KOR. JOUR. MICROBIOL. September, 1986 p. 204-210 Vol. 24, No. 3

Studies on the Organization and Expression of tRNA Genes in *Aspergillus nidulans* (VI) The Molecular Structure of $tRNA^{Arg}$ Gene of *A. nidulans*

Lee, Byeong Jae and Hyen Sam Kang

Department of Microbiology, College of Natural Sciences, Seoul National University

*Aspergillus nidulans*의 tRNA 유전자의 구조와 발현에 관한 연구 (VI) *Aspergillus nidulans*의 $tRNA^{Arg}$ 유전자의 분자구조

이병재 · 강현삼

서울대학교 자연대학 미생물학과

Abstract: One clone(pANt32) carring $tRNA^{Arg}$ gene was selected from Aspergillus total tRNA gene clones. The nucleotide sequences of this tRNA gene were determined by Maxam and Gilbert's chemical cleavage methods. The sequence of this tRNA gene is as follow; 5'GGCCGGCT-GCCCAATTGGCAAGGCGTCTGACTACGAATCAGGAGATTGCAGGTTCGAGCCCTGCGT-GGGTCA3'. This sequence conicides with the characteristics of other eukaryotic tRNA. Some consensus sequences(ACT-TA box, TATTTT and T-cluster) are found in both 5'-end and 3'-end flanking regions.

Key words: *Aspergillus nidulans*, $tRNA^{Arg}$ gene, DNA sequences.

The recombinant DNA technology and various nucleotide sequencing methods developed in 1970's have made it possible to understand the organization and expression of a certain gene, especially, eukaryotic gene (Cohen et al., 1972 ; Jackson et al., 1972 ; Maxam and Gilbert, 1977 ; Sanger et al., 1977 ; Simoncsits et al., 1977 ; Donis-Keller et al., 1977). After the first cloning of the tRNA gene of *Saccharomyces cerevisiae* (Beckmann et al., 1977), many tRNA genes of eukaryotes such as Xenopus, Drosophila, *Bombyx mori*, mouse, rat, *Caenohabditis elegans*, Neurospora, *Aspergillus* and human were cloned (Clarkson et al., 1978 ; Cortese et al., 1981 ; Roy et al., 1982 ; Hagenbuchle et al., 1979 ; Garber and Gage, 1979 ; Selker and Yanofsky, 1980 ; Lee and Kang, 1983 ; Cortese et al., 1978). As a result of the DNA sequencing for these cloned tRNA genes, it is known that intervening sequences (IVS) exist in some tRNA genes (Kang et al., 1979 ; for detail see Lee, 1986). There is no general rule about the existence of IVS in certain tRNA genes. But two significant facts are found; one is that IVS has not been found until yet in the mammalian tRNA genes, the other is that there seems to bo no IVS in the genes of $tRNA^{Arg}$ and $tRNA^{Asp}$. Thus we have chosen $tRNA^{Arg}$ gene to examine whether the existence of IVS is species or tRNA family dependent or both. The molecular structures of $tRNA^{Asp}$ have been reported in a separate paper(Kim et al., 1986).

MATERIALS AND METHODS

Isotopes and Enzyme

[α-^{32}P]-dATP was purchased from NEN and [r-^{32}P]-ATP was synthesized in our laboratory or purchased from Amersham. Various kinds of enzymes were bought from New England Biolab., Takara or BRL.

Colony Hybridization

Colony hybridization to the total tRNA gene clone of Aspergillus was performed with $tRNA^{Arg}$ labeled with [γ-^{32}P]-ATP at 5'-end according to Lee and Kang(1983).

tRNA Gene Localization

After constructing restriction enzyme maps with some hexanucleotide recognizing enzymes, Southern hybridization was carried out by the method of Southern(1975 ; 1979) with some modifications. When the agarose(1.2%) gel electrophoresis was completed, DNA was denatured by soaking the gel in two volume of denaturation solution(0.5M NaOH, 1.5M NaCl) for 15 min. at room temperature with gentle shaking, and then in neutralization buffer(1.5M NaCl, 0.5M Tris-Hcl, pH 7.4) with the same way. Blotting was done according to Maniatis et al. (1982) except the concentation of blotting solution (20×SSC). Hybridization experiment was done with the same methods as those of Southern(1980).

