QUANTITATIVE DIFFERENCES IN PROLINE tRNA CONTENT OF RAT LIVER AND GRANULATION TISSUE

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SUMMARY - The relative amount of proline tRNA in tRNA extracted from rat granulation tissue is shown to be significantly greater than in tRNA similarly prepared from rat liver. Chromatography on methylated albumin-Kieselguhr revealed no qualitative differences between proline tRNAs from the two sources. These findings are considered in terms of a possible coupling between the synthesis of specific tRNAs and the relative rates of incorporation of the related amino acids into proteins.

Recently, in an avian (1) and an insect (2) system an attempt has been made to correlate quantitative changes in the amounts of specific tRNAs with the rates of utilization of the corresponding amino acids. Similar results have not been obtained from mammalian systems for the major reason that no conditions have been found in which genome composition remained constant but the amino composition of the proteins being synthesized changed markedly.

An approach to such a system was suggested by the fact that the fibroblasts of granulation tissue make primarily proteins for intracellular use during an initial period of proliferation and later synthesize collagen in large amounts (3). Since collagen is composed of approximately 30% proline plus hydroxyproline while most proteins contain less than 5% proline, tRNA extracted from granulation tissue would be expected to be enriched in the

proline-accepting species if the intracellular tRNA content were related to the amino acid composition of proteins being synthesized.

MATERIALS AND METHODS

Granulation tissue was produced by sterile subcutaneous implantation of cellulose acetate sponges into young male Holtzman rats. After 5 or 10 days, groups of from 5 to 15 animals were killed by decapitation and then sponges and livers removed. Tissue was routinely formalin fixed, sectioned and stained with Masson's Trichrome stain to estimate grossly the extent of fibroblast proliferation and collagen production.

Transfer RNA was extracted from granulation tissue and liver by homogenization in a Waring blendor and phenol extraction as previously described (7). The tRNA was further purified by DEAE cellulose chromatography (5) and was stripped of amino acids as described by Yang and Novelli (6). The yield was approximately 0.5 mg per gram of liver or granulation tissue.

Uniformly ^{14}C labeled amino acids of specific activity 33.3mc/mM to 455mc/mM were obtained from Schwarz BioResearch and were either used as such or diluted with ^{12}C amino acid to a specific activity of 10mc/mM.

A crude mixture of aminoacyl-tRNA synthetases from rat liver was prepared and the tRNAs assayed as previously described (4). Assay systems were incubated for 10 minutes by which time the reaction had reached a plateau. The amount of ^{14}C aminoacyl-tRNA synthesized was proportional to tRNA input when tested in the range of 0.5 to 1.0 A_{260} units of tRNA/ml assay system.

Methylated albumin Kieselguhr (MAK) chromatography was performed as described by Sueoka and Yamane (8) on 3 cm x 3 cm columns. Reversed phase-Freon chromatography was performed as

described by Weiss and Kelmers (9) on a column measuring 1.5 cm x 250 cm.

RESULTS

Histological appearance. Sponges left in vivo for only 5 days showed at their periphery a loose array of thin-walled blood vessels and young fibroblasts infiltrated by lymphocytes and a few neutrophiles. Very little collagen was present. By 10 days the fibroblastic proliferation was much more extensive and collagen was abundant.

Transfer RNA quantitation. Table I indicates that levels of proline tRNA are greatly elevated in 5- and 10-day granulation tissue when compared to liver. Also, the relative proline tRNA content of granulation tissue appears to increase between the

TABLE I

Amino Acid	Acceptance ($\mu\mu$moles/A_{260})		
		Granulation tissue	
	Liver	5-day	10-day
Pro	4.7	19.5	26.7
Arg	11.9	20.0	17.3
Leu	44.9	40.9	54.5
Thr	27.7	28.4	38.5
Phe	42.3	30.7	36.6
Glu	22.1	28.1	30.9
Gly	14.3	21.6	23.2

Amino acid acceptance of tRNA preparations from rat liver 5-day, and 10-day granulation tissue. Assay systems (0.1 ml) contained 0.5 A_{260} units of liver or granulation tissue tRNAs and the indicated ^{14}C-amino acid at a concentration of 10^{-4}M. The remaining components are previously described (4).

5- and 10-day time points. In this experiment the tRNAs for 6 other amino acids were assayed and, but for arginine (see below), there was no striking difference between granulation tissue and liver.

