Modified bases in transfer RNA

VANI BRAHMACHARI and T. RAMAKRISHNAN

Microbiology and Cell Biology Laboratory, Indian Institute of Science, Bangalore 560 012, India

Abstract. Transfer RNA is uniquely enriched with modified bases. A large body of information has accumulated about the occurrence, nature and distribution of modified bases in tRNA. But similar investigations on the enzymes involved in this post-transcriptional modification have been hampered by the instability of the enzymes and lack of suitable substrates. The present review summarises briefly, the occurrence and methods of detection of modified bases, the enzymes involved in their formation and also certain suggestive evidence for the role of modification in cellular metabolism.

Keywords. tRNA; methyltransferases; methylation base modification; function.

Introduction

Transfer RNA entered the field of molecular biology research in 1958 when Crick proposed the role for an adaptor molecule in the translation of genetic messages. Thereafter the presence of such a molecule was experimentally verified and further it became clear that once the amino acid is attached to the cognate tRNA, it is the tRNA that dictates the translocation of the amino acid in the protein synthesising machinery. Thus tRNA plays a pivotal role in information transfer in the cell.

Over the years, the tRNA molecules by the virtue of their chemical stability unlike mRNA, their small size (70–80 nucleotides long) and their relative abundance turned out to be one of the widely studied and well investigated components of the cell Now we know the primary structure of more than 180 tRNA molecules from various sources as well as their generalised three dimensional crystal structure at 2·5 A resolution.

The studies on tRNA have been very fruitful with the development of various methodologies for the purification and separation of single species tRNA (Nishimura, 1971 and references therein). The methods of reversed phase chromatography (Kelmers *et al.,* 1965) and electrophoresis on polyacrylamide gel (Ikemura and Dahlberg, 1973) have made it possible to purify single species of tRNA starting from small quantities of total tRNA.

Abbreviations used: mcm^5U, 5-Methoxycarbonyl methyl uridine; $cmnm^5U$, 5-carboxy methyl aminomethyl uridine; acp^3U, 3-(3-amino-3-carboxy propyl) uridine; m^5C, 5-methylcytosine; ac^4C, N^4-acetyl cytidine; s^2C, 2-thiocytidine; i^6A, N^6-isopentenyl adenosine; ms^2i^6A, 2-methyl thio-N6-isopentenyl adenosine; t^6 A, (N-(9-β -D-ribofuranosyl purin-6 yl) carbamoyl) threonine; m^1G, 1-methyl guanine; m^2 G, 2-methyl guanine; m_2^2 G, N^2N^2-dimethyl guanine; m^7 G, 7-methyl guanine; NMR, nuclear magnetic resonance; HPLC, high pressure liquid chromatography; AMV, avian myeloblastosis virus.

Hand in hand with this development are the methods developed for sequencing of tRNA. These include various enzymatic as well as chemical methods as a result of which now it is possible to decipher the sequence starting from 5–10 μg of pure tRNA species (Nishimura and Kuchino, 1983).

It has also become clear now that tRNA participates in several cellular functions other than protein synthesis such as priming tumour virus DNA synthesis, regulation of cognate amino acid biosynthesis Operon and aromatic amino acid transport (Rich and Rajbhandary, 1976; Buck and Griffiths, 1981).

Nature of modified bases in tRNA

The presence of modified bases in RNA was first detected in transfer RNA molecules, and methylation was one of the earliest detected modifications (Borek and Srinivasan, 1966; Borek, 1963). From then on, a large variety of modified bases have been detected in tRNA. These results have been summarised, in several review articles (Srinivasan and Borek, 1964; Nishimura, 1972; Nau, 1976; Feldman, 1977; Bjork, 1983).

Macromolecules offer a variety of complex structures which serve as substrates for methylation. Moreover the sites of methylation within each base is also varied. For instance, in uracil, C5-methylation is achieved in 5-methyl uracil, and this is the site for various other post-transcriptional modifications like 5-methoxycarbonyl methyl uridine (mcm^{5}U) Uridine-5, oxyacetic acid methyl ester (mV) and 5-carboxy methyl amino-methyl uridine (cmnm^{5}U). The C4-S bond is formed to get 4-thiouridine and N3 is involved in forming 3-(3-amino-3-carboxy propyl) uridine (acp^{3}U).

In cytosine the C5-CH$_3$ is formed in 5-methylcytosine (m^5 C), N4 is involved in forming N^4-acetyl cytidine (ac^{4}C). N3 in 3-methyl cytidine (m^{5}C) and C2 in forming 2-thiocytidine (s^{2}C).

