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TRANSFER RIBONUCLEIC ACIDS FROM ELEVEN IMMUNOGLOBULIN-SECRETING MOUSE PLASMACYTOMAS

CONSTANT AND VARIABLE CHROMATOGRAPHIC PROFILES COMPARED WITH THE MYELOMA PROTEIN SEQUENCES

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Summary

In order to test the concepts that aminoacyl-tRNAs in plasmacytomas may on the one hand modulate the protein synthesized or on the other hand reflect the structure of the synthesized protein, the RPC-5 chromatographic profiles of aminoacyl-tRNAs for all 20 amino acids were studied in tRNA prepared from normal mouse liver and 11 plasmacytomas. The patterns of isoaccepting tRNA were compared with the structure of the myeloma protein being synthesized.

The elution profiles of aminoacyl-tRNAs for nine of the amino acids were constant, i.e. they were the same for liver and all plasmacytomas. Significant variability was observed in the profiles of the other 11 families of aminoacyl-tRNAs: asparagine, serine and tryptophan, had peaks of isoaccepting tRNAs found in tumors and not in liver; glutamic acid, histidine and lysine, had different patterns of aminoacyl-tRNAs in plasmacytomas which could be distinguished from the elution profile of liver; and isoleucine, proline, threonine and tyrosine, showed pattern variability in only a few of the tumors. Valyl-tRNA uniquely had one isoacceptor present in liver but absent in the tumors. This variability is thought to be associated with different posttranscriptional modification of the tRNAs rather than regulation of individual tRNA genes in response to particular amino acid sequences in secreted myeloma proteins. Similarly, the lack of correlation of isoacceptors with sequence differences makes the modulation of protein fine structure by tRNA availability unlikely.

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Abbreviations: IgA, immunoglobulin A; IgG, immunoglobulin G; Tris, tris(hydroxymethyl)aminomethane; dpm, disintegrations · min⁻¹.

Introduction

Chromatographic studies of tRNA from prokaryotes and eukaryotes have revealed a wide spectrum of isoaccepting tRNAs that far exceeds the number of triplet codewords. Some of the redundant tRNAs could be available for regulatory roles, especially since they are known to vary in number and relative amounts in different organisms, different tissues, and under different biological conditions (For reviews see Refs. 1–5). A correlation between the levels of aminoacyl-tRNAs within a cell and the amino acid composition of its proteins has been described for a number of systems: rat fibroblasts [6], hormone-treated chicken liver and oviduct [7,8], mammary gland [9], sheep reticulocytes [10], rabbit reticulocytes [11], and silk worm silk gland [12]. Furthermore, a regulatory role for tRNA in eukaryotes has been inferred in interferon-treated cells [13].

Potter et al. [14] proposed that the fine structure of immunoglobulin gene products could be regulated during translation by the presence of rare, ambiguous triplets in key areas of the mRNAs. These could be translated differently by unique, individual populations of isoaccepting tRNAs which each clone of immunocytes would develop and stabilize at the time of its final differentiation. This hypothesis was investigated by a number of workers studying a limited number of tRNAs and plasmacytomas [15–20]. These reports were conflicting, in that they did show stable, reproducible differences for a few aminoacyl-tRNAs among plasmacytomas secreting different light and heavy chains, but failed to show a regulatory role for plasmacytoma leucyl-tRNA in a reticulocyte cell-free protein synthesizing system.

Currently, the most widely accepted theories of antibody diversity point to a genetic determination through the selection of different structural genes rather than regulation at the translational level. Furthermore, an inverted alternative to translational regulation by tRNA has been presented in Garel's 'Functional Adaptation' theory [21], according to which, at any given time and in any tissue, a cell's tRNA populations are varied, through continuous selective adjustment of tRNA synthesis, to meet mRNA demands.

These theories and problems have inspired the accumulation of an impressive number of anecdotal descriptions of a few aminoacyl-tRNAs in a wide variety of organisms and tissues and in a wide variety of physiological and pathological states. However, it was felt that a complete study of aminoacyl-tRNA patterns in a carefully selected genetically and histologically similar group of tissues was badly needed to adequately examine both concepts that correlate tRNA populations with protein structure. To this end a complete study was undertaken of all the aminoacyl-tRNAs present in 11 mouse plasmacytomas, selected from the large library induced and characterized by Michael Potter [22]. Our study was designed to investigate whether the tRNA populations were 'functionally adapted' to the synthetic products of these plasmacytomas and if these extensive studies revealed evidence supporting tRNA as a modulator of protein synthesis in these tumors. In particular, we asked (1) if any aminoacyl-tRNAs were found only in tumors; (2) if any aminoacyl-tRNA profiles were correlated with the production or non-production of immunoglobulin; (3) if any profiles were correlated with the production of light or heavy chain or certain classes of

these chains; and (4) if any isoaccepting tRNAs could be correlated with the amino acid sequence of the hypervariable regions of light or heavy chain.

