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Chromatographic and Functional Comparison of Human Placenta and HeLa Cell Tyrosine Transfer Ribonucleic Acids†

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ABSTRACT: Chromatographic and functional properties of tyrosine isoaccepting transfer ribonucleic acids (tRNAs) from placenta and HeLa cells were analyzed and compared. RPC-5 chromatography separated four major isoacceptors from each source, with those from HeLa cells eluting generally later than those from placenta. There was some overlap: HeLa $tRNA_1^{Tyr}$ eluted in a position between placenta $tRNA_3^{Tyr}$ and $tRNA_4^{Tyr}$; and HeLa $tRNA_2^{Tyr}$ and placenta $tRNA_4^{Tyr}$ eluted in similar positions with the HeLa isoacceptor eluting slightly later than the placental isoacceptor. Thus there are no isoacceptors common to both sources. The function of the individual isoacceptors was compared in a rabbit-reticulocyte, cell-free, protein-synthesizing system for both the rate of incorporation of tyrosine into the polypeptide chain and the site of incorporation in α-globin. Two isoacceptors were compared simultaneously in the same reaction, and overlapping comparisons were made to relate each isoacceptor to all the others. There were no significant differences in the rates of incorporation among the isoacceptors, nor were there any differences in the sites of incorporation. All eight isoacceptors donated tyrosine equally well into the three tyrosine containing tryptic peptides of α-globin. Whatever the structural differences among the tyrosine isoacceptors are, they do not affect the function of the tRNA in this protein-synthesizing system.

Isoaccepting tRNAs isolated from genetically related sources often exhibit different chromatographic properties. Transfer RNAs from many neoplastic cells differ from those of normal cells in this respect (Taylor et al., 1968; Baliga et al., 1969; Gallagher et al., 1972; Mushinski and Potter, 1969). The precise nature of the changes which occur upon transformation varies with cell type and with specific isoacceptors. Taylor et al. (1968), for example, showed that some transformed cells contained tyrosyl-tRNA species not found in normal cells, and that other transformed cells contained different ratios of tyrosyl isoacceptors than normal cells. New species of histidine, tyrosine, and asparagine tRNAs were seen in Novikoff hepatoma when compared with normal rat liver (Baliga et al., 1969) and transformation of rat embryo or mouse cells by SV40 resulted in the production of a new aspartyl-tRNA (Gallagher et al., 1972). Because transfer RNAs occupy a central position in the flow of genetic information and are known to be involved in genetic regulation, analysis of functional differences which might result from the structural changes seems desirable.

A few studies of this type have been performed. These functional studies have been carried out in several different systems. Sharma et al. (1975) used chick oviduct magnum explants and showed that bulk Novikoff hepatoma tRNA added to the medium specifically inhibited ovalbumin synthesis, while normal rat liver tRNA had no effect. Several groups have compared incorporation of amino acids from unfractionated tRNAs into specific proteins. In one such study, Mushinski et al. (1970) showed that there was no difference between unfractionated tRNA from normal mouse liver and plasma cell tumors in the transfer of leucine into rabbit hemoglobin in a cell-free system, even though the leucine tRNAs differed chromatographically. Bridges and Jones (1973) found a slight quantitative difference in incorporation of serine into the tryptic peptides of protein produced by a cell-free system from one plasmacytoma when the unfractionated tRNAs of a different plasmacytoma were used. There were significant

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differences in the chromatographic profiles of the seryl-tRNAs from these two plasmacytomas. In an even more well-defined system, Gonano et al. (1971) found that the phenylalanine isoacceptor unique to Morris hepatoma cells did not differ from normal tRNA in incorporation in response to synthetic polynucleotide in an *Escherichia coli* cell-free system. Finally, several groups have looked at specific codon recognitions in ribosome binding assays and have found differences in codon recognition among isoacceptors and corresponding differences in site of incorporation of the amino acid into the globin chains in a rabbit-reticulocyte, cell-free system (Woodward and Herbert, 1972; Galizzi, 1969; Weisblum et al., 1965, 1967). In other cases the isoacceptors recognize all the codons for an amino acid and there is no difference in incorporation (Takeishi et al., 1972). However, even if an isoacceptor recognizes all the codons for an amino acid, it may not do so with equal efficiencies (Harada and Nishimura, 1972).