Adenine nitrogens as well as carbons can serve as sites for modification. For example N-l and N-6 form 1-methyl adenine and 6-methyl adenine, N^6-isopentenyl adenosine (i^6 A), 2-methyl thio-N6-isopentenyl adenosine (ms^{2}i^{6}A), (N-(9-β-D-ribofuranosyl purin-6-yl) carbamoyl) threonine (t^6 A) respectively.

In the case of guanine, N-l, N-2, N-7 can be methylated to form 1-methyl guanine (m^{1}G), 2-methyl guanine (m^2 G), N^{2}N^2-dimethyl guanine (m$_2^2$ G) and 7-methyl guanine (m^7 G). Hypermodification of guanine occurs in the case of base Y and its derivatives. The *Q* base or quenine is another derivative of guanine where N-7 is replaced by C-7 with a bulky side chain including a mannosyl or D-galactosyl group. Of these variety of modifications the most common is methylation of base and sugar moiety. The structure of all the various modified bases found in tRNA have been compiled by Nishimura (1979).

The nature and position of modified bases in tRNA are species-specific. Thus there are several bases which are exclusively found in eukaryotes or prokaryotes. Table 1 lists some of such bases. Thiolation, for instance is found only in prokaryotes whereas methylation of cytosine is restricted to eukaryotes. Besides, the abundance of certain modified bases at specific positions within the cloverleaf structure of tRNA varies distinctly between eukaryotes and prokaryotes. Figure 1 depicts these differences for each possible modification at a given position. Note the difference in case of

Table 1. Presence of minor nucleosides.

Exclusively in prokaryotic tRNAs	Exclusively in eukaryotic tRNAs
s^2C	m^3C
s^4U	m^5C
o^5U	mcm^5U
mo^5U	Tm
cmo^5U	m^2G
mam^5s^2U	m_2^2G
$cmam^5U$	i^6A
$cmam^5s^2U$	m^1I
$mcmo^5U$	Wye and derivatives
s^2T	
m^2A	
m^6A	
ms^2i^6A	

dihydrouridine at 16th position and 1-methyladenine at 58th position. It is clear that tRNAs from eukaryotes are modified to a greater extent than those from prokaryotes. Thus $tRNA_1^{Ser}$ from rat liver has 14 modified nucleotides whereas that from *Escherichia coli* has only seven modified nucleosides. However, the abundance of ribothymidine-54, m^7 G-46 and dihydrouridine-20 are comparable between the two groups (figure 1). Almost all bacterial tRNA contain m^5U-54, exception to this are the tRNA from Mycoplasma (Walker and Rajbhandary, 1978), Streptomyces (Kuchino *et al.,* 1982), *Staphylacoccus epidermis* (Roberts, 1974), archaebacteria (Gupta and Woese, 1980), *Micrococcus luteus* (Delk *et al.,* 1976), *Thermus thermophilus* (Watanabe *et al.,* 1976a) and *Mycobacterium smegmatis* (Vani *et al.,* 1979). The transfer RNA from *Mycobacterium smegmatis* contains 1-methyladenine which is very abundant at 58th position of tRNA from eukaryotes but not in the tRNA from prokaryotes. The tRNA from *B. subtilis* (Raettig *et al.,* 1977), *B. stearothermophilus* and archaebacteria (Gupta and Woese, 1980) are also reported to contain 1-methyl adenine. Archaebacteria are also known to lack m 7 G in their tRNA (Woese and Fox, 1977).

Methods for detection and distribution of modified bases in tRNA

There are several methods used for the detection of modified bases in tRNA. The monomers derived from tRNA samples, after enzymatic or chemical digestion are analysed by two dimensional chromatography on cellulose coated glass plates (Nishimura *et al.,* 1967). There are several solvent systems given for developing the chromatogram. The one given by Feldman and Falter (1971) is specifically suited for acid labile residues, whereas, that given by Rogg *et al.* (1976) is suited for nucleoside separation.