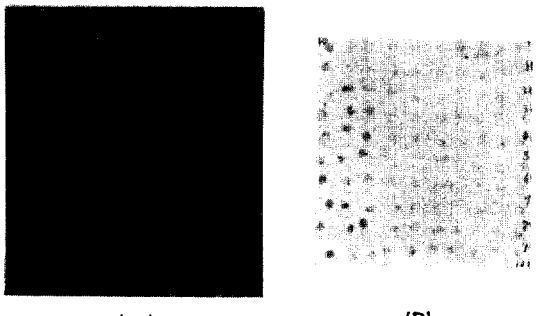

Fig.1. *Selection of $tRNA^{Arg}$ gene clone by colony hybridization*
(A), the autoradiogram. (B), DNA print. The numbers marked in DNA print stand for the colony number.

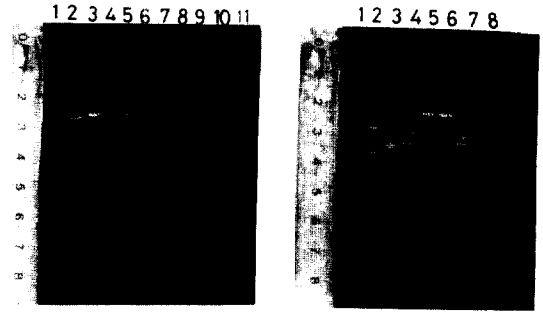

Fig.2. *Enzyme digestion pattern of pANt32.*
(Left) (1) lambda-Hind III, EcoRI (2) pANt32-PstI,SalI (3) pANt32-Bgl II, PstI (4) pANt32-Bgl II, EcoRI (5) pANt32-BamHI,PstI (6) pANt32-BamHI,Bgl II (7) pANt32-HindIII, SalI (8) pANt32-Hind III, PstI (9) pANt32-Hind III, BamHI (10) pANt32-Hind III, BamHI (11) pBR322-HinfI. (Right) (1), (8) lambda-Hind III, EcoRI (2) pANt32-SalI (3) pANt32-PstI (4) pANt32-PstI (4) pANt32-HindIII (5) pANt32-EcoRI (6) pANt32-Bgl II (7) pANt32-BamHI.

DNA Sequencing

DNA fragments to be sequenced were labeled at 3'-end with [α-^{32}P]-dATP(Maniatis *et al.*, 1982). Sequencing was done with Maxam and Gilbert's chemical cleavage method(Maxam and Gilbert, 1977 ; 1980). The reaction time at each base modification step was modified in this laboratory(Lee, 1986).

RESULTS AND DISCUSSIONS

$tRNA^{Arg}$ Gene Clone Selection and Gene Localization

Clone 32(pANT32) showed positive signal in colony hybridization of total tRNA gene bank with the 5'-end labeled $tRNA^{Arg}$ (Fig. 1). The restriction map was constructed with hexanucleotide recognizing enzymes(Fig. 2) and pANt32 contains about 3.4 kb of *Aspergillus* DNA with two sites for PstI and SalI as well as a single site for Hind III and Bgl II. Southern hybridization for above enzyme combination revealed that B fragments of Hind III-SalI, BamHI-Hind III, BamHI-Bgl II and C fragments of Hind

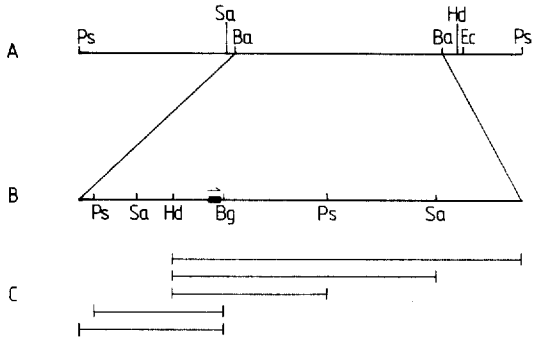

Fig.3. *Physical map of pANt32.*
The whole map of pANt32 (B) *Aspergillus* DNA inserted. The position of tRNA gene is designated as thick bar. Arrow means for the direction of transcription. (C) The fragments which have shown positive signals in hybridization.

