MEETING REPORT

tRNA: STRUCTURE AND FUNCTIONS
Report of a Pre-Symposium held in Riga, U.S.S.R., on June 19 and 20, 1970, within the scope of the VII International Symposium on the Chemistry of Natural Products *

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The four sessions of the Pre-symposium were devoted to four different aspects of the subject, these form the main divisions of the report. The Pre-symposium was opened with an introductory speech by V.A.Engelhardt.

1. Primary structure of tRNA; minor components; methods of tRNA isolation.

The introductory talk devoted to the primary structure of tRNA was given by M.Staehelin (Switzerland).

A comparison of the tRNAs whose sequences have been reported shows that they fall into three classes, class I with 3 base pairs in the D loop and a short extra arm, class II those with 3 base pairs in the D loop and a long extra arm, and class III those with 4 base pairs in the D arm. The distribution of minor nucleotides indicates that most of the modifying enzymes recognize nucleotides at specific positions in the tRNA. Apart from I and Gm, two unidentified nucleotides, a G and a C derivative, have been found in the anticodon region. In one of the serine tRNAs from rat liver a minor nucleotide was also revealed in this position. This tRNA is bound much better to ribosomes in the presence of UCU and UCA than of UCC and UCG. There are at least 6 serine tRNAs from rat liver which have very similar properties and it is quite difficult to separate them from one another. The primary sequences of these tRNAs differ in the non base-paired parts only in the anticodon region and in all base-paired regions except the D loop.

G.Dirheimer (France) described the primary structure of $tRNA^{Asp}$ from brewer's yeast (fig. 1). Analysis indicates that this $tRNA^{Asp}$ is composed of 75 nucleotide residues including 8 unusual ones. It is characterized by pUp and $GCCA_{OH}$ terminal sequences.

According to T.Ukita (Japan) yeast $tRNA^{Glu}_{III}$ has GpCpC as the 3′-terminal sequence, pUpCpCpGp as the 5′-terminal sequence and TpψpCpGp as the common sequence, it contains 7 minor nucleosides including three ψs, one each of rT, m^5C, hU and sU, it does not contain *O′* nor *N*-methylated nucleosides. 2-Thio-5-uridine acetic acid methyl ester (S) was found in an oligonucleotide of CpUpSpUpCpApCpCpGp and it was proposed that the sequence SpUpCp is the anticodon of this tRNA. The specific GpApA codon recognition by this $tRNA^{Glu}_{III}$ was reasonably explained by the suggestion that S cannot pair with G but can pair with A.

S.Nishimura (Japan) reported on the isolation from *E. coli* and rat liver of about 20 highly purified tRNAs specific for various amino acids and on the isolation and characterisation of new minor constituents, uridine-5-oxyacetic acid (*A*) and a 2-thiouridine derivative (*B*) from *E. coli* tRNAs. *A* was located in the first position of the anticodon of *E. coli* $tRNA^{Val}_{I}$ and

* This report is based mainly on the abstracts presented by the participants of the Pre-symposium. Only the speakers are mentioned and not the other authors of the presentations.

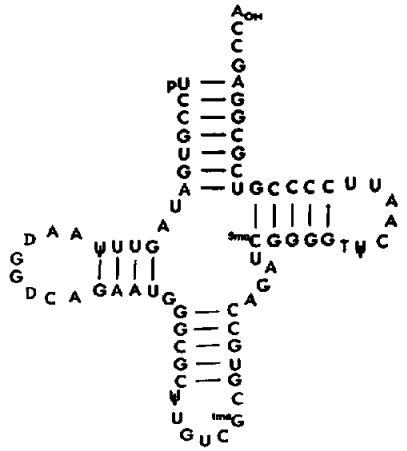

Fig. 1. Primary structure of $tRNA^{Asp}$ from brewer's yeast (G.Dirheimer).

$tRNA_I^{Ser}$. *B* was also located in the first position of *E. coli* $tRNA_{II}^{Glu}$ anticodon. A new minor nucleoside, 5-methyl-2-thiouridine was found in rat liver $tRNA_{III}^{Glu}$ and $tRNA_{II}^{Lys}$.