A column chromatographic method has been given by Katz and Dudock (1969). A sensitive automatic recording system hooked to a column of cation resin was developed
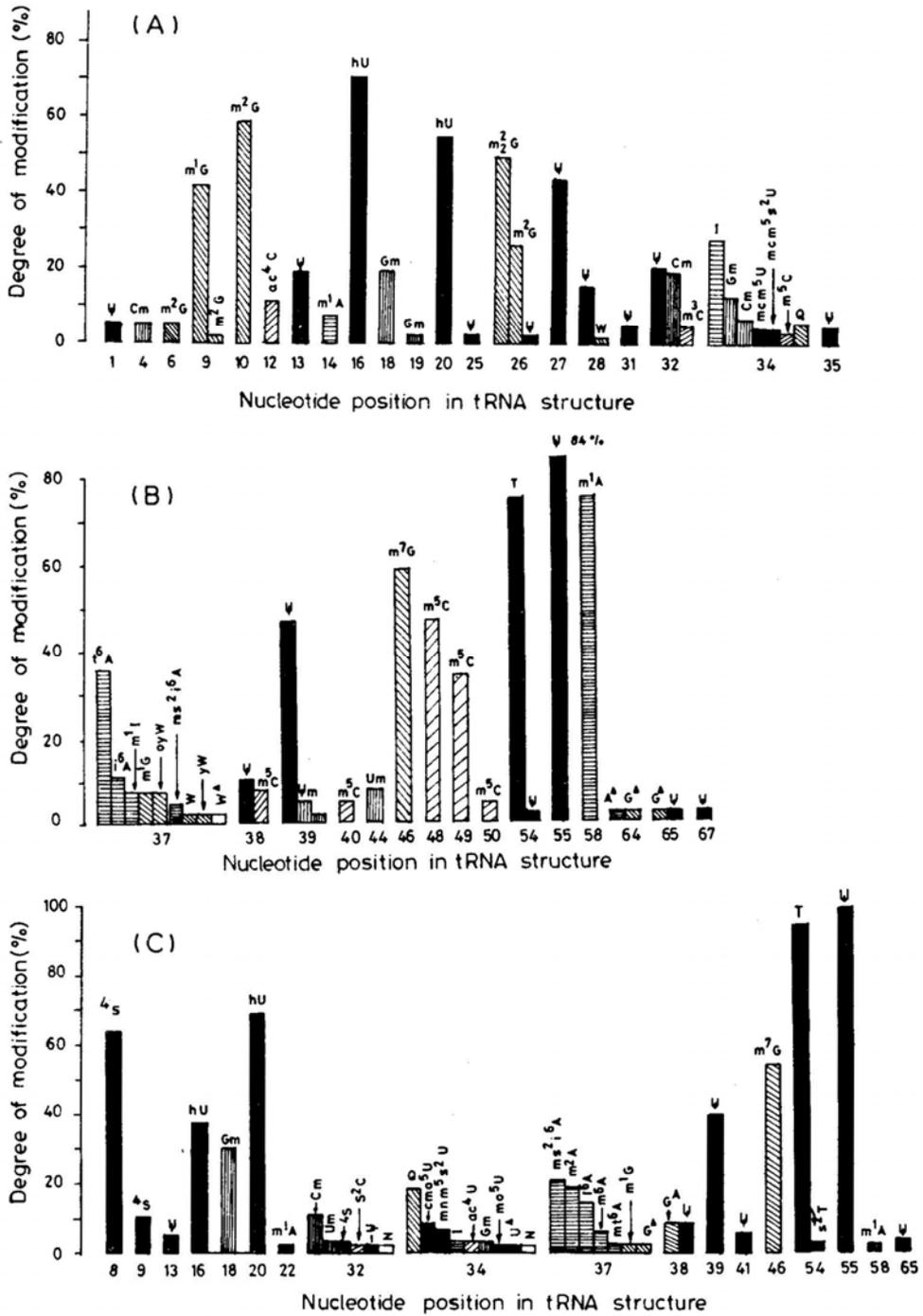
Figure 1. Abundance of modified bases in tRNA. (A) and (B) eukaryotic tRNA (C) prokaryotic tRNA. Taken from Singhal and Fallis (1979).

by Uziel *et al.* (1968). Use of Dowex column in base separation was given by Leboy (1971).

Two dimensional finger printing (Sanger *et al.,* 1965) and paper electrophoresis technique (Brownlee and Cartwright, 1977) have also been used in the detection of modified bases.

More recently nuclear magnetic resonance (NMR) has been employed in the analysis of modified bases (Kastrup and Schmidt, 1978). High pressure liquid chromatography (HPLC) has become one of the most sensitive techniques for modified base analysis. Fractionation of modified nucleosides on HPLC using a column of μ-Bondapak C18 has been reported by Davis *et al.* (1979) and Gehrke *et al.* (1978). With its high sensitivity HPLC can effectively be used for quantitation of modified residues in a given sample. The structure of modified bases has been worked out using mass spectrometry. Mass spectrometry has been used for detection of modified bases also (McCloskey, 1974; Von Mindin and McCloskey, 1973; Yamaizumi *et al.,* 1979).

