SEQUENCE STUDIES ON HUMAN PLACENTA $tRNA^{Val}$: Comparison with the mouse myeloma $tRNA^{Val}$

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SUMMARY. The major species of valine specific tRNA was isolated from human placenta, degraded to oligonucleotides, and shown to have the nucleotide sequence pG-U-U-U-C-C-G-U-A-G-U-G-Ψ-A-G-D-G-G-D-D-A-U-C-A-C-m^{2}G-Ψ-U-C-G-C-C-U-(I or C)-A-C-A-C-G-C-G-A-A-A-G-m^{7}G-D-m^{5}C-m^{5}C-C-C-G-G-U-Ψ-C-G-m^{1}A-A-A-C-C-G-G-G-C-G-G-A-A-A-C-A-C-C-A_{OH}. This human placental $tRNA^{Val}$ differs from the major species of mouse myeloma $tRNA^{Val}$ only in that it contains either I or C in the wobble position of the anticodon, and totally lacks 2'-O-methylcytosine and 5-methylcytosine in the anticodon loop.

During the course of our studies concerning the role of modified nucleotides in mammalian tRNA (1-4), we isolated several human placental tRNAs which either partially or totally lacked the modified nucleoside ribothymidine (rT) at position 23 from the 3' terminus (4-7). Recent unpublished observations in our laboratory indicate that these tRNAs are acceptable substrates for *in vitro* methylation by *E. coli* uridine methylase, although their rate of methylation is quite slow if the tRNA totally lacks rT *in vivo*. These results are contrary to our earlier report (4) which indicated that the mammalian $tRNA^{Val}$'s were not acceptable substrates for this methylation reaction under the conditions employed in the earlier study. After modification of our previous hypothesis (4) to include the more recent observations, we now believe that the presence of two 5-methylcytosines at the juncture of the minor loop (loop III) and the stem of loop IV causes an alteration in the structure of these class IV tRNAs, thereby slowing the rate of methylation of uridine to ribothymidine by the *E. coli* enzyme.

In an attempt to test this hypothesis, we have determined the complete nucleotide sequence of the major species of human placenta $tRNA^{Val}$, ($tRNA^{Val}_1$). The results of these studies, which confirm our prediction that this human tRNA should contain the two 5-methylcytosines at positions 48 and 49 (4), also illustrate the great structural similarity between isoaccepting tRNAs from different

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mammalian tissues, including humans.

MATERIALS AND METHODS. The isolation of human placenta $tRNA^{Val}_1$ to a purity of 1.2 nmoles per A_{260} unit has previously been reported in detail (6). This tRNA was further purified to apparent homogeneity by two additional chromatographic steps on RPC-5 at neutral pH (7). Conditions for digestion by ribonucleases and procedures for the resolution of the complete digestion products were identical to those reported earlier for human placenta $tRNA^{Phe}$ (3). The procedures employed for analysis of the 5' terminal nucleoside and sequential degradation of each oligonucleotide fragment were as described (8,9). The sequentially degraded and tritium post-labeled oligonucleotides were then resolved using a two-dimensional PEI-cellulose procedure which will be described in detail elsewhere (10). Briefly, the PEI-cellulose chromatogram was developed using a stepwise gradient of unbuffered lithium chloride (0.05 M to 0.60 M) in the first dimension and a stepwise gradient of ammonium formate, pH 3.5, (0.02 M to 2.50 M) in the second dimension. The 3' tritium labeled trialcohols were subsequently analyzed following *in situ* ribonuclease T_2 digestion, as described (8).

RESULTS AND DISCUSSION. Tracings of the two-dimensional PEI-cellulose TLC maps of the complete RNase T_1 and RNase A digestion products of human placenta $tRNA^{Val}_1$ are shown in Figures 1 and 2, respectively. A summary of the nucleotide sequence and molar ratios for each fragment, compared to mouse myeloma $tRNA^{Val}$, is shown in Tables I and II. Comparison of the oligonucleotides obtained from either the human or mouse $tRNA^{Val}$ indicates only slight differences in both the nucleotide sequence and molar ratios.