R.H.Hall (Canada) reported on the isolation and characterization of a hyper-modified nucleoside from the tRNA of serveral organisms, *N*-(nebularin-6-ylcarbamoyl)threonine which has been detected in a yeast $tRNA^{Ile}$ adjacent to the 3′ of the anticodon which possesses cytokinin activity like N^6-(Δ^2-isopentenyl) adenosine.

D.B.Dunn (England) reported on the modification of a method primarily introduced in 1963 to isolate minor components as nucleotides from small (5–20 mg) samples of tRNA.

D.Söll (USA) described the isolation from *Mycoplasma* sp. (Kid) of tRNAs lacking some specific modified nucleosides: ribothymidine (rT), 2′-*O*-methylnucleosides, isopentenyladenosine and thiouridine (tU). Such tRNAs were used to demonstrate that rT and tU are not required for charging by synthetases or for in vitro protein synthesis. These tRNAs were also used for the identification and partial purification of the enzyme which catalyzes the formation of isopentenyladenosine in tRNA. From *Mycoplasma* double stranded DNA 40 times enriched in regions coding for tRNA was isolated. Transcription of this DNA with *E. coli* RNA polymerase yielded 70 fold net RNA synthesis. With this RNA in vitro formation of pseudouridine and dihydrouridine catalyzed by *E. coli* extracts was observed.

The talk of A.D.Kelmers (USA) was devoted to reversed-phase chromatography (RPC) systems for the separation of tRNAs. Gramme quantities of highly purified arginine, glutamic acid, formyl-methionine, phenylalanine, and valine-tRNAs have been prepared. The separation of individual tRNAs by RPC is affected by a number of experimental variables, especially by Mg^{2+}. The tRNAs can be partially resolved into three groups by gel permeation chromatography on polyacrylamide P-100 columns. The elution sequence is the reverse of that obtained on RPC columns. It is assumed that the three-dimensional folding of the tRNA molecules may determine the chromatographic elution sequences.

F.Gonano (Italy) described chromatographic differences between $tRNA^{Phe}$ from Morris hepatoma and from normal liver. A $tRNA_X$ of unknown nature was also isolated from hepatoma but not from normal liver, it behaved abnormally on the Sephadex column and was unable bind with ribosomes.

2. Tertiary structure of tRNA

R.Bock (USA) devoted his introductory talk to the crystallization and structural studies on crystalline tRNA. Eleven species of tRNA have been crystallized in 1968–70 in England, America, Germany, Sweden, Japan and the USSR. The X-ray data collected to date give general molecular shapes and packing but is now usable only as two and three dimensional Patterson projections. The 3-d Patterson projections of tRNA with 1 or 2 molecules per asymmetric unit are not informative about the detailed folding of tRNA loops. Solution of the phasing problem will be necessary before unambiguous molecular detail can be derived from the X-ray data. The key crystallographic results were derived by analysis and comparison of the 3-d Patterson synthesis of X-ray diffraction data from *E. coli* $tRNA^{Arg}$, $tRNA^{Leu}$ and yeast $tRNA^{fMet}$.

A.Surovaya (USSR) analyzed the molecular morphology of tRNA on the basis of rotational relaxation time (ρ_h) measured from the polarization of fluorescence of Acridine Orange adsorbed on the tRNA molecules. For the $tRNA^{Val}$ and $tRNA^{Lys}$ ρ_h is 38 ± 2 nsec at 20°. The asymmetry of the tRNA molecule is not less than 3 even at a high degree of hydration.

N.G.Esipova (USSR) presented some preliminary data on the small-angle X-ray analysis of the mixed tRNAs from yeast in concentrated isotropic solutions.

F.Cramer (GFR) discussed tRNA tertiary structure. The radius of gyration (R_G) in low-angle X-ray scattering has been measured at various temperatures for $tRNA^{Phe}_{yeast}$. The R_G up to 30° is 24 Å suggesting a highly compact molecule. At 70° the R_G is 55 Å corresponding to a random coil. Two crystal forms of $tRNA^{Phe}$ (yeast) are obtained by addition of various organic solvents, one of the space group R32, the other of the space group $C222_1$. To obtain isomorphic substitution a $tRNA^{Phe}$ (yeast) minus one or two terminal nucleotides was prepared by the action of snake vernom phosphodiesterase, 5-iodo-CTP and adenosine-5′-*O*-(1-thiotriphosphate) were used to obtain respectively I and S substituted derivatives of the tRNA.