Incorporation of radioactivity into tRNA molecules in the form, for instance, of [^{32}P] -has contributed greatly to the sensitivity of detection. In cases where the incorporation of radioactivity in the form of precursors has been difficult, post-abelling techniques have been used. Randerath *et al.* (1972) have given the method for post-labelling the nucleosides with tritium and analysing by chromatography. Yet another post-labelling technique has been developed by Silberklang *et al.* (1977) wherein the RNA is digested into 3'-nucleoside triphosphates and they are 5"labelled using γ- [^{32}P]-ATP and the resulting labelled nucleoside diphosphates (^{32}P Np) are analysed. This apart, in the late 1970s several post-labelling techniques have been developed for sequencing tRNA (Lockard *et al.,* 1978; Gupta and Randerath, 1979; Kuchino *et al.,* 1979). These techniques involve the 3'-end labelling of tRNA, with ^{32}p Cp and T4 RNA ligase or 5 "end labelling using γ- [^{32}P]-ATP and T4 polynucleotide kinase.

However the localization of modified bases within the cloverleaf structure of tRNA just by analysing various tRNAs in mixture, has not been possible. The exact position of modified bases is known only while sequencing pure single species of tRNA. By adopting tRNA sequencing method of Kuchino *et al.* (1979), for post labelling and analysing tRNA mixtures, we have been able to empirically localize the modified bases within the cloverleaf structure (Nishimura, S., personal communication; Vani, 1982). The oligonucleotides derived after digestion of tRNA mixtures with formamide are labelled at 5'-ends with γ-[^{32}P]-ATP and fractionated on sequencing gels. The oligonucleotide bands can now be assigned emperically to various regions of the cloverleaf structure of tRNA. The data from one such analysis are shown in figure 2 and table 2.

The data on the nucleotide sequence of a large number of tRNA species indicates the probable positions at which modified bases can occur. A compilation of such data correlated with the nature of hydrogen bonding each of them can involve in, brings out certain interesting features of modified base distribution. From the data given in table 3, certain features of the distribution of modified bases in tRNA can be elucidated:
(i) Pseudouridine and 5-methylcytosine, show the maximum variation in their position of occurrence, and both of these bases can involve in Watson-Crick base, pairing.
(ii) Certain bases like 1-methyl guanine, 1-methyl inosine, 2-thio-cytosine, 2-dimethyl
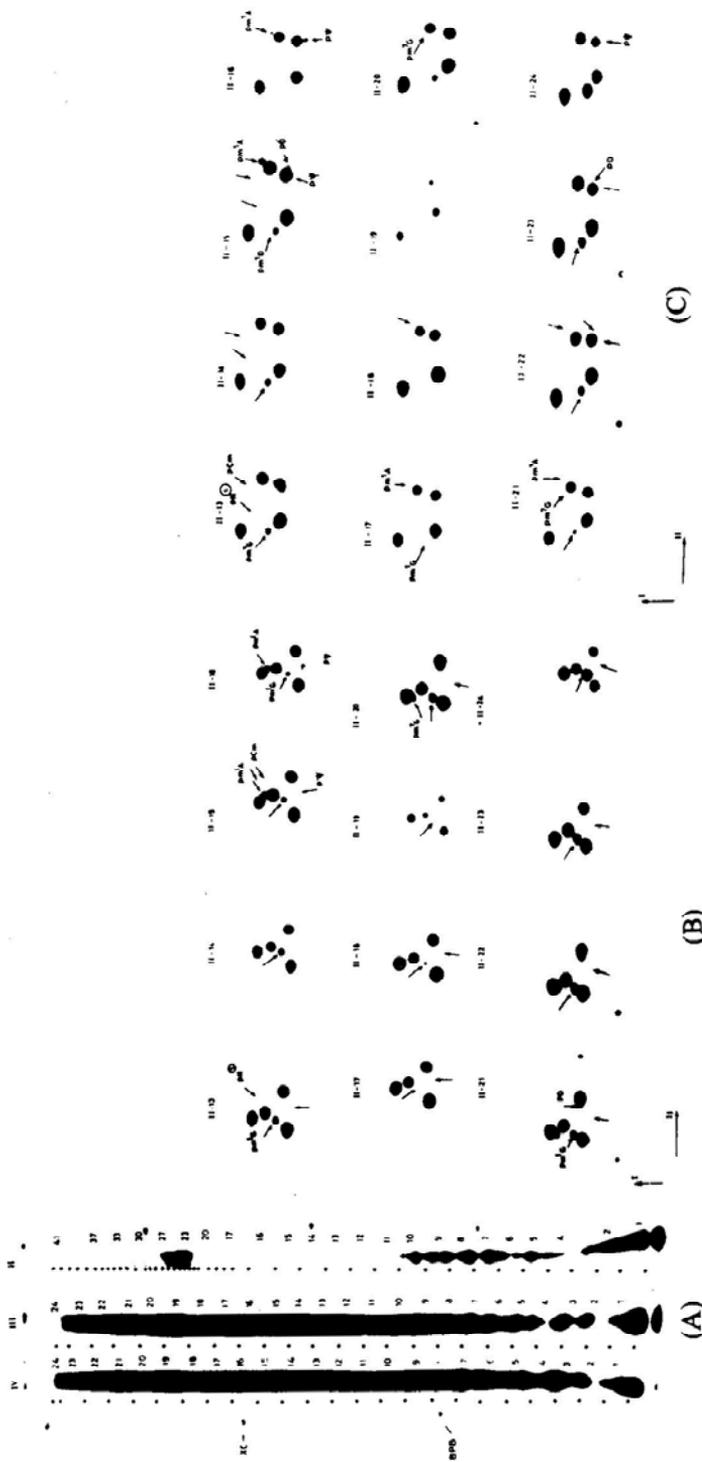
Figure 2. Localization of modified bases within tRNA structure by post labelling. (A) Analysis of oligonucleotides derived from tRNA mixtures on sequencing gels after labelling at 5′-end with γ-[³²P]-ATP. (B) and (C) Analysis of mononucleotides derived from fraction II on the gel (A). The number II-13 to II-24 correspond to the band numbers indicated on (A), lane II. (B) In solvent system A; I, Isobutyric acid: 0·5 M ammonium hydroxide 5 : 3 v/v; II, Isopropanol: concentrated HCl: Water 70 : 15 : 15 v/v/v; (C) in solvent system B; I, Isobutyric acid, ammonia, water 60 : 1 : 33 v/v/v; II, 0·1 M sodium acetate pH 6·8, ammonium sulphate and *n*-propanol 100 : 60 gms: 2 v/w/v.