Materials and Methods

Plasmacytomas. The following groups of BALB/c plasmacytomas were compared: (1) MPC 11, which secretes immunoglobulin G (IgG), and NP 2, a tumor from a mutagenized tissue cultured clone of MPC 11 in which immunoglobulin secretion had ceased [23]; (2) B47P, derived from a single cell clone of MPC 11, and B47L3NP1, a mutagenized clone of B47P which had ceased protein production (Scharff, M.D., personal communication); (3) MOPC 321 and TEPC 124, two independently induced tumors producing only kappa light chains with three amino acid differences, in two of the hypervariable regions [24]; (4) TEPC 15 and HOPC 8, independently induced plasmacytomas which secrete immunoglobulin A (IgA) myelomas proteins that bind phosphocholine and have only one amino acid difference, in the third hypervariable region of the heavy chain, [25,26]; and (5) Adj.PC 6A and Adj.PC 6A', two sublines of Adj.PC 6A, the first of which secretes an IgA with euglobulin properties and the second of which makes an IgA without euglobulin properties (unpublished observation) and Adj.PC 6C, a tumor originating in the same primary tumor mouse as Adj.PC 6A but which secretes an IgA protein with a large deletion in the constant portion of the heavy chain [27]. These tumors, their derivation and characteristics are summarized in Table I.

MPC 11, NP 2, B47P, and B47L3NP1 were grown as solid tumors from tissue culture cells cloned, described and kindly provided by Dr. Matthew D. Scharff. The other tumors were induced and characterized in the laboratory of Dr. Michael Potter. All tumors were of BALB/c origin, were grown subcutaneously in the flanks of BALB/c mice, and were transplanted or harvested when they had reached a diameter of approximately 20 mm. The growth rates of all tumors were approximately the same, requiring transplantation about every 2–3 weeks.

tRNA and aminoacylating enzymes. tRNA was prepared by homogenization of minced tissue in isotonic sucrose buffer, extraction with pH 7 buffered phenol of post-nuclear supernates, and ethanol precipitation. After deacylation at pH 8, high molecular weight rRNA was precipitated at 4°C by bringing the solution to 1 M in NaCl. The supernatant was dialyzed exhaustively against 0.1 mM MgCl₂ at pH 7–8 and then lyophilized. Further fractionation or purification was eschewed lest unequal losses of certain isoacceptors might occur and affect the final chromatographic profiles. Aminoacylating enzymes were prepared after homogenization in 2-mercaptoethanol- and sucrose-containing isotonic buffer, ribosomes removed by centrifugation, and the supernatant passed through a Sephadex® G-100 column. The excluded fractions were pooled, concentrated by ultrafiltration, mixed with an equal volume of glycerol and stored at –15°C. For details of the above procedures see Ref. 28. Both normal mouse liver, mouse plasmacytoma and rat liver synthetases were tested, and the rat liver enzyme preparation was used for aminoacylating all the tRNAs since it generally charged the most actively and produced the same aminoacyl-tRNA profiles as the mouse liver and tumor preparations. The sources and

TABLE I
TISSUES STUDIED FOR tRNA PROFILE COMPARISON AND THEIR CHARACTERISTICS

Group	Tumor	Characteristics	Possible correlation with tRNA profiles
A. Normal	1. —	BALB/c Liver	multiple secretory proteins
B. Four tumors derived from an IgG _{2b} (κ) producing plasmacytoma	2. MPC11 3. NP2 4. B47P 5. B47L3NP1	Producer Non-Producer Producer Clone Non-Producing Clone	Ig production or cessation of production, cloning of cells, mutagenesis
C. Two κ secretors with minimal V _L differences	6. MOPC321 7. TEPC124	26 27 27A 27B 27C 27D 28 Ala <u>Lys</u> Ser Val Asn Thr Tyr . . . Glx <u>Asx</u> Pro Ala <u>Gln</u> Ser Val Asx <u>Trp</u> Tyr . . . Glx <u>Ala</u> Pro	κ chain sequence variations in hypervariable regions
D. Two IgA(κ) tumors with minimal V _H differences	8. HOPC8 9. TEPC15	96 97 98 99 100 101 Tyr Tyr Gly Asn Ser Tyr Tyr Tyr Gly Ser Ser Tyr	α chain sequence variations in a hypervariable region
E. Three IgA(κ) tumors with VH identity but different C _H	10. Adi.PC 6A 11. Adi.PC 6A' 12. Adi.PC 6C	Euglobulin IgA _p Non-Euglobulin IgA _p Half molecule IgA _H (C _H ₃ deletion)	α chain variations in the constant region
F. More normal tissues	13. —	NIH/Swiss livers and brains	specialized, differentiated tissues