In this study the mammalian tyrosine isoacceptors of human placenta and HeLa cells were compared chromatographically on RPC-5 columns and functionally in a rabbit-reticulocyte, cell-free, protein-synthesizing system. Tyrosine tRNAs were chosen because it had been found previously that, when there are differences in chromatographic profile of tRNAs from genetically related sources, the tyrosine tRNAs were the ones which changed most frequently (Taylor et al., 1968). HeLa cells were chosen as the transformed line because previous work had shown substantial chromatographic differences on MAK[1] columns between its tyrosyl-tRNA and that of normal human liver (Holland et al., 1967). In addition, we had noted earlier that tyrosyl-tRNAs from some solid human breast carcinomas were very similar to those present in HeLa cells (Penhoet and Holland, 1971).

Four tyrosine isoacceptors were found by RPC-5 chromatography in both HeLa and placenta with those from HeLa eluting generally later than those from placenta. There was some overlap but none of the species from one source was identical with any species from the other source. The individual isoacceptors were compared in a rabbit-reticulocyte, cell-free system for both rate of incorporation of tyrosine and site of incorporation in the globin chain. There were no significant differences in the rates of incorporation among the isoacceptors, nor were there any differences in the site of incorporation. All eight isoacceptors donate tyrosine equally well into the three tyrosine containing tryptic peptides of α-globin.

Materials and Methods

Cells and Tissues. The HeLa cells were grown in suspension culture in Joklik-modified minimum essential medium (Grand Island Biological Co., Grand Island, N.Y.) with 5% calf serum (Irvine Scientific Co., Irvine, Calif.). They were grown to a density of 8×10^5 to 1×10^6 cells per ml and then harvested by centrifugation at 2500*g* for 10 min at 4 °C. The cells were washed twice by centrifugation with PBS. If not used immediately, the packed cells were stored frozen at −80 °C. Fresh human placentas from normal deliveries were obtained from Alta Bates Hospital, Berkeley, Calif. They were packed in ice for transport to the laboratory where they were washed with distilled water. The placentas were stored at −80 °C until used.

Preparation of tRNA and Aminoacyl Synthetases. The methods of Taylor et al. (1968) were used to prepare tRNA from HeLa cells and placenta and to prepare aminoacyl-tRNA synthetases from HeLa cells, placenta, and rabbit liver. Mammalian tRNAs were aminoacylated by the procedure of Taylor et al. (1968) using 10 μCi of L-[3,5-^{3}H]tyrosine (specific activity 31 to 54 Ci/mmol, Amersham/Searle, Arlington Heights, Ill.) or 0.5 μCi of L-[U-^{14}C]tyrosine (specific activity 483 to 522 mCi/mmol, Amersham/Searle) per 300 μl of reaction mixture. Rabbit-liver aminoacyl synthetases were used unless otherwise indicated. *Salmonella typhimurium* tRNA and aminoacyl synthetases were a gift of Dr. Harold O. Kammen. The bacterial tRNA was aminoacylated under conditions similar to mammalian tRNA except that 2 mM ATP and 1 mM CTP were used.

RPC-5 Chromatography. Chromatography was performed by the method of Pearson et al. (1971), except that no gradient was used. Tyrosyl-tRNA charged with labeled tyrosine was applied to the column in a solution consisting of 10 mM sodium acetate (pH 4.5), 10 mM magnesium chloride, 2 mM 2-mercaptoethanol (buffer A) with 0.4 M sodium chloride. The column was washed with 2 column volumes of the same solution; then the tyrosyl-tRNA was eluted with 0.55 M NaCl in buffer A. The column was washed with 1.5 M NaCl in buffer A between runs. The column was 0.6 × 30 cm and 1-ml fractions were collected.

Isolation of Tyrosyl-tRNA Isoacceptors. Tyrosyl-tRNA isoacceptors were fractionated on a preparative scale by RPC-5 chromatography on a 2.5 × 90 cm column. Fractions of 12 ml were collected and assayed for radioactivity. Fractions containing the isoacceptors were pooled separately and concentrated by DEAE-cellulose chromatography. The pooled fractions were diluted to 0.3 M NaCl and applied to 3-ml DEAE-cellulose columns. The columns were washed with 3 ml of 0.3 M NaCl in buffer A; the tRNAs were eluted with 0.7 M NaCl in buffer A. The tRNA was precipitated with 2.5 volumes of 95% ethanol at −20 °C and then collected by centrifugation and dissolved in buffer A.