Although the nanonucleotide C-C-U-C-A-C-A-C-G_p was not obtained as a RNase fragment in the mouse myeloma $tRNA^{Val}$ (11), it was present in a molar ratio of 0.35 from a similar digestion of the human placental $tRNA^{Val}_1$ (Table I). In addition, the molar ratio of the RNase T_1 fragments C-C-U-I_p and A-C-A-C-G_p were both 1.0 in mouse myeloma $tRNA^{Val}$ and 0.65 in the human placental $tRNA^{Val}$ (ref. 11 and Table I). A comparable result obtained from the RNase A digestion of these tRNAs can be seen in Table II, where the molar ratios for the fragments I-A-Cp are 0.65 in the human and 1.0 in the mouse myeloma $tRNA^{Val}$. Also noteworthy is the absence of both the ribose methylated cytosine and the 5-methylcytosine in the anticodon loop fragments of human placental $tRNA^{Val}_1$, since both of these modified nucleotides were observed in the anticodon loop fragments of the mouse myeloma $tRNA^{Val}$ (11).

Another major difference between the sequence of these two mammalian $tRNA^{Val}$'s is the lack of the partial Ψ_{27}-A_{43} and C_{27}-G_{43} base pair in the anticodon stem. Since molar amounts of both the dinucleotide m^2G-Ψ_p and the heptanucleotide G-A-A-A-G-m^7G-D_p were

Table I

Complete RNase T_1 digests of $tRNA^{Val}_{1a}$ ---Comparison between human placenta and mouse myeloma cells

[a]Spot no.	Compound	Molar ratio [b] $tRNA^{Val}_{h.p.1a}$	Molar ratio [c] $tRNA^{Val}_{m.m.}$
1	Gp	5	5
2	C-GP	2.2	2
3	[d] A-A-A-C-A-C-C-A_{OH}	0.35	1
4	D-Gp	1	1
5	U-Gp	1	1
6	U-A-Gp, Ψ-A-Gp	1 each	1 each
7	[e] C-C-U-Ip	0.65	1
8	A-A-A-Gp	1	1
9	m^1A-A-A-C-C-Gp	0.9	1
10	[e] A-C-A-C-Gp	0.65	0
	[e] A-C-A-m^5C-Gp	trace	1
11	m^7G-D-m^5C-m^5C-C-C-Gp	0.8	1
12	U-Ψ-C-Gp, Ψ-U-C-Gp	1 each	1 each
13	U-U-U-C-C-Gp	1	1
14	D-D-A-U-C-A-C-m^2Gp	0.8	1
15	[d] A-A-A-C-A-C-Cp	0.65	0
16	pGp	1.1	1
17	[e] C-C-U-C-A-C-A-C-Gp	0.35	0

[a]See Fig. 1 for the position of each spot on PEI-cellulose thin layer, spots #6 and #12 contain two fragments.

[b]The molar ratio of Gp was derived from the fragments of the complete RNase A digest of $tRNA^{Val}_{h.p.1a}$ (see Table II). All other molar ratios were determined directly from the RNase T_1 digest.

[c]Calculated from the major species of mouse myeloma $tRNA^{Val}$ (11,15)

[d]Depending on the digestion condition, A-A-A-C-A-C-C-A_{OH} is split further to A-A-A-C-A-C-Cp.

[e]The $tRNA^{Val}_{h.p.1a}$ contains 65% of C-C-U-Ip and A-C-A-C-Gp, and 35% of C-C-U-C-A-C-A-C-Gp; whereas $tRNA^{Val}_{m.m.}$ contains molar amounts of C-C_m-U-Ip and A-C-A-m^5C-Gp.

present in the RNase A digest of human $tRNA^{Val}_1$ (see Table II), and molar amounts of the tetranucleotides G-C-U-$Ψ_p$ and A-A-A-G_p were present in the RNase T_1 digest of this tRNA (see Table I), we

