Sequence of Human Glycine Transfer Ribonucleic Acid (Anticodon CCC). Determination by a Newly Developed Thin-Layer Readout Sequencing Technique and Comparison with Other Glycine Transfer Ribonucleic Acids†

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ABSTRACT: The sequence of $tRNA^{Gly}_{CCC}$ from human placenta was determined by a newly developed thin-layer readout sequencing technique [Gupta, R. C., & Randerath, K. (1979) *Nucleic Acids Res. 6*, 3443–3458]. This technique enabled us to display and identify about 93% of the major and the modified nucleotides in the RNA chain. The remaining positions were determined by methods entailing fingerprinting of 3′-terminally ^{3}H-labeled oligonucleotide derivatives and base-specific enzymatic cleavages. The complete nucleotide sequence of human $tRNA^{Gly}_{CCC}$ is pG-C-G-C(m)-C-(m^2)G-C-U-G-G-U-G-ψ-A-G-U-G-G-D-A-U-C-A-U-G-C-A-A-G-A-U(m)-U-C-C-C-A-U-ψm-C-U-U-G-C-G-A-C-m^5C-m^5C-G-G-G-T-ψ-C-G-(m^1)A-U-U-C-C-C-G-G-G-C-G-G-C-C-G-C-A-C-C-A_{OH}. The sequence of human $tRNA^{Gly}_{CCC}$ was compared with the sequences of human $tRNA^{Gly}_{GCC}$ [Gupta, R. C., Roe, B. A., & Randerath, K. (1979) *Nucleic Acids Res. 7*, 959–970] and four other eucaryotic glycine tRNAs. Human $tRNA^{Gly}_{CCC}$ appears to be unusually rich in ribose-methylated nucleosides. Another unusual feature of this tRNA, which is shared with human $tRNA^{Gly}_{GCC}$ but not with other eucaryotic glycine tRNAs, is the presence of two methylated nucleosides in its amino acid acceptor stem. The T-arm sequence of all glycine tRNAs from higher eucaryotes has been completely conserved, indicating an important as yet undetermined function of this particular region of these RNAs. In all animal glycine tRNAs sequenced to date, the base between the D stem and the anticodon stem appears to be capable of forming a secondary base pair with the first base of the variable arm, implying the presence of a sixth Watson–Crick base pair in the anticodon stem of these tRNAs.

About 110 tRNAs have been sequenced to date from various organisms, but only ~10% of these are from mammals. This has been due in part to the lack of sufficiently sensitive methods for sequence analysis of RNA, as mammalian tissues contain only small amounts of individual tRNAs (0.1–4 mg/kg of tissue). As a consequence, minor isoaccepting tRNAs from mammalian sources have not been sequenced thus far. For a compilation of sequenced tRNAs, see Gauss et al. (1979). The sequence analysis of all isoaccepting tRNAs, including the minor ones, from one particular organism represents an important step toward understanding the various interactions of tRNA with other macromolecules.

Recently, several highly sensitive radioactive derivative ("postlabeling") methods (Gupta & Randerath, 1977a,b; Donis-Keller et al., 1977; Simoncsits et al., 1977; Lockard et al., 1978; Stanley & Vassilenko, 1978; Peattie, 1979; Gupta & Randerath, 1979; Randerath et al., 1980) for sequence analysis of RNA have been developed which are applicable to the small amounts of mammalian tRNAs available. In particular, we have recently described a procedure for sequence analysis of RNA enabling one to identify the modified nucleotides and their positions in the RNA chain directly as ^{32}P-labeled derivatives (Gupta & Randerath, 1979). In this paper, we report on the application of this method to the sequence analysis of a human glycine tRNA, $tRNA^{Gly}_{CCC}$ (anticodon CCC). This tRNA was of particular interest to us for various reasons. (1) Since another human glycine tRNA ($tRNA^{Gly}_{GCC}$) had been sequenced previously in our laboratory (Gupta et al., 1979), a comparison of the structures of the two isoacceptors was of interest. (2) The structures of glycine tRNAs from several eucaryotic sources (except mammals) are known [see Gauss et al. (1979)]; thus, it was of interest to compare the human glycine tRNAs with other known glycine tRNAs. (3) Eucaryotic glycine tRNAs are especially rich in 5-methylcytidine (Marcu et al., 1977; Garel & Keith, 1977; Zúñiga & Steitz, 1977; Kawakami et al., 1978; Gupta et al., 1979; Roe et al., 1976), a methylated nucleoside whose formation is strongly inhibited by the antineoplastic drug 5-azacytidine (Lu et al., 1976; Lu & Randerath, 1979). Glycine tRNAs that are deficient in 5-methylcytidine may be useful in investigations on the function(s) of this methylated nucleoside (Harris & Randerath, 1978).

Materials and Methods

Materials. The sources of most materials used have been indicated previously (Roe, 1975; Anandaraj & Roe, 1975; Gupta & Randerath, 1979; Gupta et al., 1979). The ribose-methylated nucleoside 5′-monophosphates pAm, pCm, pUm and pGm were from P-L Biochemicals.