Table 2. Localisation of the oligonucleotides to the different regions of tRNA structure and analysis modified base composition of fraction II.

Band number	Corresponding region of tRNA	Modified base(s) present
1–8	Stem (a)	pm^1G and pCm
9–11	Stem (e)	pm^1G
12–16	Loop (iv) and Stem (e)	$p\psi$, pCm, pm^1G, pN^1, pm^1A and pD.
17–21	Stem (d), Loop III and Stem (c)	pm^1G, pm^7G, pm^1A.
22–27	Loop (II)	pm^1G, $p\psi$ and pN^3
28–31	Stem (c)	pm^1G
31–39	Stem (b) and Loop I	pm^1G, pN^2
39–44	Stem (a)	None

Nomenclature for the regions of the cloverleaf is as given by Clark (1977). pm^1G-l-methylguanosine 5'–monophosphate, pCm-2'-O-methylcytosine 5'-monophosphate, ψ *p*-pseudouridine 5'-monophosphate, pm^1A-l-methyladenine 5'-monophosphate, pD-dihydrouridine 5'-monophosphate, pm^7G-7-methylguanosine 5'–monophosphate. pN^1, pN^2, pN^3-unidentified nucleotides.

guanine, 1-methyl adenine can involve only in Hoogsteen base pairing but not Watson-Crick base pairing. Concurrently they occur only in either the loop or the junction regions which does not demand conventional Watson-Crick pairing. However m^2G in tRNA of yeast, *Bombyx mori* and mammalian tRNA occurs at the 10th position within the D-stem (Kuntzel *et al.,* 1974; Sprague *et al.,* 1977; Chen and Roe, 1978). (iii) Hyper-modified bases like t^6A, i^6A, Y, O_2YW and alkylated cytosine like m^3C occur exclusively in the looped out regions. These bases cannot be involved in either Watson-Crick or Hoogsteen type of hydrogen bonding. Thus a correlation between the region of occurrence of modified bases and their ability to form hydrogen bonding is reflected in the distribution of modified bases along the sequence of transfer RNA. However it should be noted that all modified bases can involve in stacking interactions if not hydrogen bonding, even with sequentially distant residues, thus maintaining the tertiary structure of tRNA.

Enzymes of tRNA modification/methylation

As early as 1963 Mandel and Borek demonstrated that the methyl groups of all the methylated nucleotides come from methionine, including the - CH_3 group of ribothy-midine. The enzymes which mediate this reaction *in vivo* constitute the group of S-adenosyl methionine-tRNA methyltransferases (EC 2.1.1.29–36) generally referred to as tRNA methylases.

The variety seen in tRNA modification demands the presence within the cell of a whole battery of enzymes with unique base and site specificity. The presence of six different methylases in *E. coli* was shown by Hurwitz *et al.* (1964). They identified uracil,

Table 3. Nature of the possible base pairing of modified bases present in transfer RNA.