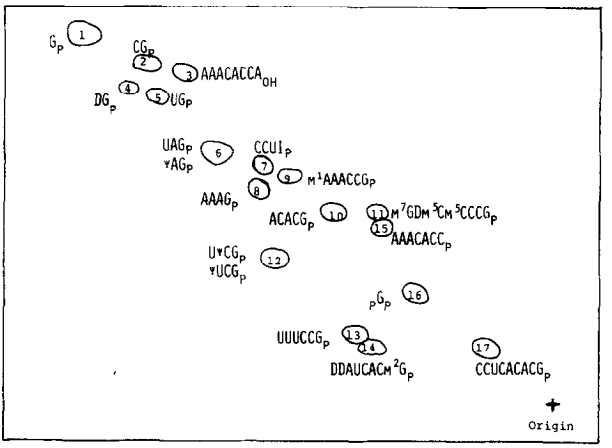

Figure 1. PEI-cellulose TLC map of RNase T_1 digest of human placental $tRNA^{Val}_1$. The first dimension was developed with increasing concentrations (0.0 - 0.5 M) of LiCl in 0.3 M Tris-HCl, 7.5 M urea (pH 7.9), followed by stepwise Li-formate (0.0 - 1.5 M) chromatography in 7.5 M urea (pH 3.5) for the second dimension.

therefore conclude that the human $tRNA^{Val}_1$ contains molar amounts of the Ψ_{27}-A_{43} base pair in the anticodon stem region rather than a partial C_{27}-G_{43} base pair replacement found in the mouse myeloma tRNA (11). A fluorogram of the two-dimensional PEI-cellulose separation for the sequentially degraded oligonucleotide G-A-A-A-G-m^7G-D_p is shown in Figure 3. The interpretation of the fluorogram is simplified by the observation that the background spots travel as a series of hyperbolic arcs which differ significantly from the mobility of the sequentially degraded oligonucleotides. This procedure, which will be discussed in a later communication (10), was used to determine the complete sequence of all oligonucleotide fragments (chain length of greater than three) obtained from either RNase T_1 or A digestions of the human placental $tRNA^{Val}_1$.

From the data presented in Tables I and II, it is possible to write the complete nucleotide sequence for human placental $tRNA^{Val}_1$ in the classical, cloverleaf form (12) as shown in Figure 4. While the primary structure of human placenta $tRNA^{Val}$ is similar to that of mouse myeloma $tRNA^{Val}$, it is possible to deduce this unique sequence based only on the assumptions that the modified nucleotides

Table II

Complete RNase A digests of $tRNA^{Val}_{1a}$ --- Comparison between human placenta and mouse myeloma cells

[a] Spot no.	Compound	Molar ratio [b] $tRNA^{Val}_{h.p.1a}$	[c] $tRNA^{Val}_{m.m.}$
none	[b] Adenosine	1	1
1	m^5Cp, Cp	2, 10.35	2, 10
2	Dp, Up, Ψp	1, 4, 1	1, 4, 1
3	A-Cp	[e] 3.35	2
	$A-m^5Cp$	trace	1
4	A-Up	1	1
5	G-Cp	2.1	2
6	G-Up, G-Ψp, m^2G-Ψp	1 each	1 each
7	A-G-Dp	1	1
8	I-A-Cp	[e] 0.65	1
9	$G-m^1A-A-A-Cp$	1.1	1
10	A-G-Up	1	1
11	G-G-Dp	0.9	1
12	G-G-Up	0.9	1
13	$G-A-A-A-G-m^7G$-Dp	0.9	1
14	G-G-G-Cp	[d] 0.7	1
15	G-G-A-A-A-Cp	0.9	1
16	pG-Up	1	1

[a] See Fig. 2 for the position of each spot on PEI-cellulose thin layer, spot #1 contains two fragments and spots #2, #6 contains three fragments. Adenosine can not be detected on the anion-exchange thin layer.

[b] The molar ratios of adenosine and nucleoside monophosphates were derived from the fragments of the complete RNase T_1 digest of $tRNA^{Val}_{h.p.1a}$ (see Table I). All other molar ratios were determined directly from the RNase A digest.

[c] See footnote of Table I c.

[d] Some nucleotide material was lost due to streaking of compound on chromatogram (3).

[e] See footnote of Table I e.

occur in their usual positions (13), that the normal rules of base pairing apply, and that the structure can be written in the classical