TABLE II
AMINO ACIDS USED IN THE ACYLATING REACTION

L-Amino acid	Specific activity (mCi/nmol)	Commercial source	$\mu\text{Ci}/100 \mu\text{l}$ of incubation mixture
[³ H]Ala	5 500	Schwarz/Mann	55
[¹⁴ C]Ala	156		2.75
[³ H]Arg	1 300 or 7 000		40 or 20
[¹⁴ C]Arg	312		2
[³ H]Asn	12 000		80
[¹⁴ C]Asn	220	New England Nuclear	2.8
[³ H]Asp	23 000		25
[¹⁴ C]Asp	208	Schwarz/Mann	2.75
[³ H]Cys	47 000		20
[¹⁴ C]Cys	295		4
[³ H]Gln	26 000	Amersham/Searle	20
[¹⁴ C]Gln	213	New England Nuclear	2
[³ H]Glu	19 000	Schwarz/Mann	40
[¹⁴ C]Glu	246		1
[³ H]Gly	23 000		5
[¹⁴ C]Gly	106		0.5
[³ H]His	3 100		40
[¹⁴ C]His	312		2
[³ H]Ile	8 000		30
[¹⁴ C]Ile	309		3
[³ H]Leu	6 000		55
[¹⁴ C]Leu	312		2.75
[³ H]Lys	8 000		10
[¹⁴ C]Lys	300		1
[³ H]Met	700 or 500		25 or 17.5
[³⁵ S]Met	2 870		22.5
[³ H]Pro	2 500		20
[¹⁴ C]Pro	260		1
[³ H]Ser	1 600		27.5
[¹⁴ C]Ser	156		2.75
[³ H]Phe	6 000		60
[³ H]Phe	1 500	New England Nuclear	0.6
[¹⁴ C]Phe	460	Schwarz/Mann	3
[³ H]Thr	2 900		20
[¹⁴ C]Thr	208		1
[³ H]Trp	1 000	Amersham/Searle	55
[¹⁴ C]Trp	52		2.75
[³ H]Tyr	8 000	Schwarz/Mann	27.5
[¹⁴ C]Tyr	460		2.75
[³ H]Val	6 000		60
[¹⁴ C]Val	260		3

activities of the radioactive amino acids used are listed in Table II.

Aminoacylation reactions. Aminoacylation conditions were optimized for each amino acid and for each enzyme preparation using normal mouse liver tRNA in the assay. Conditions were chosen primarily on the basis of maximum yield of counts of radioactive aminoacyl-tRNA and reproducibility of tRNA profiles, but it was not always possible to work at saturating conditions for each isotopically labeled amino acid. The concentration of enzyme yielding the highest plateau level of aminoacyl-tRNA and the length of incubation at 37°C were the first variables optimized. Synthetase preparations with endogenous