TABLE II

TRANSFER RNA CONTENT OF RAT LIVER AND 10-DAY GRANULATION TISSUE

Amino Acid	% of total accept.		Ratio	P
	Liver	Gran. tissue	Gran. tissue/liver	
Pro	1.8	3.6	2.00	0.01<P<0.02
Arg	3.7	5.7	1.51	0.05<P<0.1
Gly	8.2	10.6	1.29	*
Tyr	2.7	3.1	1.15	*
Asp	7.9	8.5	1.08	*
Asn	11.9	12.4	1.04	*
Glu	3.6	3.7	1.03	*
Ser	6.5	6.4	0.98	*
Gln	9.4	9.2	0.98	*
Ala	9.5	9.1	0.96	*
His	2.4	2.3	0.96	*
Lys	2.7	2.5	0.93	*
Ileu	2.8	2.5	0.89	*
Val	4.8	3.9	0.81	*
Thr	9.7	7.4	0.76	*
Phe	5.1	3.7	0.73	0.05<P<0.1
Leu	7.7	5.4	0.70	*

Compiled data in which each amino acid acceptance was determined in from 3-5 separate experiments at an amino acid concentration of 2×10^{-6}M. P denotes probability that difference between liver and 10-day granulation tissue is due to chance alone. (*) denotes P> 0.2. Since not all amino acids were assayed in each experiment, the percentage of total was calculated by assuming that in a given preparation each tRNA made up a constant proportion of the total acceptance and then normalizing to a calculated value for total acceptance. Total acceptance of both liver and granulation tissue tRNA preparations was from 400-500 $\mu\mu M/A_{260}$.

In Table II data on amounts of tRNAs for 17 amino acids is presented in terms of percentage of total amino acid acceptance capacity. This mode of presentation is designed to minimize the effects of differences in purity and minor variations in assay conditions between experiments. Values for percentage of total acceptance were evaluated using Student's t-test and only the difference in proline tRNA between liver and granulation tissue

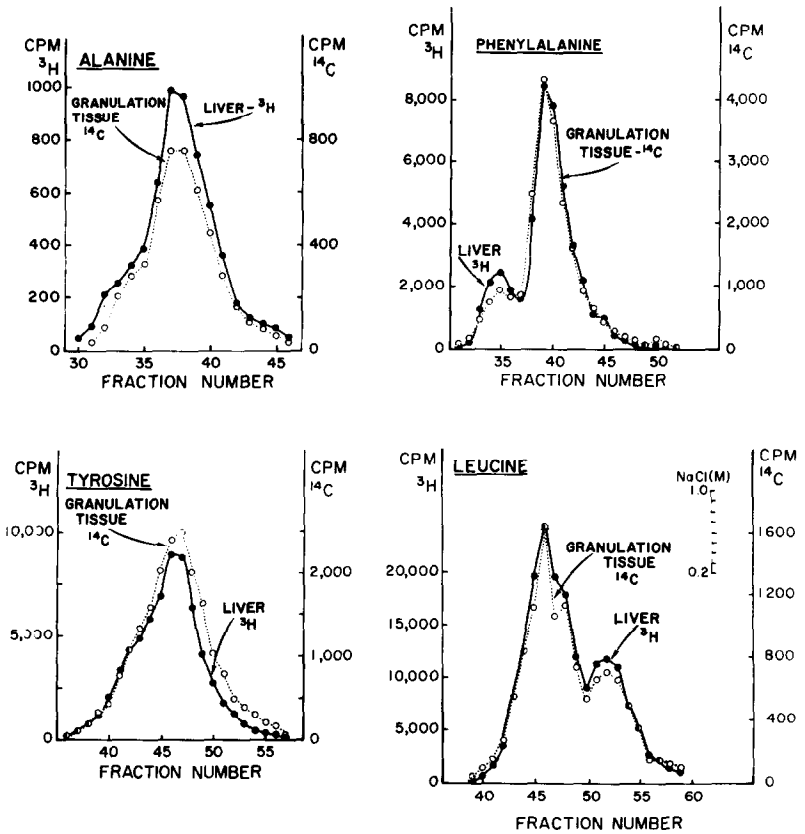

Figure 1. One milligram amounts of tRNA from granulation tissue and liver were separately acylated with either ^{3}H or ^{14}C amino acid in a scaled up system identical to that used for assays. After phenol extraction and ethanol precipitation the samples were mixed and applied to a MAK column. The column was washed with 50 ml of a buffer containing 0.2 M NaCl and 0.05 M Na-phosphate (pH 6.7) at a flow rate of 1.0 ml/min. The tRNA was eluted with a 200 ml linear gradient of 0.2 to 1.0 M NaCl in the same buffer. Fractions (2.5 ml) were precipitated by addition of 2.5 ml of cold 10% trichloroacetic acid and 200 µg of carrier DNA. The precipitate was collected on membrane filters which were dried and counted in toluene scintillation fluid.