Isolation of $tRNA^{Gly}_{CCC}$. Partially purified $tRNA^{Gly}_{CCC}$ was isolated from human placenta by phenol extraction at pH 4.5 in 0.14 M sodium acetate buffer, followed by adsorption on DEAE-cellulose (Roe, 1975) and several column chromatographies (Anandaraj & Roe, 1975). For subsequent further purification, column chromatography on RPC-5 was used. $tRNA^{Gly}$ (7.4 mg) obtained by Aminex A-28 chromatography (Anandaraj & Roe, 1975) was dissolved in 4 mL of buffer A (0.01 M Tris-HCl, pH 7.6, 0.01 M $MgCl_2$, and 0.001 M $Na_2S_2O_3$) containing 0.3 M NaCl, applied to an RPC-5 column previously equilibrated with buffer A containing 0.3 M NaCl, and eluted with an 800-mL linear gradient of 0.3–1.2

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M NaCl in buffer A, at a flow rate of 4 mL/5 min. After the A_{260} for alternate 4-mL fractions had been determined, aliquots (10 μL) of every fifth tube were used in a standard aminoacylation reaction to determine the elution profile [see Roe (1975) for the assay conditions]. Peak 1 (see Results) was used in the present study. The tRNA was further purified by electrophoresis at 1000 V (constant), ~12 mA, on a 20% polyacrylamide, 7 M urea, 50 mM Tris, 50 mM boric acid, and 1 mM EDTA (pH 8.3) slab gel (40 × 20 × 0.2 cm) and extracted from the gel (Chia et al., 1973).

Thin-Layer Readout Technique for Sequence Analysis. This was done as described (Gupta & Randerath, 1979), with the following modifications. (1) Five micrograms of the tRNA was heated in 10 μL of water in an open tube in a boiling water bath for 45 s. (2) The gels were 0.035 cm rather than 0.06 cm thick, and 2–3-μL aliquots were applied to each well. (3) The labeled hydrolysate (Gupta & Randerath, 1979) contained 1–2 μg/μL each of the tracking dyes bromophenol blue (BP) and xylene cyanol FF (XC). (4) Shorter fragments (chain length up to 40 nucleotides) were resolved on a 20% polyacrylamide slab gel (50 × 30 × 0.035 cm); three 2-μL aliquots of the labeled digest (Gupta & Randerath, 1979) were electrophoresed for 4.5, 9, and 14 h at 2500 V (constant), ~15 mA. (5) PEI–cellulose "prints" (Gupta & Randerath, 1979) of ladders obtained from the 20% gels were treated with RNase T_2 as follows. One to two microliters of enzyme solution (0.2 unit/μL in water) was applied to a third (Gupta & Randerath, 1979) of each band of the ladder. After this had been repeated once, the treated strip was covered with Teflon tape and incubated as described (Gupta & Randerath, 1979).

The enzymatically released ^{32}P-labeled 5′-terminal nucleotides were identified by contact transfer to a PEI–cellulose acceptor sheet and chromatography in 0.55 M ammonium sulfate and 1.75 M ammonium formate, pH 3.5, followed by autoradiography (Gupta & Randerath, 1979).

Modified nucleotides were identified on the basis of their known chromatographic behavior (Gupta & Randerath, 1979), except for ribose-methylated residues. Since the chromatographic behavior of the latter residues, which are obtained as dinucleoside triphosphates (pN^{1}m-N^{2}p) by RNase T_2 treatment, was not known, these compounds were degraded to nucleoside 5′-monophosphates and identified by subsequent chromatography as follows. The dinucleoside triphosphate spot(s) was cut from a chromatogram that had been developed in the ammonium formate system (Gupta & Randerath, 1979) and treated in situ with nuclease P_1 (0.1 μg/μL) containing 50 mM Tris-HCl, pH 7.3 (Gupta & Randerath, 1979). (Spots from ammonium sulfate chromatograms could not be used because of interference by the salt with the subsequent chromatography.) Several (up to 10) cutouts were then soaked in methanol (50 mL) for 5 min, dried, and treated in situ with a solution of snake venom phosphodiesterase (0.1 μg/μL) in 50 mM Tris-HCl, pH 8.7, and 5 mM $MgCl_2$ at 38 °C for 2 h or overnight. The cutouts were again soaked in methanol and dried, and nonradioactive markers of pAm, pCm, pUm, and pGm (10–15 nmol each) were applied to the center of the cutout, which had been prewetted by application of 2 μL of methanol to counteract the hydrophobicity of the enzyme-treated layer. The nucleotides were contact-transferred (Gupta & Randerath, 1979) to a PEI–cellulose thin layer (20 cm long) and separated in acetic acid–formic acid as described (Gupta et al., 1976b), except that the chromatogram was predeveloped with methanol to the origin instead of water to avoid disturbance of the separations due to the hydrophobic nature of the donor spot. Nonradioactive compounds were located under UV light, and radioactive compounds were located by autoradiography.

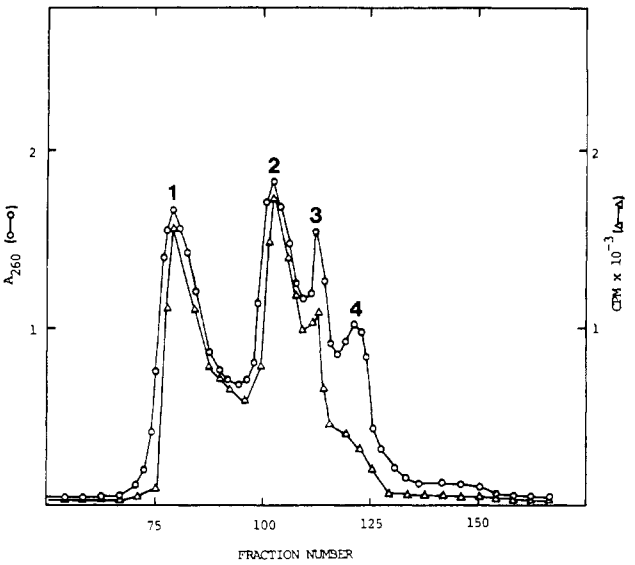

FIGURE 1: RPC-5 chromatography of 7.4 mg of partially purified $tRNA^{Gly}$ of human placenta (Anandaraj & Roe, 1975). After the A_{260} of alternate fractions had been determined, aliquots of every fifth fraction were assayed in a standard aminoacylation reaction (Roe, 1975). Peak 1 was used in the present work.