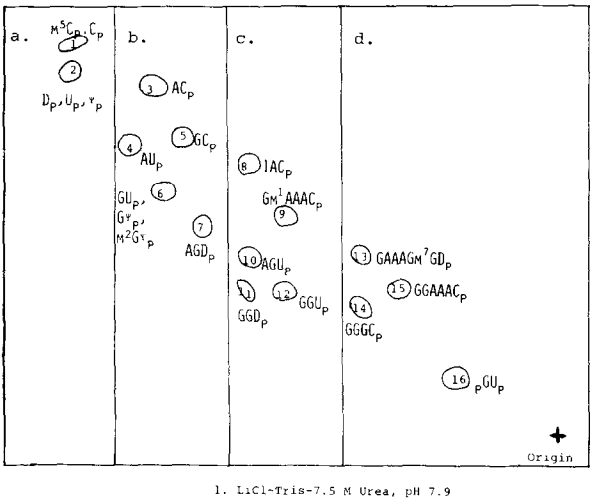

Figure 2. PEI-cellulose TLC map of RNase A digest of human placental $tRNA^{Val}_1$. The solvents used in both dimensions were identical to those employed in Figure 1, except that the plate was cut into four strips prior to development in the second dimension with a stepwise Li-formate gradient (a. 0.0 - 0.2 M; b. 0.0 - 0.4 M; c. 0.0 - 0.7 M; d. 0.0 - 1.0 M) containing 7.5 M urea (pH 3.5).

cloverleaf form. Although analysis of the partial fragments is required to unequivocally establish all the overlaps (manuscript in preparation), the structure as shown in Figure 4 was deduced using the following logic.

Since the 5' and 3' terminal fragments are pGp and A-A-A-C-A-C-C-A_{OH} (T-3), respectively, the fragment U-U-U-C-C-Gp (T-13) can be placed adjacent to the pGp by complementary base pairing while the 3' side of this stem can be completed by complementary base pairing with the fragment G-G-A-A-A-Cp (P-15). As human placenta $tRNA^{Val}$ is an acceptable substrate for the E. coli uridine methylase, although at a low rate, the tetranucleotide U-Ψ-C-Gp (T-12) can be positioned in loop IV. The addition of the two overlapping fragments G-m^1A-A-A-Cp (P-9) and m^1A-A-A-C-C-Gp (T-9) now completes this seven membered loop. By placing m^7G-D-m^5C-m^5C-C-C-G_p (T-11) in the minor loop (loop III) and lower strand of loop IV, and by complementary base pairing the upper stem of loop IV using the tetranucleotide fragment G-G-G-Cp (P-14), the nucleotide sequence of this stem region can be obtained. The minor loop and a portion of

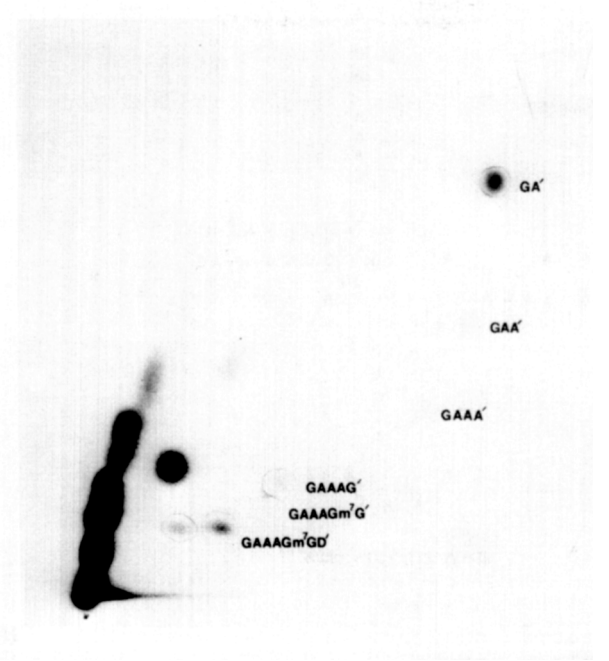

Figure 3. Two-dimensional PEI-cellulose TLC of a partial snake venom phosphodiesterase/bacterial alkaline phosphatase degradation of the heptanucleotide G-A-A-A-G-m^7G-D_p. Analysis was by tritium postlabeling, and fluorography as described (8).

the anticodon stem can be written by overlapping G-A-A-A-G-m^7G-Dp (P-13) in the minor loop and base pairing this fragment to the complementary Ø-U-C-Gp (T-12). The anticodon loop, written by placing C-C-U-C-A-C-A-C-Gp (T-17) or C-C-U-Ip (T-7) and A-C-A-C-Gp (T-10) so that the universal uridine is adjacent to the 5' side of the anticodon containing U-A-C or I-A-C respectively, can be completed by complementary base pairing with the overlapping dinucleotides C-Gp (T-2) and G-Cp (P-5).