Nature of Modified nucleoside	Position(s) of occurrence in tRNA	Nature of base pair Watson–Crick	Hoogsteen
s^4U	8_j	+	+
o^5U	34_l	+	+
ψ	$27_s/28_s/32_l/38_l/39_s/40_s/55_l/65_s$	+	+
U_m	32_l	+	+
D	17_l	+	+
s^2T	54_l	+	+
mo^5U	34_l	+	+
T	54_l	+	+
s^2C	32_l	−	+
m^5C	$34_l/38_l/40_s/48_v/49_s/50_s$	+	+
ac^4C	$34_l, 12_s$	+	+
m^3C	32_l	−	−
m^2A	37_l	+	+
m^1A	$9_j/14_l/58_l$	−	+
m^6A	$14_l/37_l$	−	−
i^6A	37_l	−	−
m^1G	$9_j, 37_l$	−	+
m_2^2G	26_j	−	+
m^2G	10_s	−	+
G_m	39_s	+	+
m_2^2G	26_j	−	+
m^7G	46_v	+	−
Q	34_l	+	−
yW	37_l	−	−
O_2yW	37_l	−	−
m^1I	37_l	−	+

s–stem, l–loop, j–junction, v–variable loop, (+)–possible, (−)–not possible.

cytosine, adenine tRNA methylases and three enzyme fractions which methylated guanine–one being specific for 7-position and two for N-l of guanine.

The purification of modifying enzymes has been difficult since they are present in low amounts. Further, the lack of suitable tRNA substrates for each enzyme and the instability of the enzymes have hampered the extensive characterization of the enzymes. Most enzymes so far characterised are known to be composed of one polypeptide in the active state, except tRNA (m^5U) methyltransferase from *S.feacalis* which is composed of two identical subunits (Bjork, 1983). For this methylation reaction methyl tetrahydrofolate is used as methyl group donor in *S.feacalis* as well as *B. subtilis*. The tRNA (s^4U) synthetase is also known to be composed of at least two subunits (Abrell *et al.,* 1971).

Methylases have been partially purified from several systems. Aschoff *et al.* (1976) have carried out the purification of 7-methyl guanine methylase from *E. coli*. They have used tRNA[fmet] of *B. subtilis* which lacks m^7G, as the substrate. From *E. coli* strains, tRNA m^1G-methyl transferase has been purified by Hjalmarsson *et al.* (1983) and quenosine

inserting enzyme by Okada and Nishimura (1979). The supK methylase has been studied by Pope and Reeves (1978). The association of methylase with virions was first reported by Gantt *et al.* (1971) in avian myeloblastosis virus (AMV). Purification of this enzyme was done by Taylor and Gantt (1979) to about 1000 fold. They have shown that the enzyme methylates G-10 of E. *coli* B tRNA ^{Phe} and has a molecular weight of 77,000.

In the purification of methylases, apart from ion exchange and gel filtration techniques, blue sepharose column chromatography has been employed by Greenberg and Dudock (1980) and it has been possible to carry out the purification of tRNA-m^{1}A methyltransferase I from *Mycobacterium smegmatis* by affinity chromatography on AMP-sepharose column (Vani and Ramakrishnan, 1984a). Studies on substrate specificity and partial purification of the 1-methyl adenine methyl transferase from rat liver were carried out by Kuchino and Nishimura (1974). The recognition sequence of the enzyme has been suggested to be *G-T-ψ-C-G-A-A-U-C* in the *TψC* loop region. Glick and Leboy (1977) have also investigated the nature of 1-methyl adenine methylase of rat liver and shown that it methylates A-58.1-Methyl adenine methylase and 2-methyl guanine methylase from HeLa cells were partially purified and shown to methylate A-58 and G-27 of E. *coli* (Spermulli *et al.,* 1974). At least one enzyme for each modification has been detected in all the systems studied to date. However there are reports indicating the presence of more than one enzyme for the same modification but with different target specificity. Smolar *et al.* (1975) have shown that in yeast there exist two tRNA (m^{1}G) methyltransferases; one enzyme catalyzing the formation of m^{1}G at position 37 and the other at position 9. There is also a similar report on tRNA (m^2 G) methyltransferase from rat liver (Kraus and Staehelin, 1974). They showed that one of them recognises a sequence like Y–G-Cp, the otherY-G-Up. It is interesting to note that these two enzymes have been reported to have different sensitivity to *S*-adenosyl homocysteine (Glick *et al.,* 1975).

The occurrence of more than one enzyme species has also been reported for tRNA (m^{1}A) methyltransferase from rat brain cortex (Salas and Dirheimer, 1979) and *Mycobacterium smegmatis* (Vani and Ramakrishnan, 1984a). In *M. smegmatis,* two activities of tRNA (m^{1}A) methyl transferase have been demonstrated, one methylating both E. *coli* and yeast tRNA, and the other methylating only the E. *coli* tRNA. These two activities have different sensitivity to methylation inhibitors like *S*-adenosyl homocysteine and an analog SIBA (5'S-isobutyl-thioadenosine).

