not with U or C, whereas an unmodified U can base-pair with any of these four nucleotides (1). Since the codons AUA and AUG are read as methionine in ascidian (*H. roretzi*) mitochondria (6), a $tRNA^{Met}$ should exist which is capable of decoding both of these codons. Most metazoan mitochondrial genomes characterized so far have a single $tRNA^{Met}$ gene that presumably can translate methionine codons. However, these tRNA genes have the anticodon CAT. We have found that the anticodon first residue (C at the DNA level) is modified to f^5C in $tRNAs^{Met}$ of both bovine liver and nematode mitochondria (4), indicating that the $tRNA^{Met}$ should translate not only the AUG but also the AUA codon (Takemoto *et al.*, submitted). The only known exception is the case of the mt genome of a mollusc, blue mussel (*Mytilus edulis*), which possesses a second $tRNA^{Met}$ gene with the anticodon TAT in addition to the usual one with the anticodon CAT (17).

Yokobori *et al.*. recently determined the whole sequence of the ascidian mt genome and found that $tRNA^{Met}$ exists as a single gene whose anticodon first position is occupied by T instead of the usual C (Yokobori *et al.*, in preparation). In the present work, we clarified that the $tRNA^{Met}$ has $cmnm^5U$ at the first position of the anticodon, which is able to recognize A as well as G in the third position of the codons AUA and AUG (1). This is the first reported instance of a single $tRNA^{Met}$ in a metazoan mitochondrion possessing a modified U at the first position of the anticodon.

Recognition of $tRNA^{Met}$ by methionyl-tRNA synthetase The anticodon CAU is known to be a major determinant for methionyl-tRNA synthetase in almost all genetic systems, including that of *E. coli* (2). However, the *H. roretzi* mt $tRNA^{Met}$ with a modified U ($cmnm^5U$) at the anticodon first position was also found to be recognized by methionyl-tRNA synthetase of *H. roretzi* mitochondria. Since our preliminary results showed that the enzyme could also charge bovine mt $tRNA^{Met}$ with methionine, it might be that the ascidian (*H. roretzi*) enzyme changed its recognition system for $tRNA^{Met}$. A

possible alternative explanation may lie in another unique feature of the ascidian (*H. roretzi*) $tRNA^{Met}$ — the fact that the nucleotide 3'-adjacent to the anticodon is occupied by an unmodified U. In general, purine or its derivatives are found at this position in most tRNAs (15). Replacement of the purine by U in the *H. roretzi* mt $tRNA^{Met}$ may have affected the three dimensional structure of the anticodon loop, perhaps thereby enabling the $tRNA^{Met}$ to be recognized by methionyl-tRNA synthetase.

Structural characteristics of ascidian mt $tRNA^{Met}$ Another characteristic feature of the ascidian mt $tRNA^{Met}$ is that it contains no contiguous G-C pairs at the bottom of the anticodon stem, a feature which has been regarded as typical of the initiator tRNAs so far studied (7, 21) and which is thought to be important for recognition by initiation factor 3 (IF3) (16). At least two contiguous G-C pairs are conserved at the bottom of the anticodon stem in other metazoan mt $tRNAs^{Met}$, except for the case of *Mytilus edulis* $tRNAs^{Met}$ which has the anticodon TAT (7, 15). Since the other *M. edulis* mt $tRNA^{Met}$ with the anticodon CAT has conserved G-C pairs, it is considered that the two species of *M. edulis* mt $tRNA^{Met}$ probably have different functions: $tRNA^{Met}_{TAT}$ may be used as an elongator, and $tRNA^{Met}_{CAT}$ as an initiator. Since the *H. roretzi* mt genome contains no other $tRNA^{Met}$ gene, the single $tRNA^{Met}$ may function as both the elongator and the initiator. It is also possible, of course that a second unknown $tRNA^{Met}$ of non-mitochondrial origin may be present in ascidian mitochondria.

Another characteristic of *H. roretzi* mt tRNAs is that most of them have a truncated T arm (Yokobori *et al.*, in preparation). The T arm is a major determinant for EF-Tu in most genetic systems (19,20). However, the nematode mt system in which almost all the mt tRNAs lack the T arm, has a unique EF-Tu whose C-terminal is extended by several tens of amino acid residues (Watanabe *et al.*, submitted). These findings raise interesting questions on the interaction between tRNA and EF-Tu, which may be answered by further analysis of the *H. roretzi* mt translation system.