aminoacylation greater than 5% of the maximum plateau value were discarded. Then isotope, ATP, and Mg^{2+} concentrations and pH were individually varied until the reaction was maximal and dependent upon tRNA concentration. Most of the amino acids required the following reaction conditions: 100 mM Tris-HCl, pH 7.6, 10 mM MgCl₂, 14 mM 2-mercaptoethanol, 0.25 mM each of 19 amino acids minus the radioactive one, 5 mM neutralized Na₂ATP, radioactive amino acid as indicated in Table II, 0.75–2.0 mg · ml⁻¹ of tRNA, and 0.2–0.6 A₂₈₀ of enzyme. Exceptions to these conditions were: (1) 10 mM Na₂ATP was used with cysteine, glutamic acid, glycine, methionine, and tryptophan; (2) 20 mM MgCl₂ with methionine; (3) pH 7.2 with alanine and pH 7.8 with tyrosine. Low efficiency of aminoacylation with glutamic acid was attributed to dilution of the isotope with [¹²C]glutamic acid which had been created by deamidation of a portion of the excess of glutamine in the '19-minus glutamic acid' solution. When an '18-minus glutamine and glutamic acid' solution was substituted, aminoacylation with glutamic acid improved markedly. Plateaus of aminoacylation were generally reached after 25 min at 37°C, and the reactions were stopped by addition of 3–4 volumes of cold 0.4 M NaCl, pH 4.5, column buffer (see below). The labeled aminoacyl-tRNA was extracted by repeated shaking with an equal volume of buffer-saturated phenol. Aminoacyl-tRNAs were precipitated with 3 volumes of ethanol, stored overnight at -15°C, centrifuged to collect the precipitate which was then dissolved in 100 µl of 0.4 M NaCl in 10 mM sodium acetate and magnesium acetate, pH 4.5. Free radioactive amino acids were removed from the aminoacyl-tRNAs by chromatography on 5-ml columns of Sephadex® G-25, and the aminoacyl-tRNAs were stored at -15°C. Total amino acid acceptance varied considerably with different enzyme and tRNA preparations for several amino acids. The large number of combinations of amino acids and tRNA preparations precluded an extensive study of total acceptance for each amino acid, so the analysis was restricted to an examination of the qualitative and reproducible differences among RPC-5 chromatographic profiles.

RPC-5 chromatography. ³H-labeled and ¹⁴C-labeled aminoacyl-tRNAs to be compared were mixed and chromatographed on a 0.6 × 30 cm RPC-5 column [29] which was developed at 400 lb/inch² at 38°C with a 150 ml gradient of NaCl in 10 mM sodium acetate and magnesium acetate and 1 mM 2-mercaptoethanol, pH 4.5. The linear NaCl gradients used to resolve the isoaccepting aminoacyl-tRNAs for the various amino acids were selected from trial chromatographic runs performed to maximize resolution. The gradients chosen are listed in Table III. After loading the sample, columns were washed with 60–80 ml of 0.4 M NaCl, developed with the appropriate gradient, and flushed with 20 ml of 2 M NaCl in the same buffer. Fractions of 1.6 ml were collected directly in glass scintillation vials; 12 ml Aquasol® were added to each vial, and the vials (100/column run) were counted twice in a liquid scintillation counter with external standardization. Disintegrations per minute (dpm) for each isotope were calculated from the averaged counts and a plot made by a PDP-11 computer and Cal Comp plotter such as illustrated in Fig. 2.

In all cases where different patterns were observed during cochromatography, the possibilities that the differences were due to the particular isotope or incomplete aminoacylation were ruled out by repeating the chromatography

TABLE III

STARTING AND ENDING NaCl CONCENTRATIONS CHOSEN FOR GRADIENT ELUTION OF AMINOACYL-tRNAs FROM RPC-5 COLUMNS

Aminoacyl-tRNA	NaCl molarity
Ala, Ile	0.50-0.60
Met	0.50-0.65
Asp, Cys, Gln, Glu, Gly, Pro	0.50-0.70
Asn, His, Val	0.55-0.65
Leu, Lys, Thr, Tyr	0.55-0.70
Arg	0.55-0.75
Trp	0.60-0.70
Ser	0.60-0.75
Phe	0.65-0.70

with reversed labels and aminoacylation with different enzyme preparations.

Reproducibility of column profiles. It had been previously demonstrated in our laboratory that, at least for several aminoacyl-tRNAs, the RPC-5 column profiles of tumor tRNAs remained virtually unchanged over the course of many transplant generations and over a period of years [18]. In the work reported here, we made multiple preparations of tRNA from most of the tumors, and there were very few differences noted when various aminoacyl-tRNA column profiles were compared for different preparations.

The cloned lines B47P and B47L3NP1, which had to be grown up from tissue culture several times but were maintained as subcutaneous tumors for long periods as well, gave less consistent profiles of glutamyl-tRNA than the other plasmacytomas. Repeated tRNA preparations were undertaken and tRNA patterns that were present in more than one preparation were preferred to those that seemed anomalous.

The deamidation of asparagine and glutamine may play important roles in influencing reproducibility in asparagine and glutamic acids-tRNA patterns. Tiny amounts of a late eluting peak, now thought to be aspartyl-tRNA, can be seen in some asparaginyl-tRNA profiles (cf. TEPC 15 and Adj.PC 6A'-non Eu in Fig. 3), and were not considered in the profile evaluation. The difficulties with glutamic acid have been described above.