A.D.Mirzabekov (USSR) said that the self-assembly of $tRNA^{Val}_I$ (yeast) quarters is mainly due to formation of complementary Watson-Crick base-pairs. The mode of association of $tRNA^{Val}_I$ quarters conforms to the tRNA " clover leaf" model only. The conformation of dissected and native $tRNA^{Val}_I$ are similar inasmuch as the circular dichroism (CD) spectrum of the mixture of 3′ and 5′ halves and that of unsplit tRNA are identical. K_m and V_{max} of the enzymatic acylation of the dissected and native $tRNA^{Val}_I$ molecules are rougly the same.

L.Kisselev (USSR) described a selective modification of the $tRNA^{Val}_I$ (yeast) by $^{14}CH_3ONH_2$ under conditions when the 3-d structure of the tRNA was stable. It was found that C_{75} and C_{76} in the 3′-end fragment, C_{37} and C_{39} in the anticodon loop and C_{17} in the D-loop were modified, C's in the S- and TψC-loops were completely inaccessible. C_{17} was found to be non-essential for acceptor capacity; on the contrary, C_{37} and C_{39} in the anticodon loop are important for the acceptor activity of this $tRNA^{Val}$.

M.A.Grachev (USSR) described the chemical modification of $tRNA^{Val}_I$ (yeast) with CME-carbodiimide. Two fractions of CME-carbodiimide modified $tRNA^{Val}_I$ were obtained; the first contained up to 20 CME-carbodiimide residues per mole and exhibited no acceptor activity. The second contained 6 residues per mole and had 80% acceptor activity.

H.Zachau (GFR) presented evidence that m′A is protected in $tRNA^{Phe}$ (yeast) against $NaBH_4$-reduction while it is easily reduced when the CCA-half of the tRNA is treated with $NaBH_4$. The pG-terminus seems to be buried while dissected parts of the anticodon region are easily phosphorylated with polynucleotide kinase. Indications of the presence of two conformations of tRNA with respect to the ease of phosporylation of the terminal nucleotide were presented.

N.Sueoka (USA) discussed the difference between native and denatured states of the $tRNA^{Trp}$ (*E. coli*). According to preliminary analysis the main difference is associated with the position of the D-region in the three-dimensional structure of the $tRNA^{Trp}$.

W.Guschlbauer (France) discussed the difference between acylated and non-acylated tRNA species revealed by CD in a strong magnetic filed.

M.Grunberg-Manago (France) reported that the binding of some oligonucleotides to tRNA during the aminoacylation was changed. The results suggest that a portion of the molecule, containing at least one G–G pair is exposed during the charging process.

G.Matsuka (USSR) had found that a drop in the acceptor activity for tyrosine, glycine, leucine and methionine takes place in tRNA isolated from animal tissues during starvation. The acceptor activity however can be restored by a short heating of tRNA in the presence of Mg^{2+}.

3. Interaction of tRNAs with aminoacyl tRNA ligases.

In an introductory speech A.Bayev (USSR) discussed two models of the tRNA recognition site: a compact one (the site is made by a nucleotide cluster) and a dispersed one (the component nucleotides are distributed between some remote parts of the molecule). He said that published data produce the impression that the localization and structure of the recognition sites in all specific tRNAs are different. The anticodon as a functional entity is not a part of the recognition site, however some exceptions are possible, and $tRNA^{Val}_I$ (yeast) seems to be one of them. All methods of elucidation of the recognition site are based on the same principle. One modifies, destroys or eliminates single monomers or their parts in the tRNA polynucleotides chain and detects how these events affect the tRNA acceptor function. The method of "dissected molecules" is now widely used for tRNA investigation.

A.D.Mirzabekov (USSR) reported on the $tRNA^{Val}_I$ studied by the "dissected molecule" method. The exci-

sion of the dinucleotide pG_IGp of the CCA-stem, the dinucleotide $A_{36}Cp$ of the anticodon or sequences F_{10-19} of the D arm and/or F_{58-63} of the T arm inactivates $tRNA_I^{Val}$. Moreover the inactive incomplete molecules lacking pG_IGp, $A_{36}Cp$ or F_{10-19} as well as individual 3′- and 5′-halves do not inhibit markedly the enzymatic aminoacylation of native $tRNA_I^{Val}$. It is assumed that pG_IGp and $A_{36}Cp$ are at least parts of two different recognition sites of $tRNA_I^{Val}$.