III-PstI contain tRNA gene(Fig. 4). $tRNA^{Arg}$ gene was located within the C fragment(about 400 bp) of HindIII-BglII(Fig. 3).

DNA Sequencing and Identification of $tRNA^{Arg}$ Gene

We decided to determine the whole sequence of HindIII-BglII C fragment. Because of the limit of resolution on the gel, it is believable to read up to 300 bases in 60cm long gel. It is, therefore, necessary to compare the sequence of both strands with each other to obtain a faithful sequences. Therefore, DNA was labeled at the 3'-end of HindIII site and sequenced to the rightward at first and next labeled at the 3'-end of Bgl II site and sequenced to the leftward. Labeled DNA with [α-^{32}P]-dATP was digested with PstI which makes it easy to elute a proper fragment because of its asymmetric digestion pattern. PstI also showed low artifact labeling effect due to its 5'-recessed end.

Fig.5 shows 8% DNA sequencing gel in which the $tRNA^{Arg}$ gene is included. About 300 bp from Bgl II site and 350 bp from Hind III could be sequenced and the sequences were confirmed by comparing its complementary sequences. The $tRNA^{Arg}$ gene was found between 64th base and 136th base downward from Bgl II site of this fragment (Fig.6). This sequence coincides with its cognate RNA sequence(Lee and Kang, 1986).

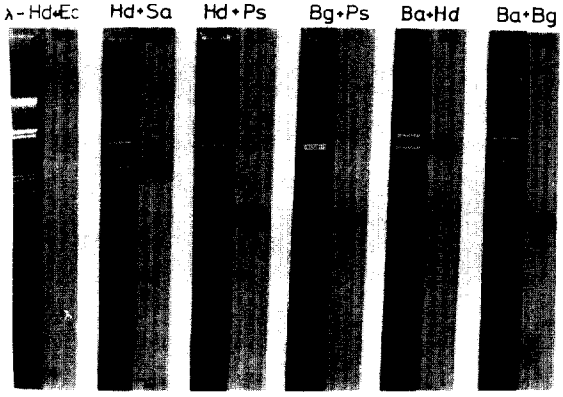

Fig. 4. *Southern hybridization of pANt 32 with* [^{32}P]-$tRNA^{Arg}$
Ba;BamHI, Bg;BglII, Ec;EcoRI, Hd;Hind III, Ps;PstI, Sa;SalI.

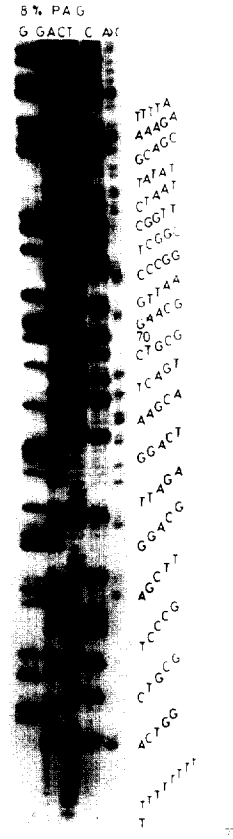

Fig.5. *DNA sequencing gel(8%-7M urea PAG).*
DNA was isotopically labeled at BglII site and sequenced. tRNA gene is contained in this gel.

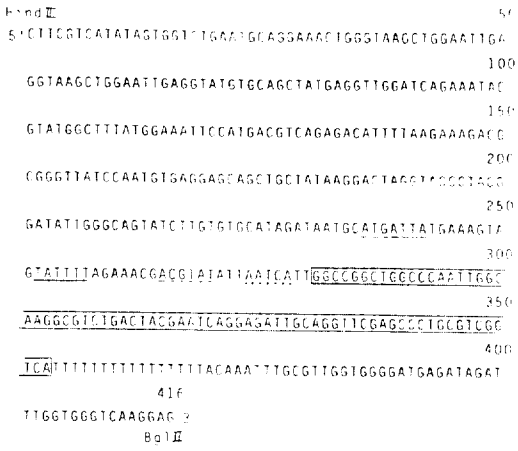

Fig.6. *DNA sequences of pANt32 BglII, HindIII C fragment*
tRNA gene is indicated in box. Some consensus sequences at the 5'-flanking region are indicated as follows; possible ACT-TA box(---), AT-rich sequence such as TATTTT, TTC(----) and repeated sequence to the structural gene(·····).