Computer analysis. The double-label chromatographic profiles facilitate the visual comparison of any two aminoacyl-tRNA profiles. To compare and evaluate the over 500 profiles generated in this study, the data from all the profiles were compiled and analyzed using an IBM-370 computer. This consisted of (1) the creation of composites of the liver and 11 plasmacytoma profiles of isoacceptors for each amino acid suitable for visual comparison and (2) the calculation of a numerical index of similarity for all possible pairs of profiles for each amino acid. Both required a program to normalize and align the profiles from different column runs so that comparisons would not be hindered by small variations in column elution. This alignment made possible the construction of a vertical composite of the 12 profiles for each amino acid which could be plotted by the Cal Comp plotter. These composites are presented for all 20 amino acids in Figs. 1 and 3-5 where the plasmacytoma profiles are lined up un-

der the liver profile in the same order as that in Table I. The similarity index presented in this report is the linear correlation coefficient *, r , calculated from corresponding points in the two profiles being compared. This coefficient is 1.0 for identical profiles and decreases toward 0.0 as the profiles become more dissimilar. Other measures of similarity have been applied to these profiles, and the details of this and other methods of comparing profiles will be presented elsewhere. For the calculations and composite construction, the initial 10–20 fractions, which frequently contain a broad peak of background from deacylation, and the final 5–10 fractions, which often show a narrow peak of radioactivity from the 2 M NaCl flush, are not included, since these variable peaks are not thought to represent true peaks of aminoacyl-tRNA. Examples of these two peaks can be seen in Fig. 2 which shows an original, double-label chromatogram without deletion of any portion of the profiles.

Results

Minor quantitative variations

The aminoacyl-tRNAs for nine amino acids manifested very similar profiles in tRNA from liver and the 11 plasmacytomas examined. Composites of these RPC-5 profiles are shown in Figs. 1A and 1B. Fig. 1A contains the profiles of alanyl-, glutamyl-, glycyl-, methionyl-, and phenylalanyl-tRNAs which showed virtually no differences both among tumors and when the tumors were compared with liver (correlation coefficient, $r = 0.92$). The profiles shown in Fig. 1B show a few, minor differences which are summarized below. Arginyl-tRNAs from the different sources have some differences in the second, large peak, although the relative amounts of the two peaks did not change appreciably. We suspect that these variations may reflect some unresolved heterogeneity. Aspartyl-, cysteinyl-, and leucyl-tRNAs had small quantitative differences in the amounts of minor peaks following the major peak of isoaccepting tRNA.

Valyl-tRNA

The final composite in Fig. 1B illustrates that the valyl-tRNAs from all the tumors examined in this study have very similar elution profiles. Poor resolution on RPC-5 columns prevents us from enumerating the number of isoacceptors of valyl-tRNAs present in tumors and in liver. The most interesting finding is the presence of a prominent first eluting peak in the liver profile, which is rudimentary at best in all the tumor profiles. By this criterion the elution profiles of liver valyl-tRNAs can be distinguished from all the tumors' profiles ($r(\text{liver vs. HOPC } 8) = 0.90$ while $r(\text{HOPC } 8 \text{ vs TEPC } 15) = 0.99$). The presence of a liver-specific peak is summarized in the double-label chromatogram shown in Fig. 2.

* The correlation coefficient,

$$r = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{ns_x s_y}$$

where x_i = dpm of aminoacyl-tRNA_x in fraction i, y_i = dpm of aminoacyl-tRNA_y in the same fraction i, s_x and s_y are the standard deviations of the two sets of data, and n = number of fractions being compared.