In Vitro Protein Synthesis. Rabbit reticulocytes were prepared by the procedure of Gilbert and Anderson (1970) and the lysate was prepared by the procedure of Housman et al. (1970). The in vitro protein-synthesizing reaction contained 20 mM Tris-HCl (pH 7.5), 50 mM KCl, 3 mM $MgCl_2$, 1 mM ATP, 0.2 mM GTP, 1 mM dithiothreitol, 10 mM phosphocreatine, 0.12 mg/ml creatine kinase, 80 μM each of 19 amino acids (except tyrosine), 40 μg/ml hemin, and 450 μl/ml reticulocyte lysate. When tyrosyl-tRNA was present as the only source of label, unlabeled tyrosine was added to 0.1 or 1.0 mM. Tyrosyl-tRNA, tRNA, and/or free labeled tyrosine were also added to the reaction. All ingredients were mixed at 0 °C and the reaction was started by bringing the temperature to 30 °C.

Rate of Incorporation. Aliquots of the reaction mixture (usually 50 μl) were taken at various times and added to 3 ml of acid–acetone (0.2 ml of HCl–100 ml of acetone). The resulting precipitates were centrifuged at 500*g* for 10 min, the supernatants decanted, and the pellets dissolved in 0.2 ml of 1 N NaOH. These were reprecipitated with 2.0 ml of 10% Cl_3CCOOH, heated to 95 °C for 10 min, and then cooled at 0 °C for 10 min. The precipitates were centrifuged at 500*g* for 10 min, the supernatants decanted, and the pellets dissolved in 0.5 ml of NCS (Amersham/Searle), heating to 50 °C if necessary. The solutions were added to 10 ml of toluene scintillation fluid containing 4 g/l. 2,5-diphenyloxazole and 0.05 g/l. *p*-bis[2-(5-phenyloxazolyl)]benzene (New England Nu-

[1] Abbreviations used are: MAK, methylated albumin–kieselguhr; PBS, phosphate buffered saline (137 mM NaCl, 2.68 mM KCl, 8.10 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 0.90 mM $CaCl_2$, 0.49 mM $MgCl_2$); Tris, tris(hydroxymethyl)aminomethane; DEAE, diethylaminoethyl; NCS, Nuclear-Chicago solubilizer.

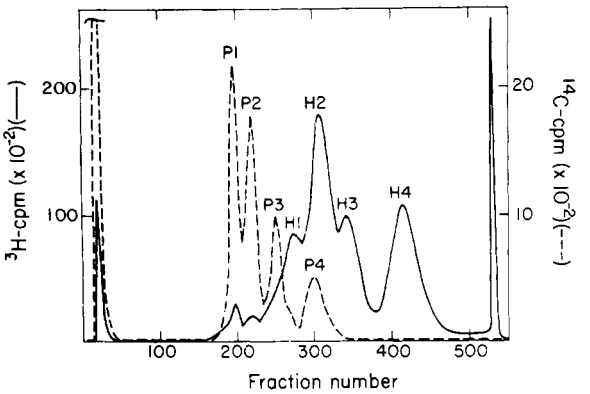

FIGURE 1: RPC-5 chromatography of human placental tRNA aminoacylated with [^{14}C]tyrosine and HeLa cell tRNA aminoacylated with [^{3}H]tyrosine. The samples were mixed and applied to a 0.6 × 25 cm RPC-5 column in 0.4 M NaCl in buffer A. The column was washed with 40 ml of the same solution, then 170 ml of 0.55 M NaCl in buffer A, and finally 25 ml of 1.5 M NaCl in buffer A. The flow rate was 1 ml/min and the fraction size was 0.5 ml.