Gel Readout Technique for Sequence Analysis. The tRNA (15 μg) was 5′-^{32}P-labeled as described by Silberklang et al. (1977), except that the RNA concentration was 1.5 μg/μL. The labeled RNA was purified on a 15% polyacrylamide denaturing gel and partially digested with alkali, RNases T_1 and U_2 (Donis-Keller et al., 1977) and RNases A and Phy_1 (Gupta et al., 1979; Randerath et al., 1980). The products were resolved on sequencing gels (Donis-Keller et al., 1977; Gupta et al., 1979), and the cleavage patterns were displayed by autoradiography.

Analysis of the Fragments in Complete RNase T_1 and RNase A Digests. Complete digestions of the tRNA (4–12 μg) with RNase T_1 or A plus alkaline phosphatase and subsequent ^{3}H-labeling of the 3′ ends of the oligonucleotides were performed as described (Gupta et al., 1979; Randerath et al., 1980). The products were fingerprinted on PEI–cellulose thin layers, as indicated in Figure 4, and extracted from the layer (Gupta et al., 1976a, 1979). For determination of the molar ratios of individual oligonucleotides, the radioactive spots were cut from separate fingerprints derived from about 0.2 μg of tRNA and counted directly in 5 mL of scintillator (3 g of Omnifluor and 250 mL of Triton X-100 dissolved in 1 L of xylene). For determination of the nucleotide sequence each compound was subjected to base composition analysis (Gupta et al., 1976a), 3′-terminal (Randerath et al., 1974) and 5′-terminal (Gupta et al., 1976a) analysis, and sequence analysis (Gupta & Randerath, 1977a,b).

Results

Chromatography on RPC-5 resolved the $tRNA^{Gly}$ isoacceptors into four species (Figure 1). Peak 1 was used in the present study. Peak 2 was shown to be $tRNA^{Gly}_{GCC}$ (Gupta et al., 1979). The minor species have not as yet been analyzed. The $tRNA^{Gly}_{CCC}$ fraction obtained accepted 1.2 nmol of glycine per A_{260} unit. Further purification on a denaturing polyacrylamide gel showed a major band in addition to several minor faster moving bands, suggesting that the tRNA obtained from the RPC-5 column was partially nicked. In the present work, only the major band was analyzed.

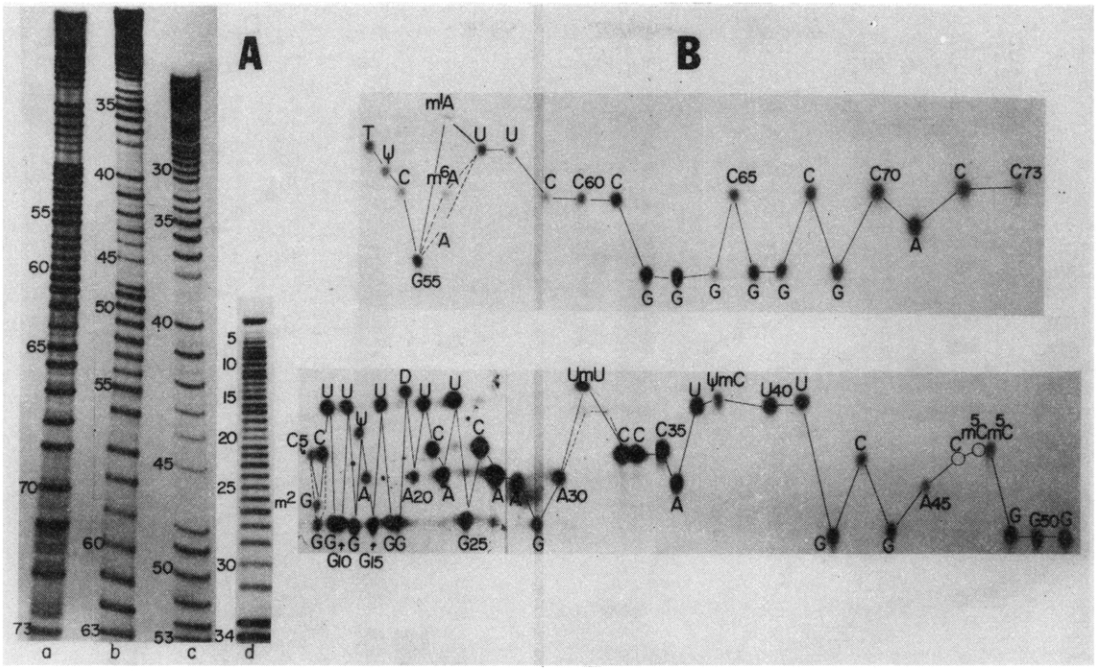

FIGURE 2: Thin-layer readout (Gupta & Randerath, 1979) of positions 5–73 of human $tRNA^{Gly}_{CCC}$. (A) Polyacrylamide gel patterns obtained by brief heating of the RNA in water, 5′-^{32}P-labeling of the fragments, gel electrophoresis, and autoradiography; ladders a–c were obtained by electrophoresis for 4.5, 9, and 14 h, respectively, on a 20% polyacrylamide sequencing gel; ladder d was obtained by electrophoresis for 12 h on a 12% polyacrylamide gel. (B) Readout of the 5′ termini obtained after transfer of fragments from the gels to PEI–cellulose layers. After in situ RNase T_2 treatment of the fragments, the 5′-terminal 5′-^{32}P-labeled nucleoside 3′,5′-diphosphates were identified by PEI–cellulose thin-layer chromatography in 0.55 M ammonium sulfate and autoradiography. C46 and m^5C47, represented by circles, were visualized after prolonged exposure (not shown) as the corresponding fragments were obtained in low yields. Broken lines indicate that two termini were found for some positions (see text). Fragment 4 (ladder d) was found to give two weak spots, C and Cm-C, after prolonged exposure (not shown).