W.Chambers (USA) said that comparison of structural changes in active and inactive molecules after irradiation with 254 nm light demonstrated an inactivation target in the acceptor stems of yeast $tRNA_{Iab}^{Ala}$. This suggestion has been verified with "halves" and "quarters" obtained by partial hydrolysis of yeast $tRNA_{II}^{Ala}$ with RNase T_I. Only combinations capable of forming the acceptor stem had acceptor activity specific for alanine only.

As reported by H.Zachau (GFR) the removal of the anticodon and D-loop from $tRNA^{Phe}$ (yeast) did not abolish the acceptor activity. The complex formed by the ¾ of one molecule and the half of the other is also active.

According to S.Nishimura (Japan) the controlled digestion of *E. coli* $tRNA^{fMet}$ by RNase T_I results in a cleavage at the D-loop. Recombination of the two halves brings about full acceptor activity. The pC-halves were then digested from the 3′-terminus by snake venom phosphodiesterase. The results showed that 40% of the initial methionine acceptor activity were still present after the elimination of the looped-out sequence, but the further removal of one nucleotide from the stem region decreased the activity to 10% and of two or more completely abolished it.

According to A.Mehler (USA) the arginine activating enzyme from *E. coli* was able to recognize specifically some elements of two regions of its tRNA. The recognition of relatively small fragments suggests that it is a sequence of bases that is recognized, not a 3-d structure which may well be important for the catalytic function of tRNA. Elements of different portions of the tRNA molecule, near the 3′-terminus and possibly from the 5′-terminus are both recognized by the enzyme, they are bound independently and, it is suggested, they induce conformational changes in the enzyme, since they bring about a significant increase in stability and changes in the binding of arginine.

M.Grunberg-Manago (France) presented some preliminary data according to which the addition of a mixture of 3′- and 5′-halves to intact $tRNA^{Phe}$ increases its acceptor activity. She suggested that in some cases more than one site on the enzyme molecule is able to bind tRNA.

U.Lagerkvist (Sweden) reported that valyl-tRNA synthetase from yeast forms a stable and specific enzyme-substrate complex with $tRNA^{Val}$ from the same source. The loss of an average of 7 nucleotide residues at the 3′-hydroxyl end of $tRNA^{Val}$ by digestion with snake venom phosphodiesterase did not affect the ability of the $tRNA^{Val}$ to form a stable complex with the enzyme.

Study of the complex between $tRNA^{Phe}$ and phenylalanyl tRNA synthetase (PRS) from yeast was presented by J.-P.Ebel (France). Homogeneous PRS with an average M.W. of 220,000 and subunit structure A_2B_2 was isolated from baker's yeast. A stable and specific complex between the $tRNA^{Phe}$ and the PRS has been isolated by sucrose gradient centrifugation. One molecule of $tRNA^{Phe}$ is combined with the tetrameric molecule of the enzyme, the interaction is strongly dependent on the pH. The tRNA without the pCpCpA end is able to form the complex with PRS.

A.V.Parin (USSR) reported on the selective and reversible inhibition of the acylating ability of the tryptophanyl tRNA synthetase (TRS) from rat liver by β-mercaptoethanol. A stable complex between ^{14}C-ATP and highly purified TRS from beef pancreas was isolated. Electron microscopic analysis of "polymeric" forms of TRS formed in the presence of tryptophan has revealed single rod-like particles with the minimum diameter of 125±10 Å as well as their paracrystalline 3-d aggregates of length up to 1 μm. A 40–45 Å periodicity along the rod was noticed. The reflections corresponding to 40 Å and approximately 490 Å were revealed in the optical diffraction patterns.

J.-P.Waller (France) reported that under certain conditions treatment of *E. coli* methionyl-tRNA synthetase with trypsin resulted in the appearance of a product of M.W. 60,000. The product is active both in the ATP–PiPi exchange and tRNA charge reactions. The optimum of Mg^{2+} concentration for the trypsin-treated enzyme differs from that of the untreated one in the tRNA charge reaction. He suggested that the product represents a dimer of two incomplete subunits lacking fragments of M.W. 15,000.