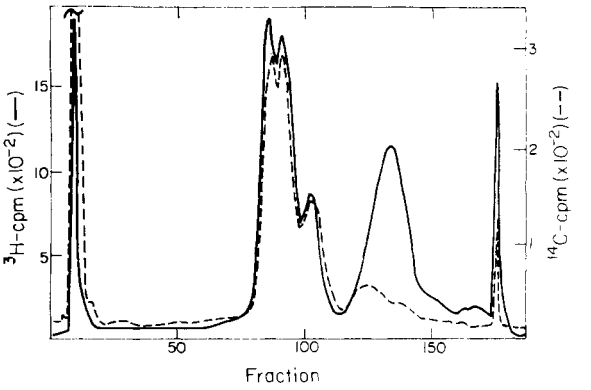

FIGURE 2: RPC-5 chromatography comparing human placental tRNA aminoacylated with [^{3}H]tyrosine with human liver tRNA aminoacylated with [^{14}C]tyrosine. The column size was 0.6 × 25 cm. The samples were mixed and applied to the column in 0.4 M NaCl in buffer A. The column was washed with 20 ml of the same solution, then with 150 ml of 0.55 M NaCl in buffer A, and finally with 20 ml of 1.5 M NaCl in buffer A.

clear, Boston, Mass.). The concentrations of labeled tyrosyl-tRNAs in the reaction mixture were determined by cold Cl_3CCOOH precipitation: an aliquot of the reaction mixture was added directly to 3 ml of 10% Cl_3CCOOH at 0 °C as soon as the ingredients of the reaction were mixed at 0 °C. The precipitate was centrifuged at 200*g* for 5 min, the supernatant decanted, and the pellet dissolved in 0.5 ml of NCS. The NCS solution was mixed with 10 ml of the same toluene scintillation fluid.

The samples were counted in either a Nuclear Chicago Mark II or a Packard Tri-Carb scintillation counter. External standards were used to measure quenching. Since the [^{3}H]-tyrosine and the [^{14}C]tyrosine differed in specific activity and efficiency of counting, it was necessary to convert all the data to molar amounts to compare rates and concentration. The procedure involved determining the counting efficiencies from the quench data, correcting for ^{14}C spillover, and converting to moles. The molar amounts for each time point were plotted and the rate of incorporation was calculated by least-squares analysis of the linear portion of the curve.

The concentrations of differently labeled tRNAs were rarely the same so the rates could not be compared directly. Since the tRNAs added to the reaction were at saturating levels, the rate of incorporation of labeled tyrosine into protein would be proportional to the concentration if the tRNAs were identical. To test for differences, the ^{14}C/^{3}H ratio of the rates was compared with the ^{14}C/^{3}H ratio for the concentrations and any difference was expressed as a percentage of the average of the two values. This allowed comparison of experiments with different ratios of label. The method of calculation involved subtracting the ^{14}C/^{3}H ratio of the concentration from the ^{14}C/^{3}H ratio of the rates. This arbitrarily assigns a negative value when the rate of incorporation of the [^{3}H]tyrosine is greater in proportion to its concentration than the [^{14}C]tyrosine.

Site of Incorporation. The in vitro protein-synthesizing reaction was stopped at 20 min by adding the mixture to 10 ml of 40 mg/ml rabbit hemoglobin at 0 °C. The method of peptide analysis was that of Weisblum et al. (1965), except that 0.1 M NH_4HCO_3 was used to buffer the trypsinization reaction and that the peptides were separated on a 0.9 × 20 cm Aminex A5 column (Bio-Rad Laboratories, Richmond, Calif.), using a linear 700-ml gradient from 0.2 M pyridinium acetate (pH 3) to 2.0 M pyridinium acetate (pH 5). The 4-ml fractions were assayed for both ninhydrin color and radioactivity. For the ninhydrin assay, 200-μl aliquots of each fraction were mixed with 0.5 ml of ninhydrin solution (2.0 g of ninhydrin, 0.3 g of hydrindantin, 75 ml of methyl Cellosolve, and 25 ml of 4 M sodium acetate, pH 5.5) and heated at 100 °C for 15 min. The fractions were then cooled and diluted with 3 ml of 50% 1-propanol. The absorbance was measured at 570 nm.

The remainder of each fraction was assayed for radioactivity by evaporating the sample at 60 °C in a vacuum oven, dissolving the nonvolatile material in 1 ml of water, and adding 10 ml of Triton X-100–toluene scintillation fluid (2 volumes of toluene and 1 volume of Triton X-100 plus 4 g/l. 2,5-diphenyloxazole and 0.05 g/l. *p*-bis[2-(5-phenyloxazolyl)]-benzene) for scintillation counting.