Figure 2 exemplifies the readout of major and modified nucleotides between positions 5 and 73 of the tRNA. Chromatographic analysis of the 5′-terminal nucleotides of the labeled fragments (Figure 2A) in ammonium sulfate (Figure 2B) and ammonium formate, pH 3.5 (spot pattern not shown), gave identical results. All fragments showed distinct single termini, except for fragments 6, 31, and 56, each of which gave two termini (Figure 2B) due to partial methylation at these positions as indicated by broken lines in Figure 2B. In addition, m^6A was obtained at position 56 due to partial conversion of m^1A to m^6A under the experimental conditions.

The jumps between fragments 31 and 33 and fragments 38 and 40 are due to the presence of ribose-methylated residues at positions 31 and 38, respectively (Gupta & Randerath, 1979). The 5′-terminal nucleotides of fragments 31 and 38 were identified as pUm-Up and pψm-Cp, respectively, as follows. Digestion with nuclease P_1 and snake venom phosphodiesterase liberated ^{32}P-labeled pUm and pψm, respectively, as identified by thin-layer chromatography (Gupta et al., 1976b). The 3′-terminal nucleotides were identified by sequence analysis of the RNase T_1 and RNase A fragments (see below).

C46 and m^5C47 were detectable only after prolonged exposure (not shown) because chain lengths 46 and 47 gave rather weak bands on the original gel (Figure 2A).

Figure 3 exemplifies autoradiograms of two sequencing gels which enabled us to read 54 nucleotide positions of the RNA chain; this included six modified nucleotides (Cm4, m^2G6, ψ13, D19, ψ53, and m^1A56) which were read as the corresponding major nucleotides. The data obtained were in complete agreement with those from the thin-layer readout technique (Figure 2). The remaining positions could not be identified because of the absence of enzymatic cleavage products particularly in RNase A and RNase Phy_1 digests (Figure 3B) and band compression (Figure 3B, OH^- lane).

Parts A and B of Figure 4 depict fractionations of radioactive oligonucleotide-3′ dialcohols obtained by complete RNase T_1 and RNase A digestions of $tRNA^{Gly}_{CCC}$, respectively, and subsequent 3′-terminal ^{3}H-labeling of the oligonucleotides. The products in either digest were well resolved except A-C′/A-U′, G-C′/m^2G-C′, and C-U-G′/ψ-A-G′. These compounds were further resolved by contact transfer and rechromatography in 0.2 N acetic acid (dinucleotides) and 0.15 M LiCl (trinucleotides). The mobilities decreased in the orders A-C′ > m^2G-C′ > G-C′ > A-U′ and C-U-G′ > ψ-A-G′, respectively.

The sequences of smaller oligonucleotides (chain lengths ≤ 3) were determined by base composition (Gupta et al., 1976a) and terminal analysis (Randerath et al., 1974; Gupta et al., 1976a). The sequences of most of the larger fragments were established by methods based on base-specific enzymic cleavage (Gupta & Randerath, 1977a,b). The positions of modified residues in the nucleotide chain were determined by base composition analysis in conjunction with 3′- and 5′-terminal analysis. ψm-C′, G-Cm-C′, C(m)-C-(m^2)G′, A-C-m^5C-m^5C-G′, A-A-G-A-Um-U′, and A-Um-U-C-C-C-A-U-ψm-C-U-U-G′ required additional information to place the modified nucleotides: base analysis (Gupta et al., 1976a) of ψm-C′, G-Cm-C′, and A-A-G-A-Um-U′ showed the absence of internal pyrimidines and no pyrimidine nucleoside trialcohol was obtained from the 3′ terminus. Because RNase T_2 did not release the 3′ terminus as a nucleoside trialcohol from any of the three compounds, the nucleotide adjacent to the 3′ terminus was ribose-methylated. The 3′-terminal trialcohols were, however, released by nuclease P_1 phosphatase digestion (Gupta & Randerath, 1977a) and identified by thin-layer chromatography (Randerath et al., 1974). In an attempt to identify the ribose-methylated nucleotides by a previously described procedure (Gupta et al., 1976c) involving 5′-^{32}P-labeling of the alkali-resistant dinucleotides, only ψm-C′ was