H.M.Duranton and F.Chapeville (France) described an enzymatic binding of valine to the 3′-end of RNA from Turnip Yellow Mosaic Virus (TYMV) in the presence of *E. coli* extract, ATP, and Mg^{2+}. Not more than one mole of valine can be bound per mole of TYMV-RNA. Valine is bound to the 2′-3′ position of the terminal nucleoside adenosine. The enzyme involved in the charge reaction is Val-tRNA synthetase.

Kinetic analysis performed by D.Knorre (USSR) has shown that some results reported in literature on a direct effect of tRNA on the activation stage cannot be in fact interpreted unequivocally. It has been shown for valyl and glycyl tRNA synthetases that hydroxamate formation proceeds in the presence of tRNA via an intermediate aminoacyl-tRNA formation. In the case of ATP–pyrophosphate exchange the reaction rate can well be limited by the dissociation stage and the effect of tRNA can be due to its allosteric influence on the dissociation, for example, of pyrophosphate rather than by an effect on the activation rate.

B.P.Gottikh (USSR) described a new method of chemical aminoacylation of tRNA. The trifluoroacetates of amino acids reacted with carbonyldiimidazole forming appropriate imidazolides of amino acid trifluoroacetates. These compounds were reacted with tRNA in an aqueous imidazole buffer, pH 7, for 5–7 hr at 4°. According to chemical and biochemical tests the aminoacylation of the acceptor end was 40–60%.

E.I.Budowsky (USSR) noticed that the final level of ^{14}C-valine incorporation in tRNA on a preparative scale was not reproducible and was only 30–60% of that in the analytical test. An improvement of reproducibility and an increase of valyl-tRNA yield could be achieved by addition of glass beads.

4. Interaction of tRNA with ribosomes

H. Matthaei (GFR) in an introduction suggested the ribosome to have three sites A, B, C for coded binding of aa-tRNA in addition to the donor site D for the following reasons: (1) G factor-free *E. coli* initiation complexes formed with intact phage fr RNA, purified 30 S and 50 S ribosomal subunits, initiation factors F1, F2 and F3, elongation factor T, and GTP, bind exclusively fMet-, Ala,- Ser-, and AsN-tRNA, These correspond to the amino-terminal sequence of the coat protein of phage fr. Alkaline hydrolysis of full initiation complexes and subsequent electrophoresis detected AsN, Ser, Ala, fMet and fMet-Ala but no tri- or tetrapeptides. It is concluded that Ser- and AsN-tRNA are bound to the additional sites B and C, respectively. (2) More than one molecule of Phe-tRNA per average poly U-coded *E. coli* ribosome can be bound under optimal conditions. (3) aa-tRNA saturation curves of homopolynucleotide-coded bacterial and mammalian ribosomes display three rather straight slopes sufficient for an extrapolation which indicates three approximately equal apparent binding capacities, corresponding to the three decoding sites.

B.Clark (England) discussed recent in vitro experimental evidence indicating that Met-tRNA can serve as an initiator in eukaryotic cytoplasm. In both types of cells Met-$tRNA_m$, but not Met-$tRNA_f$ serves as a source of methionine for internal residues during polypeptide chain biosynthesis. The formyl group is necessary in bacteria for the initiation factor–GTP specification of the initiator tRNA during the formation of the first peptide bond while it is not needed in mammalian cells.

J.Jonak (Czechoslovakia) devoted his talk to isolation of the 50 S subunits – oligolysyl-tRNA (I) and 30 S subunits – oligolysyl-tRNA-poly A (II) by sucrose gradient centrifugation. The transfer to puromycin (Pur) from complex I takes place only after the addition of poly A and 30 S subunits. The transfer to Pur from complex II can take place only after addition of 50 S subunits. G-factor and GTP has no effect on the transfer. It is suggested that during a certain intermediary phase the binding site for peptidyl-tRNA is composed of a part of the decoding site and of a part of the donor site and thus exists in a certain hybrid state.

I.Rychlik (Czechoslovakia) described the capacity of the acceptor site of the ribosomal peptidyl transferase to discriminate between peptidyl and aminoacyl-tRNA.

P.Szafranski (Poland) reported that properly prepared *E. coli* ribosomes can exhibit great ambiguity in poly U translation. This effect dissappears during the storage of ribosomes in 1 M NH_4Cl buffer at 2° most likely due to changes in ribosomal structure caused by 1 M NH_4Cl treatment.