Results

Chromatographic Studies. Figure 1 shows the RPC-5 chromatographic profiles of HeLa and human placental tyrosyl-tRNAs. Both have four major tyrosine isoacceptors resolved by this technique, with the HeLa isoacceptors eluting mostly later than those of placenta. Each set of isoacceptors is numbered one to four in the order of elution from the RPC-5 column. HeLa $tRNA_2^{Tyr}$ elutes in the same position as placental $tRNA_4^{Tyr}$ but the peak of the HeLa isoacceptor elutes slightly later than the placental one so they are not identical. The profiles remain the same when the labels are reversed or if the tRNA is aminoacylated in the presence of the 19 other, unlabeled, amino acids. Identical patterns are also produced if synthetases from the homologous tissue, rather than from rabbit liver, are used to aminoacylate the tRNA.

When separated isoacceptors fractionated on the preparative RPC-5 column were rechromatographed with unfractionated tyrosyl-tRNA from the same source, the isoacceptors ran as single peaks in the proper location. There was no interconversion of one isoacceptor to another.

Placenta was chosen as the source of human tRNA to compare with HeLa cell tRNA because it is easily obtainable. Since there was some concern that this might not represent "normal" human tissue, the placental tRNAs were compared with the tyrosine tRNAs of human liver. As seen in Figure 2 the first three peaks are identical while the liver $tRNA_4^{Tyr}$ is reduced in quantity compared with placental $tRNA_4^{Tyr}$.

In Vitro Protein Synthesis. The purpose of the in vitro protein synthesis experiments was to determine whether the structurally different tRNAs function similarly in protein

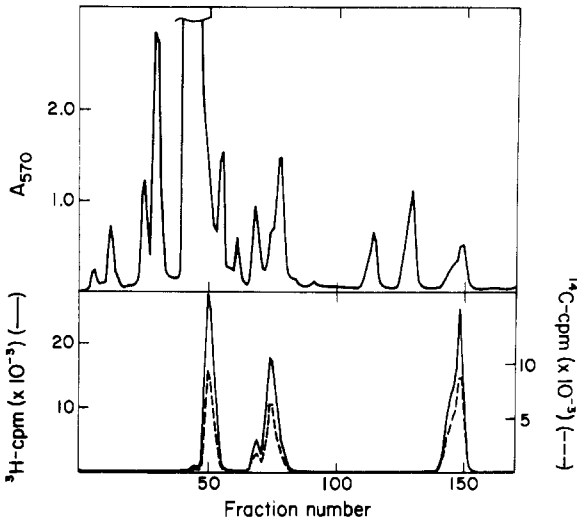

FIGURE 3: Aminex A5 chromatography of the tryptic peptides of rabbit α globin. The in vitro protein synthesizing system contained 4.52 nmol/ml [^{14}C]tyrosine and 56.4 pmol/ml of a mixture of [^{3}H]Tyr-tRNA^{Tyr} isoacceptors 1 to 4 isolated by RPC-5 chromatography. After 20 min, 1.65 ml of the reaction mixture was added to carrier rabbit hemoglobin, and the tryptic peptides of α globin were prepared and chromatographed on an Aminex A5 column as described in Materials and Methods.

synthesis. Two functions which can be studied in vitro are the rate of incorporation of amino acid into protein from the tRNA, and the specific site of incorporation in the polypeptide chain. To meet the requirements for the functional studies, the rabbit-reticulocyte, protein-synthesizing system must incorporate amino acids aminoacylated to exogenous tRNA, and incorporate them in the correct position in the polypeptide chain. This was tested by adding [^{14}C]tyrosine and [^{3}H]Tyr-tRNA$_{1\text{-}4}^{Tyr}$ (HeLa) to the same protein-synthesizing reaction and then comparing the tryptic peptides of α-globin on an Aminex A5 column as detailed in the legend to Figure 3. The first peak of radioactivity corresponds to the peptide αT4, the next two peaks represent αT6a and αT6b, and the last peak represents αT15 (Mushinski et al., 1970). Combining the counts for αT6a and αT6b, the percentages of the total counts in each peptide are 32-31-36 for [^{3}H]tyrosine and 30-31-39 for [^{14}C]tyrosine. Thus the incorporation from added, aminoacylated tRNA is the same as from the endogenous tRNA aminoacylated with the [^{14}C]tyrosine added to the reaction. These ratios are also close to the uniform distribution of label expected for a protein-synthesizing system which reinitiates.