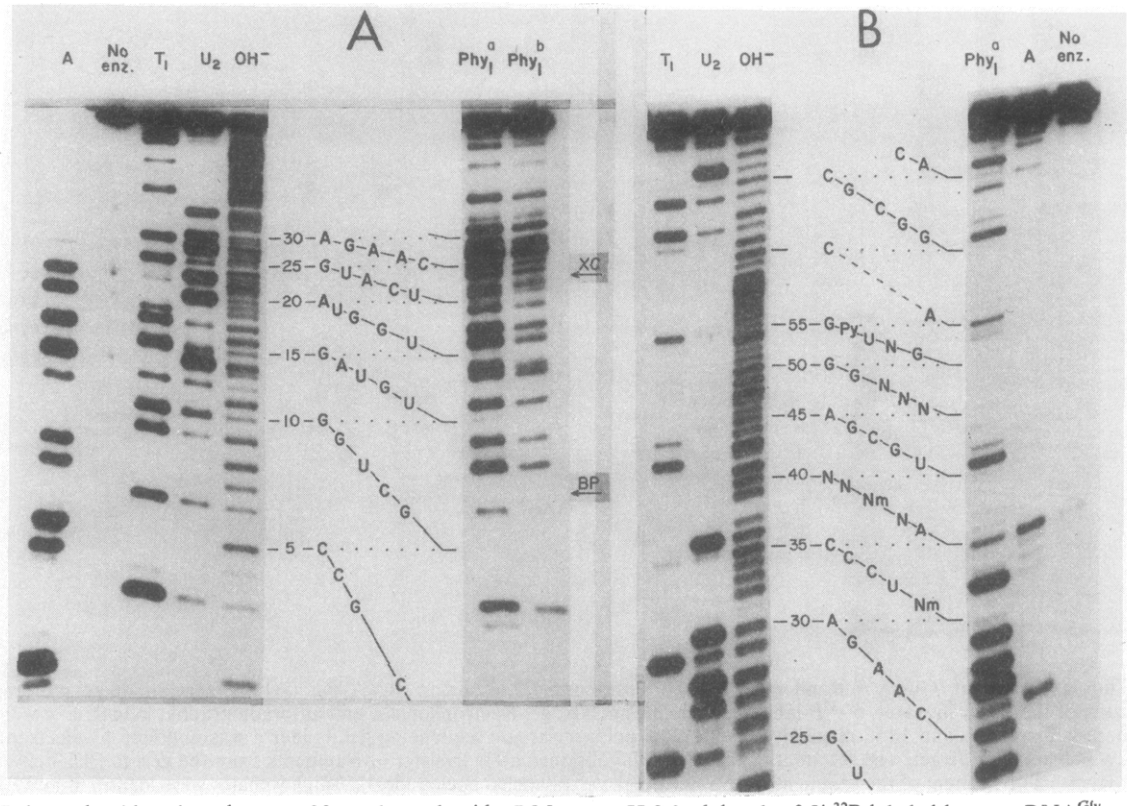

FIGURE 3: Polyacrylamide gel readouts on 20% polyacrylamide–7 M urea, pH 8.3, slab gels of 5′-^{32}P-labeled human $tRNA^{Gly}_{CCC}$. $Phy_1{}^a$ and $Phy_1{}^b$ are partial RNase Phy_1 digests at 38 and 50 °C, respectively, in the presence of 7 M urea. N denotes a position which was not enzymatically cleaved.

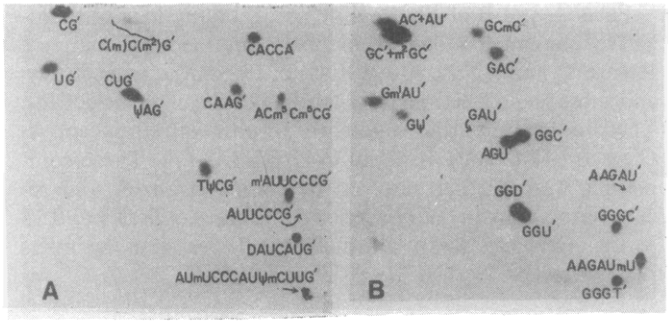

FIGURE 4: PEI–cellulose map of 3′ terminally ^{3}H-labeled oligonucleotide dialcohols obtained by digestion with RNase T_1 (A) and RNase A (B) of human $tRNA^{Gly}_{CCC}$, followed by chemical ^{3}H-labeling. First dimension (LiCl gradient) was from right to left; second dimension (ammonium formate, pH 2.6, gradient) was from bottom to top. Detection was by fluorography (Randerath, 1970). CG′, UG′, etc. are oligonucleotide-3′ dialcohol derivatives of CpG, UpG, etc. ψmC′ had migrated onto the wick attached to the top of the chromatogram shown in (B). The origins of the maps are not shown because this part of the chromatogram had been cut off prior to fractionation of the oligonucleotide derivatives (see the text).

found to become labeled, while no labeling of Cm-C′ and Um-U′ was obtained under the described conditions. Because the dinucleotide dialcohols obtained by RNase T_2 digestion of G-Cm-C′ and A-A-G-A-Um-U′ migrated slightly faster than markers of C-C′ and U-U′, respectively, on PEI–cellulose thin layers in 0.2 N acetic acid, the ribose-methylated residues appeared to be Cm and Um. Direct evidence for this was obtained by thin-layer readout sequencing of the tRNA (Figure 2).

The spots labeled C(m)-C-(m^2)G′ (Figure 4A) were found to be composed of mixtures of Cm-C-m^2G′ and Cm-C-G′ (faster spot) and C-C-m^2G′ and C-C-G′ (slower spot) on the basis of the following evidence. Base composition analysis (Gupta et al., 1976a) and sequence analysis (Gupta & Randerath, 1977a) showed that the faster and the slower compounds were Nm-C-(m^2)G′ and C-C-(m^2)G′, respectively; the ribose-methylated residue in the former was identified as Cm because G-Cm-C′ was obtained by RNase A digestion and ^{3}H-labeling (Figure 4B). Cm-C-(m^2)G′ contained 46% m^2G, as determined by base composition analysis, while C-C-(m^2)G′ contained 26% m^2G. The content of Cm was 75% as deduced from the molar ratio of the G-Cm-C′ spot (Figure 4B). On this basis, the total content of m^2G in C(m)-C-(m^2)G was calculated to be 41%.

In A-C-m^5C-m^5C-G′, the two m^5C residues were placed as shown because G-A-C′ but not G-A-m^5C′ was present in the RNase A digest (Figure 4B).