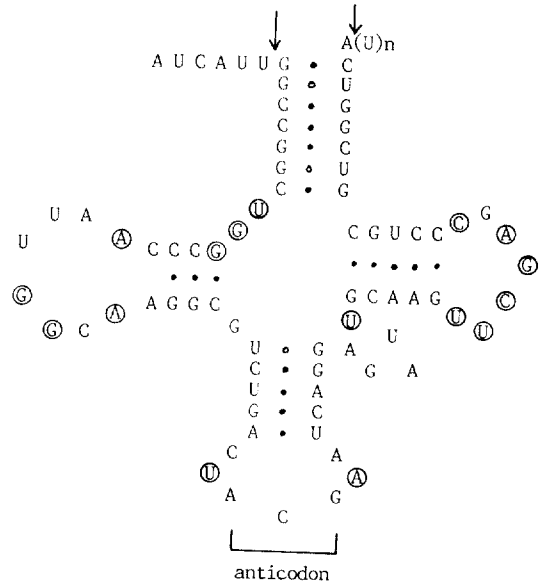

Fig.7. *Possible secondary structure of the primary transcripts*
Watson-Crick base pair is designated as closed circle(·) and Crick's wobble base as open circle( ). Arrows indicate the splicing point and conserved bases are surrounded with circle.

Analysis of tRNA^{Arg} Gene Sequence

Possible Secondary Structure of tRNA^{Arg}

tRNA has a secondary structure of clover-leaf shape by means of its internal base pairing. There are 5 Watson-Crick base pairing in amino acyl stem and 3 in dihydrouracil stem, 4 in anticodon stem and 5 in TψC stem(Fig. 7). All the non Watson-Crick bases of amino acyl stem and anticodon stem are composed of Crick's G.U wobble base pair. Especially, the two in amino acyl stem have G base on the 5'-side and U base on the 3'-side. This type of base pairing known to be more similar to that of Watson-Crick's than contrary style(Sundaralingam ., 1979). The fourth position of DHU stem is A.C mismatched type and almost all eukaryotic tRNAs with anticodon of ACG are this type. This characteristics are appeared in other tRNAs(Dillon, 1978). It seems, therefore, that the base pairing in DHU stem is dependent both on the family of tRNA and on the phylogeny, i.e. the base pairing of eukaryotes shows different type from that of prokaryotes.

All conserved bases are occurred and tRNA^{Arg} gene specific consensus sequences

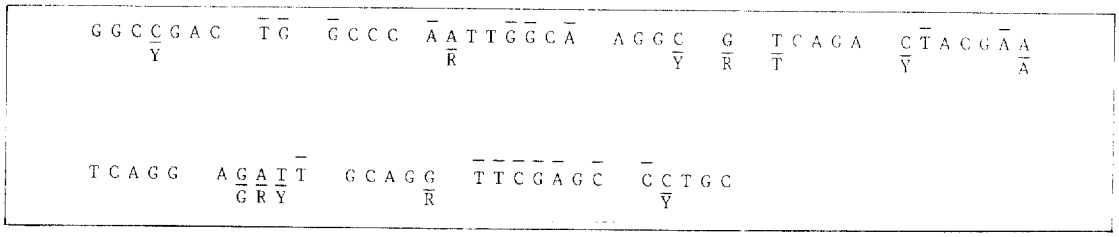

Fig.8. *tRNA^{Arg}specific consensus sequences*
Underlines stand for the consensus sequences and upper lines are the position of conserved bases. R; purine, Y; pyrimidine.

which are appeared specifically in $tRNA^{Arg}$ at the same positions are also occurred(Fig. 8). The -CCA sequence at the 3′-terminal is not found which is also the characteristics of eukaryotic tRNA genes.