Rate of Incorporation. The amount of variation in the rate of incorporation assay was determined by comparing the rate of label incorporation from differently labeled, but identical, tyrosyl-tRNAs in the same reaction. The rates of incorporation should be identical since the tRNAs were at saturating levels. Nineteen such comparisons were performed. The 19 reactions showed an average difference of 4% and a standard deviation of ±19%. Unfractionated HeLa tRNA was also compared with *Salmonella typhimurium* tRNA since this comparison was expected to show a large difference in rates. The results of two such comparisons were identical: there was a 151% difference in rates of incorporation with the HeLa tRNA incorporating tyrosine faster than the bacterial tRNA.

Comparisons of the rates of incorporation from both unfractionated tRNAs and isolated isoacceptors are shown in Tables I and II, respectively. There is no significant difference in the rate of incorporation of tyrosine from the unfractionated tRNAs. The isoacceptors were compared systematically so it

TABLE I: Rate of Incorporation of Tyrosine from Unfractionated tRNA.[a]

		^{14}C/^{3}H Ratio		
[^{14}C]Tyr-tRNA	[^{3}H]Tyr-tRNA	Rate	Concn	% Difference
Placenta	HeLa	2.12	1.97	+7
Placenta	HeLa	1.06	1.18	−11
HeLa	Placenta	1.51	1.42	+6

[a] The rate studies were performed as described in Materials and Methods. As indicated therein, the percent difference was calculated as: $\{[(R_1/R_2) - (C_1/C_2)]/([(R_1/R_2) + (C_1/C_2)]/2)\} \times 100$.

TABLE II: Rate Comparisons of Individual Isoacceptors.[a]

		^{14}C/^{3}H Ratio		
[^{14}C]Tyr-tRNA	[^{3}H]Tyr-tRNA	Rate	Concn	% Difference
pla 1[b]	pla 2	2.00	1.92	+4
pla 2	pla 1	2.79	2.45	+13
pla 2	pla 1	3.39	2.66	+24
pla 3	pla 2	3.22	2.83	+13
pla 4	pla 3	0.352	0.363	−3
pla 4	pla 3	3.70	2.92	+22
pla 4	pla 1	3.21	3.53	−10
pla 1	pla 4	4.73	5.78	−20
pla 3	pla 4	2.81	3.71	−27
HeLa 1	HeLa 2	1.59	1.73	−8
HeLa 2	HeLa 1	1.33	0.907	+37
HeLa 3	HeLa 2	2.54	1.92	+27
HeLa 4	HeLa 3	1.35	1.09	+22
HeLa 4	HeLa 1	4.92	3.93	+22
HeLa 1	HeLa 4	4.61	4.41	+5
HeLa 1	pla 1	3.64	3.53	+3
pla 4	HeLa 4	2.96	3.29	−10

[a] The in vitro protein synthesizing reactions were carried out and analyzed as described in Materials and Methods. The differences were calculated as indicated in Table I. [b] pla 1 means tRNA$_1^{Tyr}$ (placenta).

was not necessary to compare each isoacceptor with every other one: each isoacceptor was compared with the adjacent isoacceptor on the RPC-5 column, then isoacceptors separated the greatest on RPC-5 chromatography, and finally some HeLa isoacceptors were compared with some placental isoacceptors. There are no significant differences among the isoacceptors. The greatest difference seen, +37% for the [^{14}C]Tyr-tRNA$_2^{Tyr}$ (HeLa) vs. [^{3}H]Tyr-tRNA$_1^{Tyr}$ (HeLa) comparison, is less than twice the standard deviation. The experiment with labels reversed shows only a −8% difference, further indicating that the difference is not significant.