was found to be significant at the 5% level. There is a suggestion in this study and also in the data of Table I of a relative increase in arginine tRNA in the granulation tissue but this is not statistically significant.

MAK and reversed phase-Freon chromatography. Co-chromatography of liver and 10-day granulation tissue tRNA acylated with phenylalanine, alanine, leucine and tyrosine was performed with the results shown in Figure 1. Elution patterns for these amino-acylated tRNAs are nearly identical and are similar to those previously reported for normal rat liver (10). MAK and reversed phase-Freon chromatography of these tRNAs acylated with proline revealed only slight qualitative differences which are not considered significant. (Figure 2).

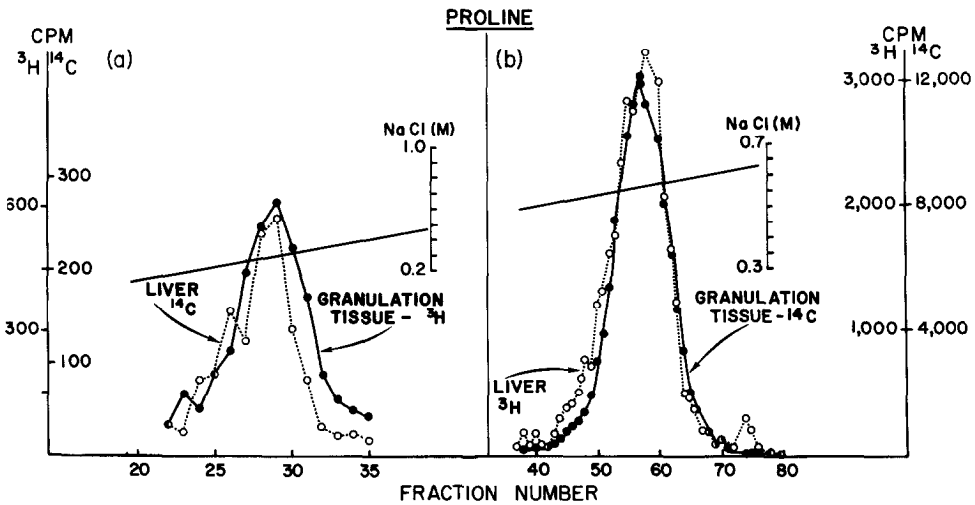

Figure 2. (a) MAK chromatography of tRNAs acylated with proline was performed as described in Figure 1. (b) Reversed phase-Freon chromatography was performed on tRNAs which had been acylated with proline as described above. After phenol extraction of the reaction mixtures and ethanol precipitation, the tRNAs were redissolved, applied to the column and eluted with a 1000 ml linear gradient of 0.35 M to 0.65 M NaCl in buffer containing 0.01 M Na-acetate (pH 4.5) and 0.01 M $MgCl_2$. Fractions (10.0 ml) were collected, precipitated with carrier and an equal volume of 10% trichloroacetic acid, collected on membrane filters, dried and counted in toluene scintillation fluid.

DISCUSSION

The major finding of this study is that the relative amount of proline tRNA is not the same in all mammalian tissues. Specifically, it is at least twice as abundant in granulation tissue as in liver. Since both of these tissues are composed of several cell types, it is likely that individual cells have tRNA populations which are even more divergent quantitatively.

It is tempting to suggest that this relative increase in proline tRNA reflects the disproportionate requirement for proline in collagen synthesis.

The elevated level of proline tRNA in 5-day granulation tissue suggests that preparation for collagen synthesis begins very early in these fibroblasts, or that this property may be a permanent characteristic of cells which have differentiated along these lines. The present data do not indicate whether or not fully differentiated fibroblasts can actually alter their tRNA populations to suit their changing metabolic needs. In order to resolve this question it will be necessary to examine tRNA populations of fibroblasts at different stages in tissue culture where the rate of collagen production can be controlled (11).

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