In A-Um-U-C-C-C-A-U-ψm-C-U-U-G′, the ribose-methylated nucleotides at positions 2 and 9 were identified as Um and ψm, respectively, because A-A-G-A-Um-U′ and ψm-C′ were obtained by RNase A digestion of the RNA and ^{3}H-labeling (Figure 4B).

The minor products A-U-U-C-C-C-G′ (Figure 4A), G-A-U′ (Figure 4B), and A-A-G-A-U′ (Figure 4B) all were present at a molar ratio of about 0.06, indicating slight undermethylation of m^1A and Um.

The fragments G-Cm-C (Figure 4B) and C(m)-C-(m^2)G (Figure 4A) were connected to extend the sequence from position 5 (Figure 2) to position 3 to deduce the complete sequence of the tRNA. The 3′ terminus of the RNA was obtained by 3′-terminal analysis of C-A-C-C-A′ (Figure 4A). Positions 1 and 2 were determined as G and C, respectively, by 5′-^{32}P-labeling of the tRNA, followed by base-specific enzymatic digestions and thin-layer chromatography of the products as previously described (Gupta & Randerath, 1977a,b). On the basis of these results, an unambiguous sequence of human $tRNA^{Gly}_{CCC}$ was deduced, as shown in Figure 5.

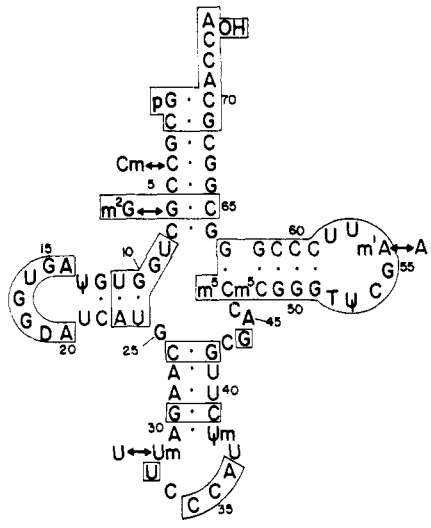

FIGURE 5: Primary structure of human $tRNA^{Gly}_{CCC}$ in cloverleaf form. The enclosed regions indicate nucleotides that are common to both human $tRNA^{Gly}_{CCC}$ and $tRNA^{Gly}_{GCC}$ (Gupta et al., 1979).

Discussion

Since human $tRNA^{Gly}_{CCC}$ is the first tRNA whose sequence has been determined by the newly developed thin-layer readout technique (Gupta & Randerath, 1979), we shall briefly comment on this technique and then compare the structural features of human $tRNA^{Gly}_{CCC}$ with the structures of other eucaryotic glycine tRNAs.

The thin-layer readout technique for sequencing of RNA was developed for two main reasons. (1) Recently developed readout sequencing techniques based on base-specific cleavages (Gupta & Randerath, 1977a,b; Donis-Keller et al., 1977; Simoncsits et al., 1977; Lockard et al., 1978; Peattie, 1979; Randerath et al., 1980) do not enable one to locate and identify modified nucleotides in the RNA chain. Because most RNAs contain modified nucleotides, this must be regarded as a major deficiency of the gel readout procedures for sequencing RNA. The thin-layer readout procedure provides a means of identifying modified nucleotides directly as ^{32}P-labeled derivatives. (2) Highly base-paired regions are resistant to controlled enzymatic hydrolysis, which makes it impossible to read the sequence in some regions of the RNA because the corresponding bands are missing in the gel. However, cleavage of phosphodiester bonds in base-paired regions occurs more readily under conditions of partial chemical hydrolysis (Stanley & Vassilenko, 1978; Gupta & Randerath, 1979; Peattie, 1979) of the RNA.

As shown in Figure 2, the thin-layer readout technique enabled us to display 93% of the sequence of human $tRNA^{Gly}_{CCC}$. On the basis of the gel readout technique, on the other hand, we were able to identify correctly only 48 of the 74 nucleotides of the RNA (Figure 3), i.e., about 65% of the sequence. The remaining nucleotides either were misidentified (because of the presence of posttranscriptional modifications) or failed to give bands on the gel (because of tight base pairing). Insufficient enzymatic cleavage was particularly apparent in the T arm and adjacent regions (nucleotides 46–65) (see Figure 3B), a region of tRNA known to be involved in tight secondary and tertiary base-pairing interactions [see Rich & RajBhandary (1976)].

Those nucleotides that could not be identified by the readout techniques were analyzed by a simple ^3H fingerprinting technique which has been reviewed recently (Randerath et al., 1980) and therefore will not be discussed here. For example, additional evidence for C46 and m^5C47, which, for as yet undetermined reasons, gave weak bands on the thin-layer readout film (Figure 2A) and could not be visualized at all on the gel readout films (Figure 3B), was provided by 3′-terminal analysis of the RNase A fragment G-A-C′ (Figure 4B) and by base composition analysis of the RNase T_1 fragment A-C-m^5C-m^5C-G′ (Figure 4A).

The slight modifications (see Materials and Methods) of the published procedure (Gupta & Randerath, 1979) were found useful in the sequence analysis of human $tRNA^{Gly}_{CCC}$; they may be applicable to other RNAs also. Thus, heating the RNA in water at 100 °C for 45 s afforded a more uniform cleavage of the phosphodiester bonds than did heating at 80 °C for 6 min, and thinner (0.035-cm) gels gave sharper bands than did 0.06-cm thick gels. The use of 20% polyacrylamide gels to resolve the shorter fragments resulted in greater distances between adjacent bands. This was found particularly important in the analysis of 3′-terminal fragments which may be somewhat contaminated on 12% gels with overdigestion products or other impurities derived from traces of contaminating RNAs. The treatment of individual bands of the ladder with RNase T_2, in addition, enabled us to obtain single termini from the shorter fragments (Figure 2B).