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REFERENCES

1. Osawa,S.; Jukes,T.H.; Watanabe,K.; Muto, A. *Microbiol. Rev.*, **1992** ***56***, 229-264.
2. Sakamoto,K.; Kawai,G.; Niimi,T; Satoh,T.; Sekine,M.; Yamaizumi,Z.; Nishimura,S.; Miyazawa,T.; Yokoyama,S. *Eur. J. Biochem.*, **1993** ***216***, 369-375.

3. Kawai,G.; Yokogawa,T.; Nishikawa,K.; Ueda,T.; Hashizume,T.; McCloskey,J.A.; Yokoyama,S.; Watanabe,K. *Nucleosides Nucleotides.*, **1994** ***13***, 1189-1199.
4. Moriya,J.; Yokogawa,T.; Wakita,K.; Ueda,T.; Nishikawa,K.; Crain,P.F.; Hashizume,T.; Pomerantz,S.C.; McCloskey,J.A.; Kawai,G.; Hayashi,N.; Yokoyama,S.; Watanabe,K. *Biochemistry*, **1994** ***33***, 2234-2239.
5. Watanabe,Y.; Tsurui,H.; Ueda,T.; Furushima,R.; Takamiya,S.; Kita,K.; Nishikawa,K.; Watanabe,K. *J. Biol. Chem.*, **1994** ***269***, 22902-22906.
6. Yokobori,S.; Ueda,T.; Watanabe,K. *J. Mol. Evol.*, **1993** ***36***, 1-8.
7. Sprinzl,M.; Steegborn,C.; Hubel,F.; Steinberg,S. *Nucleic Acids Res.*, **1996** ***24***, 68-72.
8. Ueda,T.; Ohta,T.; Watanabe,K. *J. Biochem.*, **1985** ***98***, 1275-1284.
9. Sambrook,J.; Fritsch,E.F.; Maniatis,T.(Ed.) *Molecular Cloning*, Cold Spring Harbor Laboratory Press **1989** *2nd ed.*
10. Tsurui,H.; Kumazawa,Y.; Sanokawa,R.; Watanabe,Y.; Kuroda,T.; Wada,A.; Watanabe,K.; Shirai,T. *Anal. Biochem.*, **1994** ***221***, 166-172.
11. Donis-Keller,H. *Nucleic Acids Res.*, **1980** ***8***, 3133-3142.
12. Kuchino,Y.; Hanyu,N.; Nishimura,S. *Methods Enzymol.*, **1987** ***155***, 379-396.
13. Matthews,D.E.; Hessler,R.A.; Denslow,N.D.; Edwards,J.S.; O'Brien,T.W. *J. Biol. Chem.*, **1982** ***257***, 8788-8794.
14. Schwartzbach,C.J.; Spremulli,L.L. *J. Biol. Chem.*, **1989** ***264***, 19125-19131.
15. Ohtsuki,T.; Kawai,G.; Watanabe,Y.; Kita,K.; Nishikawa,K.; Watanabe,K. *Nucleic Acids Res.*, **1996** ***24***, 662-667.
16. Schmitt,E.; Guillon,J.M.; Meinnel,T.; Mechulam,Y.; Dardel,F.; Blanquet,S. *Biochimie*, **1996** ***76***, 543-554.
17. Hoffmann,R.J.; Boore,J.L.; Brown,W.M. *Genetics*, **1992** ***131***, 397-412.
18. Wolstenholme,D.R. *Int. Rev. Cytol.*, **1992** ***141***, 173-216.
19. Nissen,P.; Kjeldgaard,M.; Thirup,S.; Polekhina,G.; Reshetnikova,L.; Clark,B.F.C.; Nyborg,J. *Science*, **1995** ***270***, 1464-1472.
20. Clark,B.F.C.; Nyborg,J. *Current Opinion in Structural Biology*, **1997** ***7***, 110-116.
21. RajBhandary,U.L.; *J. Bacteriol*, **1994** ***176***, 547-552