The influence of polyamines and salts on methylases have been investigated in several cases. Leboy (1971) showed that spermine, spermidine, putrescine and salts like ammonium acetate and magnesium acetate have differential effects on different base specific methylations. Leboy and Glick (1976) showed that tRNA m^2-G-methyltransferase I had optimum concentrations of spermidine about ten times and of putrescine about twice as much as was required for tRNA-m^{2}G methyltransferase II activity. Monovalent cations like potassium, sodium and ammonium are also known to have an effect on tRNA methylase activity (Agris *et al.,* 1975).

All known tRNA modifications, except the formation of *Q*-base, occur at polynucleotide level. The *Q*-base is inserted into tRNA by tRNA transglycosylase, which replaces guanine with quenine (Okada and Nishimura, 1979). In most RNA transmethylation, reactions *S*-adenosyl methionine (AdoMet) is the methyl donor. However in certain grampositive bacteria like *B. subtilis* and *Streptococcus feacalis* tRNA (m^{5}U)

methyltransferase utilizes, 5,10-methylene tetrahydrofolate as the methyl donor (Kersten *et al.,* 1975; Delk and Rabinowitz, 1975). However, it is interesting to note that *B. subtilis* tRNA lacking m^5U accepts methyl groups from tRNA (m^5C) methyl-transferase from *E. coli* using AdoMet as the methyl donor (Arnold *et al.,* 1976). Similarly, *E. coli* tRNA deficient in m^5U can act as substrate for the enzyme from *B. subtilis* (Kersten *et al.,* 1975). These observations indicate that although the enzyme from these two sources need different cofactors, they have very similar specificity and mechanism of recognition. In fact the studies of Gambaryan *et al.* (1979) have shown that the initial binding of tRNA methyltransferases is rather non-specific, meaning that they recognise a general structural feature of tRNA and subsequently methylate a given base at a defined position in a highly specific manner. These observations were made by using immobilized tRNA.

The maturation of tRNA molecules is a highly ordered process. The size reduction and modification are intimately related processes during the maturation of tRNA precursors. The level of certain tRNA modifying enzymes within the cell is regulated by factors like growth rate. For instance the level of tRNA (m^5U) methyltransferase increases with increasing growth rate, while that of tRNA (m^1G) methyltransferase and tRNA (mnm^5s^2U) forming enzyme remain almost constant (Ny and Bjork, 1977,1980; Ny *et al.,* 1980). Temperature is known to influence the extent of modification of tRNA in certain species like *Bacillus thermophilus* and *Thermus thermophilus* (Agris *et al.,* 1973; Watanabe *et al.,* 1976b).

Functions of modified bases in tRNA

The search for a function for the specific alkylation of tRNA bases has been on since the discovery of modified bases in tRNA. But this particular aspect has somehow been elusive to any unique generalisation.

One of the correlations drawn is between the nature of the residue at the position 37 in the tRNA structure and the first base of the codon (Nishimura, 1972, 1979). For instance, hydrophobic nucleosides such as i^6A or its derivatives are almost always found in tRNAs recognising codons starting with uracil and the tRNA interacting with codons starting with adenine have hydrophilic residues at this position. However, similar correlation is not found with codons starting with guanine or cytosine. Thus it has been suggested by Nishimura (1972,1979) that probably the hypermodification is necessary to strengthen the fidelity of translation. It has been observed (Gefter and Russell, 1969) that tRNA^{Tyr} from *E. coli* deficient in ms^2i^6A binds less efficiently to the ribosomes. In yeast the antisuppressor mutations sin 1 and mod 5-1 reduce the activity of serine-inserting UGA suppressor and the tyrosine-inserting UAA suppressor respectively and it was found that both these strains completely lack the i^6A normally present in position 37 of the suppressor tRNAs (Laten *et al.,* 1978; Janner *et al.,* 1980). Both mutants however grow relatively well, showing only 10% reduction in growth rate. *E. coli* tRNA ^{phe} carrying i^6A37 in place of ms^2i^6A37 is known to be less active in ribosome binding (Hoburg *et al.,* 1979). Yeast tRNA ^{Phe} lacking its usual component yW at 37 fails to bind to ribosomes and carry out poly(U) directed *in vitro* translation (Thiebe and Zachau, 1968). Conformational studies with i^6A and t^6A monomers have

indicated that the side chains might block the two *N*-sites of adenine for hydrogen bonding resulting in the inability to form Watson–Crick base pairing (Bugg and Thewalt, 1972; Parthasarathy *et al.,* 1974). These observations support the hypothesis that these kinds of modifications promote and maintain single stranded structure in the anticodon loop.