The sequences of many eucaryotic glycine tRNAs, i.e., human $tRNA^{Gly}_{GCC}$ (Gupta et al., 1979), silkworm $tRNA^{Gly}_{GCC}$ (Garel & Keith, 1977; Zúñiga & Steitz, 1977), wheat germ $tRNA^{Gly}_{GCC}$ (Marcu et al., 1977), yeast $tRNA^{Gly}_{GCC}$ (Yoshida, 1973), silkworm $tRNA^{Gly}_{U*CC}$[1] (Kawakami et al., 1978), and human $tRNA^{Gly}_{CCC}$ (present work), are now known. Thus, more is known about the sequences of eucaryotic glycine tRNAs than about the sequences of any other family of eucaryotic tRNA isoacceptors; cf. the compilation of tRNA sequences by Gauss et al. (1979). A comparison of the various eucaryotic glycine tRNA isoacceptors appeared of interest therefore. We shall first compare the structure of human $tRNA^{Gly}_{CCC}$ with that of human $tRNA^{Gly}_{GCC}$ (Gupta et al., 1979) and then discuss the noteworthy structural features of all eucaryotic glycine tRNAs.

In Figure 5, the enclosed regions indicate nucleotides common to both human glycine tRNAs. If differences in posttranscriptional modifications are excluded, the two RNAs exhibit a homology of 67.6%. Like all other eucaryotic glycine tRNAs, human $tRNA^{Gly}_{CCC}$ has a ribose-methylated pyrimidine nucleoside in position 4. Two additional ribose-methylated nucleosides in the anticodon arm are found only in $tRNA^{Gly}_{CCC}$. In contrast to other eucaryotic glycine tRNAs, the human RNAs contain m^2G in position 6. While m^2G has been found mostly in position 10 of tRNAs, its occurrence in position 6 does not appear to be rare in mammalian tRNAs, since, in addition to the mammalian glycine tRNAs, mammalian methionine (Petrissant & Boisnard, 1974; Piper, 1975) and leucine (E. Randerath, R. C. Gupta, R. J. Rhines, and K. Randerath, unpublished experiments) tRNAs have m^2G in position 6. The human glycine tRNAs are the only tRNAs known to have two methylated nucleosides in the amino acid acceptor stem. They are rich in m^5C, as has been found also for glycine tRNAs from other higher eucaryotes (Garel & Keith, 1977; Zúñiga & Steitz, 1977; Marcu et al., 1977; Gupta et al., 1979). The reasons for the resistance to methylation of C46 in $tRNA^{Gly}_{CCC}$ are unknown; this position is completely methylated to m^5C in human $tRNA^{Gly}_{GCC}$ (Gupta et al., 1979).

In Figure 6, sequences of all known eucaryotic glycine tRNAs have been compared. The following points appear noteworthy.

[1] U* denotes a mixture of two unknown modified uridines (Kawakami et al., 1978).

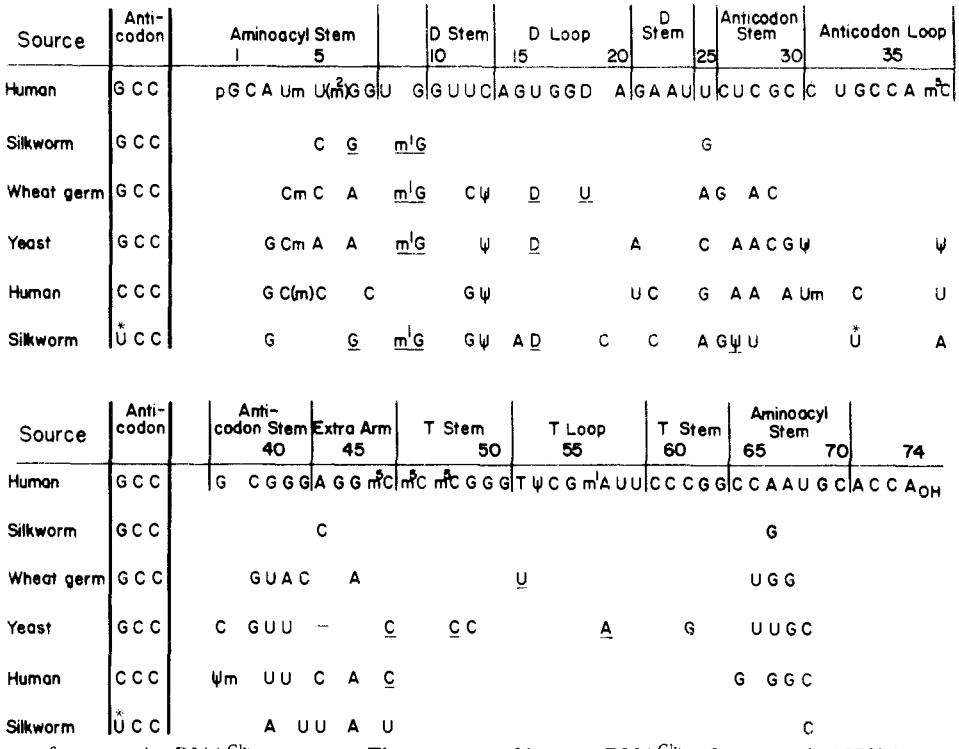

FIGURE 6: Comparison of eucaryotic $tRNA^{Gly}$ sequences. The sequence of human $tRNA^{Gly}_{GCC}$ (Gupta et al., 1979) is presented at the top. In the other tRNAs, only those nucleotides are shown which are different from the corresponding nucleotides in human $tRNA^{Gly}_{GCC}$. The underlined nucleotides indicate differences in posttranscriptional modifications only. Position 43 in yeast $tRNA^{Gly}_{GCC}$ is absent. Silkworm $tRNA^{Gly}_{U^*CC}$ contains an additional C residue in the D loop.