Sequences Related to Transcription

IVS does not exist in $tRNA^{Arg}$ gene. But this fact only is not sufficient to conclude that the existence of IVS is dependent on tRNA gene family because IVS was found in aspartic tRNA gene of *Aspergillus*.

Traboni et al.(1982) reported that the consensus sequences of internal control region(ICR) of box A is -RRYNNARY-GG and that of box B is GTCRANNC(R=purine; Y=pyrimidine; N=any nucleotide). In the $tRNA^{Arg}$ gene, the sequences correspond to box A and Box B are GGCCCAATTGG and GTTCGACC, respectively. These sequences show good agreement with the proposed consensus sequences.

Four to twenty bases of T-cluster(or stretch) are found in the 3′-flanking sequence of tRNA gene and these T-cluster may play some roles in termination of transcription(Korn and Brown, 1978 ; Silvermann et al., 1979 ; Sekiya et al., 1982 ; Sekiya et al., 1981). There are two kinds of T-cluster. One is short T-cluster which is 4-5 T-residues (Makowski et al., 1983), and the other is long T-cluster composed of more than 10 T-residues (Hu et al., 1983). The 3′-flanking sequences of *Aspergillus* tRNA gene has the long T-cluster(17 bp) as in some other organisms, but the very peculiar aspect is that in this gene, T-cluster begins just at the end of coding region(+74 base). There is, yet, no report on this kind of example.

There is no general theory on the role of 5′-flanking region in eukaryotic tRNA gene, because of little homology of sequence in this region between tRNA genes. Two contrary opinions(positive role vs negative role) are pitted until now about the role of 5′-flanking sequence in transcription. But the groups who insist the positive role of 5′-flaking region in transcription emphasize that there are AT-rich regions like ACT-TA box(Indik and Tartof, 1982) and TATAC, TATTTT or TTC(Fournier et al., 1984 ; Raymond and Johnson, 1983). In the $tRNA^{Arg}$ gene of Aspergillus, sequences similar to ACT-TA box are shown at −10, −39, −89 position and TATTTT sequence exist at −29th position(Fig. 6).

It has been reported that alternating purine-pyrimidine tracts(d(CA/TG)n) that can form Z-form DNA *in vitro* in the 5′-flaking region or in the space between two internal promoters inhibit the transcription. when microinjected into Xenopus oocyte(Santoro et al., 1984) There is no such sequence in the 5′-flanking region of $tRNA^{Arg}$ gene. From above results. we can assume that the 5′-flanking region of *Aspergillus* $tRNA^{Arg}$ gene would play a positive role. It has been observed in some tRNA genes that the sequence of coding region repeats at 5′-flanking region and the transcription initiates at purine nucleotide somewhere in this region(Santos and Zasloff, 1981). The 5 bases of −7 region are repeated in +37 region(see Fig. 6). Thus, transcription of $tRNA^{Arg}$ gene may initiate on A uncleotide of −3 or −6 position. These assumptions can be verified directly by *in vitro* or *in vivo* transcription experiments. We are now processing *in vitro* transcription test and sooner or later we can clarify a little more information about the mechanism of transcription of eukaryotes.

적 요

$tRNA^{Arg}$ 유전자를 갖고 있는 클론(pANt32)을 ***Aspergillus***의 총 tRNA 유전자 클론뱅크로 부터 선별해 냈다. Maxam과 Gilber의 DNA 염기순서 결정법으로 이 tRNA 유전자의 염기순서를 결정해 본 결과 다음과 같은 결과를 얻었다. 5′GGCCGGCTGGCCCAATTGGCAAGGCGTCTGACTACGAATCAGGAGATTGCAGGTTCGAGCCCTGCG-

TCGGTCA3 . 다른 진핵세포의 유전자에서 나타나는 여러 특징들이 나타났으며, 유전자의 주쇄부 분에 있어서 현재까지 제안된 몇몇 공통염기순서(consensus sequence)들이 나타남을 알 수 있었다.