Mutation *his T,* leading to the absence of pseudouridine at the position 38–39 is known to lead to the loss of regulation of histidine operon (Roth *et al.,* 1966). Further it has been revealed that tRNA^{His} lacking ψ reads the seven histidine codons present in a row in the histidine leader mRNA inefficiently (Johnston *et al.,* 1980). Yet another link between amino acid biosynthesis and tRNA base modification was shown recently by Bjork (1980). All mutants defective in the common pathway of aromatic amino acids are deficient in cmo^{5}U and mcmO^{5}U in their tRNAs. It has also been demonstrated that the deficiency of thiomethyl group of ms^{2}i^{6}A in tRNA stimulates the transport of aromatic amino acids (Buck and Griffiths, 1981).

In yeast trm-1 mutation leading to the deficiency of m$_2$ ^{2}G at position 26 in tRNA leads to a slight reduction in growth rate. Further the tRNA^{Ser} from mutants has reduced efficiency in *in vitro* charging (Bjork and Kjellin-Straby, 1977). Specific chemical reduction of m^{7}G 46 disrupts the C13-C22-m^{7}G46 interaction leading to a slightly less ordered tRNA structure (Arcari and Hecht, 1978). *E . coli* mutant trm *B* defective in m^{7}G formation is indicated to show slower growth than trm *B*$^+$ and heterologous *in vitro* methylation producing m^{7}G or m^{2}G results in an altered kinetics of aminoacylation (Hoburg *et al.,* 1979; Roe *et al.,* 1973). The effect of modification on aminoacylation of tRNA has not been obvious in all systems studied. However in rats the undermethylated tRNA, extracted after feeding a diet containing ethionine, shows reduced levels of aminoacylation; when injected into Xenopus oocytes, these tRNAs are found to have reduced charging capactiy (Ginzburg *et al.,* 1979). Studies with *E. coli* and yeast undermethylated tRNA species have led to the conclusion that methylated tRNA is better substrate in a wider range of heterologous systems (Peterkofsky, 1964). Similar studies with reference to the presence of 1-methyladenine have also shown that the presence of this modification render the tRNA a better substrate for aminoacylation in heterologous system (Vani and Ramakrishnan, 1984b). The presence of m^{5}U seems to influence the elongation factor directed *A*-site binding (Kersten *et al.,* 1981). The effect of ribothymidine present in mammalian tRNA on *in vitro* protein synthesis in the presence of certain of the elongation factors has been shown by Roe and Tsen (1977). Kersten *et al.* (1981) have shown that when ribothymidine is absent in tRNA there is misincorporation of leucine during poly(U) directed protein synthesis *in vitro,* the effect is pronounced on misincorporation by tRNA$_4$ ^{Leu} and does not induce any other tRNA ^{Leu} to misread. The stability of tRNA^{Met} is higher, when s^{2}m^{5}U54 is present as compared to that in the presence of m^{5}U54 which is further lowered when only U54 is present (Davenloo *et al.,* 1979).

Transfer RNA modification might serve as a fine control mechanism for modulating translational efficiency. During the development of slime mold *Dictyostelium discoideum* and the amphibian *Rana catibeinna,* changes occur in the tRNA modification pattern (Palatnik *et al.,* 1977; Klee *et al.,* 1978; Dingermann *et al.,* 1977). In *D. discoideum* the levels of m^{5}U, and m^{5}C decrease during development from vegetative cells to spore formation (Dingermann *et al.,* 1977) and m^5 U containing tRNA species

are preferentially used in protein synthesis (Dingermann *et al.,* 1980). Further, changes in the formation of Q-base also occurs during development of *D. discoideum* (Palatnik *et al.,* 1977). Transfer RNA from tumour tissue has exclusively guanine instead of Q-base in the Wobble position of tRNA^{Tyr}, tRNA^{His}, tRNA^{Asp}and tRNA^{Asn}. Recently studies with tobacco mosaic virus have shown that tRNA^{Tyr} containing Q-base prevents read-through whereas that with guanine in place of quenine leads to the synthesis of a read-through product, a 160 *K* protein instead of 110 *K* protein (Bienz and Kubli, 1981).

During *Drosophila* development the content of Q-base changes dramatically but this is influenced by factors such as growth medium and temperature of cultivation (White *et al*., 1973; Worsnick and White, 1977). Thus the pattern of synthesis of certain modified bases changes during developmental processes in eukaryotes.

The application of modern techniques of molecular biology coupled with the studies using well-defined mutants defective in tRNA modification will certainly lead to better understanding of the formation and function of modified nucleosides in RNA. The process is complex and is interlinked with several aspects of cellular metabolism at various levels. Thus the presence of modified nucleosides seems to be necessary for the subtle tuning and coordination of tRNA function as well as specific interactions with several protein factors.

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