Site of Incorporation. In addition to a study of the rates of incorporation of tyrosine from fractionated isoacceptors, the sites of incorporation in the α-globin chain were studied to determine if there were any preferences among the isoaccepting species. The reactions for the peptide analyses were the same as the reactions used to determine rates, except that fewer comparisons were needed. The results are summarized in Table III. There are no significant differences among the isoacceptors. Although there are some significant departures from the expected equal distribution of label among the three peptides, when this occurs both labels show the same distribution.

TABLE III: Comparison of Site of Incorporation by Purified Isoacceptors.[a]

tRNA	cpm in Peptides				% of Total cpm in Peptides			
	αT4	αT6	αT15	Other	αT4	αT6	αT15	Other
^{14}C HeLa 2[b]	401	505	692	63	24	30	42	4
^{3}H HeLa 1	17 191	19 283	28 693	1394	26	29	43	2
	(43)[c]	(38)	(41)	(22)				
^{14}C HeLa 3	485	508	689	39	28	30	40	2
^{3}H HeLa 4	15 659	15 574	21 818	1037	29	29	40	2
	(32)	(31)	(32)	(26)				
^{14}C HeLa 1	1 004	1 044	1 265	112	29	31	37	3
^{3}H HeLa 2	43 379	47 159	56 952	685	29	32	38	1
	(43)	(45)	(45)	(6)				
^{14}C pla 2	1 000	965	1 032		33	32	34	
^{3}H pla 1	25 951	24 143	27 443		34	31	35	
	(26)	(25)	(27)					
^{14}C pla 3	1 019	859	1 004	526	30	25	30	15
^{3}H pla 2	25 956	21 327	26 905	6105	32	27	34	8
	(26)	(25)	(27)	(12)				
^{14}C pla 4	1 254	998	1 183	60	36	29	34	2
^{3}H pla 3	28 972	21 912	27 500	403	37	28	35	1
	(23)	(22)	(23)	(7)				
^{14}C pla 4	1 062	353	1 145		42	13	45	
^{3}H pla 3	26 464	6 834	28 756		43	11	46	
	(25)	(19)	(25)					

[a] The peptides were prepared and analyzed as described in Materials and Methods. The counts listed as "other" were usually observed to elute from the Aminex A5 column before αT4, although not always in the same place relative to the ninhydrin peaks. [b] ^{14}C HeLa 2 means [^{14}C]tyrosyl-tRNA$_2^{Tyr}$ (HeLa). [c] Numbers in parentheses are the ^{14}C/^{3}H ratios of the individual peaks.

Discussion

Four major tyrosine isoacceptors were found in both HeLa cells and human placenta with the HeLa isoacceptors eluting generally later than the placental isoacceptors. Although there was some overlap, none of the isoacceptors from one source was identical with any of the isoacceptors from the other source. The placental tyrosine isoacceptors are qualitatively the same as those from human liver. The patterns are reproducible under a number of conditions, including the use of homologous or heterologous synthetases, so that they are a true representation of the tyrosine tRNAs present in both sources. These are more peaks than found by previous workers using different techniques. Holland et al. (1967) found one broad peak for human tissues and two for HeLa cells using MAK chromatography. Gallo and Pestka (1970) found three tyrosine isoacceptors in normal human lymphoblasts using RPC-2 chromatography. The proportions of those species are similar to the first three isoacceptors found in placenta in this study.

At the present time we do not know the structural basis for the chromatographic differences exhibited by these tRNAs. Since both placenta and HeLa have four major isoacceptors, it is tempting to speculate that each of the HeLa isoacceptors corresponds to one of the placental isoacceptors, differing only by some modification to one of the bases. It is even possible that all the isoacceptors are derived from modifications to the same gene product.

Structurally different isoacceptors may or may not function identically in protein synthesis. Differences in the ribosome recognition areas of the tRNA may result in different affinities for the ribosomes and affect the relative rates at which the isoacceptors can donate their amino acids to the peptide chain. In cases where all the isoacceptors for one amino acid are altered, as in the HeLa–placenta comparison, the rate of protein synthesis in general can be altered. The most likely change is a decrease in rate since any step can become rate limiting, but only a modification to the existing rate-limiting step can increase the rate.

Codon recognition may also differ among structurally different isoacceptors. Even though an isoacceptor recognizes two or more codons, it may not do so with equal efficiencies (Harada and Nishimura, 1972). Thus structural alteration might not abolish codon recognition but just change the efficiency of recognition.