ACKNOWLEDGEMENTS

We would like to thank Mr.Kim. Y.J. and Miss Jeong B.W. for their technical assistance. This research was supported partly by grant from Korea Science and Engineering Foundation(1984-1986).

REFERENCES

1. Beckmann, J.S., P.F. Johnson and J. Abelson, 1977. Cloning of yeast tRNA genes in *Escherichia coli*. *Science* **196**, 205-208.
2. Clarkson, S.G., V. Kurer and H.O. Smith, 1978. Sequence organization of a cloned $tRNA^{Met}$ fragment from *Xenopus laevis*. *Cell* **14**, 713-724.
3. Cohen, S.N., A.C.Y. Chang and L. Hsu, 1972. Nonchrosomal antibiotic resistance in bacteria: Genetic transformation of *Escherichia coil* by R-factor DNA. *Proc. Natl. Acad. Sci. USA* **69**,2110-2114.
4. Cortese, R.,D. Melton, T. Tranquilla and J.D. Smith, 1978. Cloning of nematode tRNA genes and their expression in the frog oocyte. *Nucleic Acids Res.* **5**, 4593-4611.
5. Cortese, R.,R. Harland and R. Cortese, 1980. Transcription of tRNA genes *in vivo:* single stranded compared to double -stranded templates. *Proc. Natl. Acad. Sci. USA* **77**, 4147-4551.
6. Dillon, L.S. 1978. The Genetic Mechanism and the Origin of Life, pp 229-348 Plenum Press.
7. Donis-Keller, H.,A. Maxam and W. Gilbert, 1977. Mapping adenines, guanines, and pyrimidines in RNA. *Nucleic Acids Res.* **4**, 2527-2538.
8. Fournier, A.,N.A. Guerin, J. Corlet and S.G. Clarkson, 1984. Structure and *in vitro* transcription of glycine tRNA gene from *Bombyx mori*. *EMBO J.* **3**, 1547-1552.
9. Garber, R.L. and L.P. Gage, 1979. Transcription of a cloned *Bombyx mori* $tRNA^{Ala}$ gene: Nucleotide sequence of the tRNA precursor and its processing *in vitro*. *Cell* **18**, 817-828.
10. Hagenbuchle, O.,D. Larson, G.I. Hall and K.U. Sprague, 1979. The primary transcription product of a silkworm alanine tRNA gene: identification of *in vitro* sites of initiation, termination and processing. *Cell* **8**, 1217-1229.
11. Hu, J.C., B.D. Cote, E. Lund and J.E. Dahlberg, 1983. Isolation and characterization of genomic mouse DNA clones containing sequences homologous to tRNA and 5S rRNA. *Nucleic Acids Res.* **11**, 4909-4821.
12. Indik, Z.K. and K.D. Tartof, 1982. Glutamate tRNA genes are adjacent to 5S RNA genes in Drosophila and reveal a conserved upstream sequence(the ACT-TA box). *Nucleic Acids Res.* **10**, 4159-4117.
13. Jackson, D.A., R.H. Symons and P. Berg, 1972. Biochemical method for inserting new genetic information into DNA of simian kvirus **40** : circular SV40 DNA molecules containing lambda phage genes and the galactose operon of *Escherichia coil*. *Proc. Natl. Acad. Sci. USA*69, 2904-2909.
14. Kang, H.S., R.C. Ogden, G. Knapp, C.L. Peebles and J. Abelson, 1979. Structure of yeast tRNA precuresors containing intervening sequences. *ICN-UCLA Symp. Mol. Cell. Biol.* **14**, 69-84.
15. Kim, Y.J., B.J. Lee and H.S. Kang, 1986. The organization and transcription of *Aspergillus nidulans* tRNA genes. *Korean Biochem. J.* Accepted.
16. Korn, L.J. and D.D. Brown, 1978. Nucleotide sequence of *Xenopus borealis* oocyte 5S DNA; comparison of sequences that flank several related sukaryotic genes. *Cell* **15**, 1145-1156.