(1) The loop sequences have been largely conserved. Only the 3′-terminal position of the anticodon loop exhibits a high degree of variability. Conservation of loop sequences has been observed also in eucaryotic cytoplasmic initiator tRNAs and eucaryotic phenylalanine tRNAs but not in other tRNA isoacceptors [see Gauss et al. (1979)].

(2) The T arm (nucleotides 47–63) appears to constitute the longest conserved sequence in the eucaryotic glycine tRNAs; only yeast $tRNA^{Gly}_{GCC}$ shows a G═C→C═G base pair change while this sequence, which does not occur in any other sequenced tRNA, has been totally conserved in the glycine tRNAs from higher eucaryotes. Procaryotic glycine tRNAs and other eucaryotic tRNA isoacceptors do not exhibit a particularly high degree of conservation of the T-arm sequence. The resistance of the T arm to the fixation of natural mutations suggests important, as yet undetermined functions of the T arm in eucaryotic glycine tRNAs. It is of interest that the T arm is not thought to be involved in aminoacyl-tRNA synthetase binding (Rich & Schimmel, 1977); the conservation of the T-arm sequence in eucaryotic glycine tRNA may therefore indicate other functions of these tRNAs.

(3) The sequence U8-G9-G10-U11, linked by secondary hydrogen bonds to A23 and U24 (Figure 5), also appears to be unique to eucaryotic glycine tRNAs. This area of the molecule may be involved in aminoacyl-tRNA synthetase binding (Rich & Schimmel, 1977).

(4) The close homology between silkworm and human $tRNA^{Gly}_{GCC}$ has been discussed previously (Gupta et al., 1979).

(5) A relatively high degree of variability is found in the stem areas (except the T stem), as well as in positions 25 and 43. Variability in the stem areas (including the T stem) is common to a number of eucaryotic tRNA isoacceptors, but the high degree of variability at positions 25 and 43 appears unique to the family of glycine tRNA isoacceptors. It is also found in procaryotic glycine tRNAs but not in other tRNAs. In fact, all four major nucleotides have been found in position 25 but not at any other site of the eucaryotic glycine tRNAs (Figure 6). The observation that, in the animal glycine tRNAs, a change of position 25 is accompanied by a complementary change of position 43 would be consistent with the existence of an additional (sixth) secondary base pair at the base of the anticodon stem. On the basis of X-ray crystallographic studies on yeast $tRNA^{Phe}$ (Holbrook et al., 1978), bases occupying these positions are known to form tertiary base pairs. It should be possible to establish by physical techniques, such as high-resolution NMR, whether there is an additional secondary base pair in the anticodon stem of animal glycine tRNAs.

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Regulation of Hepatoma Tissue Culture Cell Tyrosine Aminotransferase Messenger Ribonucleic Acid by Dexamethasone†

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ABSTRACT: A maximally effective concentration of dexamethasone causes an 8–10-fold increase in the steady-state values of $mRNA^{TAT}$ [from 0.04 to 0.40% of total poly(A+) RNA activity], the in vivo rate of synthesis of tyrosine aminotransferase (0.02–0.19%), and tyrosine aminotransferase catalytic activity (9–90 milliunits/mg) in rat hepatoma tissue culture (HTC) cells. Concentrations of dexamethasone which result in different steady-state levels of induction of tyrosine aminotransferase result in varying, but always proportional, changes in these three functions. Finally, variant HTC cells, which have a lower basal level of tyrosine aminotransferase than wild type cells and in which tyrosine aminotransferase is not induced in response to glucocorticoids, have proportionately lower basal $mRNA^{TAT}$ levels and show no change in the latter following treatment with dexamethasone. Inasmuch as there is a linear relationship between tyrosine aminotransferase catalytic and mRNA activities at steady state under a variety of different experimental conditions, we conclude that the concentration of $mRNA^{TAT}$ is the primary determinant of the intracellular concentration of this protein. This induction is an extremely rapid process since $mRNA^{TAT}$ increases within 30 min after the addition of the inducer. Ongoing RNA synthesis is required since the induction by dexamethasone can be prevented by the simultaneous addition of actinomycin D or cordycepin. The increase in $mRNA^{TAT}$ activity occurs in the presence of inhibitors of protein synthesis such as cycloheximide and emetine, indicating that the mRNA increase is not tightly coupled to its translation or to the synthesis of another protein.

A number of systems, most of which involve enzyme induction, have been used to study glucocorticoid hormone action. Of these, one of the most intensively studied is the induction of tyrosine aminotransferase (EC 2.6.1.5; L-tyrosine:2-oxoglutarate aminotransferase). The addition of glucocorticoid hormones results in an increase in the specific rate of synthesis of the proteins in both liver and HTC cells[1] (Kenney, 1962; Granner et al., 1968). In 1968 it was predicted that tyrosine aminotransferase induction involved a "precursor"

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[1] Abbreviations used: $mRNA^{TAT}$, the messenger ribonucleic acid which codes for tyrosine aminotransferase; poly(A+) RNA, RNA containing polyadenylate residues at the 3′ terminus; HTC cells, hepatoma tissue culture cells; SAC, *Staphylococcus aureus*, Cowan strain I; $NaDodSO_4$, sodium dodecyl sulfate.

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