The approach used in this study was to compare purified tyrosine isoacceptors in the same reaction to increase the sensitivity for detecting differences in the rate of protein synthesis. In addition the site of incorporation of tyrosine into α-globin was studied to determine whether these structurally different tyrosine tRNAs insert tyrosine into specific polypeptide positions with equal efficiencies. No differences were seen among the tyrosine isoacceptors from either HeLa cells or placenta in the rate or the site of incorporation. Whatever the structural differences among the tyrosine isoacceptors are, they do not affect the function of the tRNA in this protein-synthesizing system. It therefore seems unlikely to us that transformation-dependent changes in tRNA are responsible for changes in the regulation of specific protein synthesis at the translational level. Whether tRNAs have other functions which are affected by the structural alterations is not known.

Acknowledgments

We thank Dr. Harold O. Kammen for the gift of *Salmonella* tRNA and aminoacyl-tRNA synthetases and Jan Capper and Jane Smith for laboratory assistance.

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Structural Difference between α-Paramyosin and β-Paramyosin of *Mercenaria mercenaria*†

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ABSTRACT: A method is described for extraction of α-paramyosin in amounts comparable to that formerly attained for β-paramyosin (15–25 mg/g of muscle). A modification of the procedure for sodium dodecyl sulfate gel electrophoresis is described that permits the separation on coelectrophoresis of α-paramyosin (207 000 daltons) and β-paramyosin (200 000 daltons). The α- and β-paramyosins also can be distinguished by gel electrophoresis at pH 2.3 and by differences in solubility in the region of 0.2–0.4 ionic strength at neutral pH. Evidence is presented that the segment lost from α-paramyosin during degradation to β-paramyosin came from the C-terminal end. This evidence is based on determinations of N- and C-terminal amino acids and on the size of segments obtained after chemical cleavage at the sites of Cys residues. It has been observed earlier that the solubility characteristics of β-paramyosin at neutral pH are determined by the C-terminal one-third of the molecule and the present results indicate that the additional small segment of about 3.5% of the total mass that is present in the C-terminal end of α-paramyosin accounts for the marked difference in solubility of the two forms.

Paramyosin is a major protein in the thick filaments of molluscan adductor muscles. The protein is a double α-helical rod-like molecule about 1255 Å long and 20 Å in diameter (Lowey et al., 1963; Cohen et al., 1971). Stafford and Yphantis (1972) have reported that paramyosin as extracted from adductor muscles of the clam *Mercenaria mercenaria* by the conventional ethanol-denaturation procedure of Johnson et al. (1959) is in fact a proteolytically degraded form. By including 0.01 M EDTA[1] in all their buffers, Stafford and Yphantis were able to extract a form of paramyosin slightly larger in molecular weight which they called α-paramyosin of 210 000. The conventional form of 200 000 was termed β-paramyosin. The presence of a smaller form termed γ-paramyosin of 188 000 also was observed after some extractive procedures and this was presumed to be a product of more extensive degradation of α-paramyosin.

α-Paramyosin is probably the form that occurs in muscle filaments, and it may function there by virtue of having regions of different stability (Riddiford, 1966; Olander, 1971; Cowgill, 1972, 1974; Halsey and Harrington, 1973). Also, the solubility of paramyosin at physiological conditions may be important for self-aggregation and thus for assembly of the thick filaments of muscle. The extra segment of α-paramyosin may be of importance for this purpose because it has been shown that different segments of β-paramyosin possess different solubility characteristics (Cowgill, 1975) and the solubility of α-paramyosin differs from that of β-paramyosin (Merrick and Johnson, 1974). It seemed important to locate the segment on α-paramyosin that was removed upon degradation and to determine properties such as solubility that could be attributed to that small segment. Therefore it is the purpose of this paper

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[1]Abbreviations are: PTC-1, paramyosin trypsin resistant core; CCF-1 and CCF-2 are the two segments released from paramyosin by cleavage at the site of Cys residues; Nbs_2, Ellman's reagent, 5,5′-dithiobis(2-nitrobenzoic acid); EDTA, ethylenediaminetetraacetic acid; TLC, thin-layer chromatography; Tris, tris(hydroxymethyl)aminomethane.