17. Lee, B.J. and H.S. Kang, 1983. The organization and transcription of *Aspergillus nidulans* tRNA genes; (II)cloning of *A. nidulans* total tRNA genes in *Escherichia coli*. *Kor. J. Microbiol.* **21**, 229-237.
18. Lee. B.J. 1986. Studies on the organization of $tRNA^{Asg}$ gene and $tRNA^{Asp}$ gene in *Aspergillus nidulans*. ph.D. Thesis. Seoul National University.
19. Makowski, D.R., R.A. Haas, K.P. Dollan and D. Grunberger, 1983. Molecular cloning, sequence analysis and *in vitro* expression of a rat tRNA gene cluster. *Nucleic Acids Res.* **11**, 8601-8625.
20. Maniatis, T., E.F. Fritsch and J. Sambrook, 1982. Molecular Cloning, Cold Spring Harber Laboratory.
21. Maxam, A.M. and W. Gilbert, 1977. A new method for sequencing DNA. *Proc. Natl. Acad. Sci. USA* **74**, 560-564.
22. Maxam, A.M. and W. Gibert, 1980. Sequencing with base-specific chemical cleavages. *Methods in Enzymol.* vol. 65 pp 499-560. eds. Grossman, L. and K. Moldave, Academic Press, New York.
23. Raymond, G.J. and P.F. Johnson, 1983. The role of non coding DNA sequences in transcription and processing of a yeast tRNA. *Nucleic Acids Res.* **11**, 5969 —5988.
24. Roy, K.L., H. Cook R. Buckland, 1982. Nucleotide sequence of a segment of human DNA containing the three tRNA genes. *Nucleic Acids Res.* **10**, 7313 -7323.
25. Sanger, F.,S. Nicklen and A.R. Coulson, 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**, 5463-5467.
26. Santoro, C., F. Costanzo and G. Cilibert 1984. Inhibition of eukaryotic tRNA transcription by potential Z-DNA sequences. *EMBO J.* **3**, 1553-1559.
27. Santos, T. and M. Zasloff 1981. Comparative analysis of human chromosomal segments bearing non allelic dispersed $tRNA^{Met}$ genes. *Cell* **23**, 699-709.
28. Sekiya, T.R., K. Nishimura, K. Mathuda, Y. Taya and S. Nishimura, 1982. A rat tRNA gene cluster containing the genes for $tRNA^{Pro}$ and $tRNA^{Lys}$; analysis of nucleotide sequences of the genes land the surrounding regions. *Nucleic Acids Res.* **10**, 6411-6419.
29. Sekiya, T., Y. Kuchino and S. Nishimura, 1981. Mammalian tRNA genes: nucleotide sequences of rat genes for $tRNA^{Gln}$, $tRNA^{Gly}$, and $tRNA^{Gln}$. *Nucleic Acids Res.* **9**, 2239-3350.
30. Selker, E. and C. Yanofsky, 1980. A phenylalanine tRNA gene from *Neurospora crassa:* conservation of secondary structure involving an intervening sequence. *Nucleic Acids Res.* **8**, 11033-11043.
31. Silverman, S.,O. Schimidt, D. Soll and B. Hoverman, 1979. The nucleotide sequence of a cloned Drosophila arginine rRNA gene and its *in vitro* transcription in Xenopus germinal vesicle extracts. *J. Biol. Chem.* **254**, 10290-10294.
32. Simoncsits, A., G.G. Brownlee, R.S. Brown, J.R. Rubin and M. Guilley, 1977. New rapid gel sequencing method for RNA. *Nature* **169**, 833-836.
33. Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**, 123-517.
34. Southern, E.M. 1979. Gel electrophoresis of restriction fragments. *Methods in Enzymol.* vol.68 pp152-176 eds. Grossman, L. and Moldave, K. Academic Press, New York.
35. Sundaralingam, M. 1979. tRNA: Structure, Properties and Recognition. eds. Schimmel, P.R.,D. Soll and J. Abelson, N. pp 115-132 Cold Spring Harbor Laboratory.
36. Traboni, C.,D. Ciliberto and R. Cortese, 1982. A novel method for site directed mutagenesis: its application to eukaryotic tRNA gene promoter. *EMBO J.* **1**, 415-420.

(Received April 14, 1986)