Loop 1 and its stem region can be written by adding the overlapping fragments m^2G-Øp (P-6) and D-D-A-U-C-A-C-m^2Gp (T-14) to the anticodon stem, and completed by placing the remaining overlapping fragments in the order G-G-Dp (P-11), D-Gp (T-4), A-G-Dp (P-7), Ø-A-Gp (T-6), G-Øp (P-6), U-Gp (T-5), A-G-Up (P-10), and U-A-Gp (T-6). This unique ordering of fragments not only has the universal adenosines at both positions 14 and 21, and the universal uridine at position 8, but also allows for maximum base pairing in the stem region of loop I.

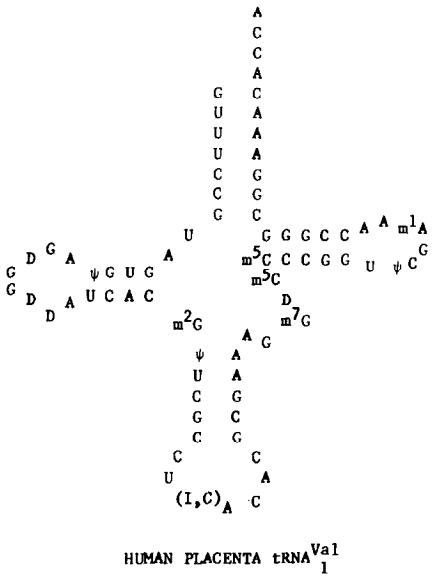

Figure 4. The nucleotide sequence of human placental tRNA$^{Val}_{1}$ arranged in cloverleaf form.

Although the human placental tRNA$^{Val}_{1}$ was isolated by ion-exchange column chromatography and is a mixture of tRNA$^{Val}_{1a}$ and tRNA$^{Val}_{1b}$, these two species differ only in the nucleotide present at the wobble position of the anticodon. Presumably, the tRNA$^{Val}_{1a}$ with the anticodon I-A-C$_p$ can recognize three of the four valine codons: G-U-C, G-U-A, and G-U-U, while the human tRNA$^{Val}_{1b}$ with the anticodon C-A-C can recognize the fourth valine codon: G-U-G. We therefore conclude that all four valine codons can be read by human placental tRNA$^{Val}_{1a,b}$. Since human placenta contains an additional valine isoaccepting species, tRNA$^{Val}_{2}$ (6), only the complete nucleotide sequence determination for this tRNA will demonstrate if any human tRNA^{Val}'s contain the additional modifications reported in the mouse myeloma tRNA^{Val} (11). Experiments aimed at determining the complete nucleotide sequence of this additional species of human tRNA^{Val} are presently in progress.

In addition, although we have demonstrated that several human tRNAs are identical to their counterparts from other mammalian sources, namely tRNA$^{Met}_{i}$ (2), tRNA^{Phe} (3) and tRNA$^{Val}_{1}$ reported herein, further structural studies of other human tRNAs are re-

quired to determine if these unique structural similarities are the rule rather than the exception.

While this manuscript was being prepared, Jank, et al. (15,16) reported the complete nucleotide sequence of rabbit liver $tRNA^{Val}_{1}$. Recent tritium postlabeling base analysis (Chen and Roe, unpublished results) indicate that the rabbit liver $tRNA^{Val}_{1}$ contains a molar amount of inosine and has a base composition similar to the human placental $tRNA^{Val}_{1a}$. In addition, through studies of the differential melting curves and temperature jump for the 30 nucleotide long 3' terminal fragment of this tRNA, Jank, et al. (16) concluded that the stem of loop IV in the intact rabbit liver $tRNA^{Val}$ might be extended by an additional A_{60}-U_{54} base pair. Since U_{54} is far less available for enzymatic conversion to rT (4,16 and our recent unpublished results) a similar A_{60}-U_{54} base pair might also be present in the human placental $tRNA^{Val}$'s. Finally, the presence of two 5-methylcytosines at the juncture of loop III and the stem region of loop IV, as postulated earlier (4), might play a role in the U→rT conversion. The increased base stacking interactions in the stem of loop IV would in turn stabilize the helicity of this region and allow for the formation of the additional base pair (A_{60}-U_{54}) proposed by Jank, et al. (16). Experiments aimed at testing this hypothesis are presently in progress.

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