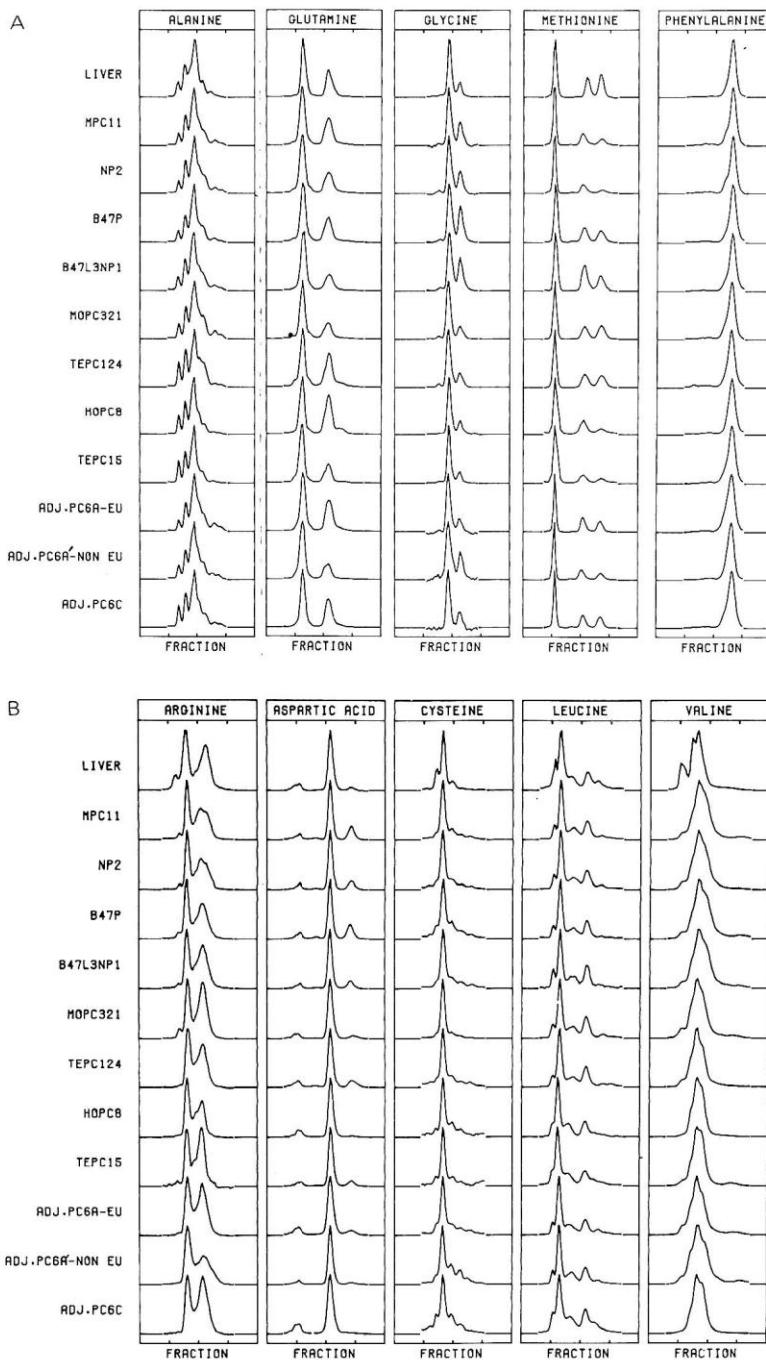


Fig. 1. RPC-5 column chromatograms of the indicated aminoacyl-tRNAs prepared from BALB/c liver or plasmacytomas as indicated using rat liver aminoacyl-tRNA synthetase as detailed in the text. Representative chromatograms from tumors and liver were lined up and plotted by computer. (A) Five composite profiles of aminoacyl-tRNAs with minor quantitative differences. (B) Five composites with a greater degree of variation among profiles. Valyl-tRNA is presented in greater detail in Fig. 2.

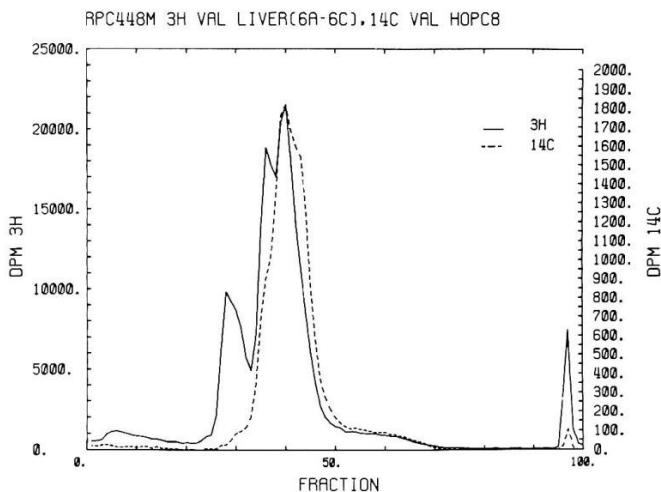


Fig. 2. Double-label RPC-5 chromatogram of ^3H -labeled Val-tRNA (—) from mouse liver (pooled from animals bearing plasmacytomas Adj.PC 6A and Adj.PC 6C) cochromatographed with ^{14}C -labeled Val-tRNA (----) from plasmacytoma HOPC 8. Note the prominence of the first peak in liver which is not seen in this or any of the tumors studied (cf. Fig. 1B).

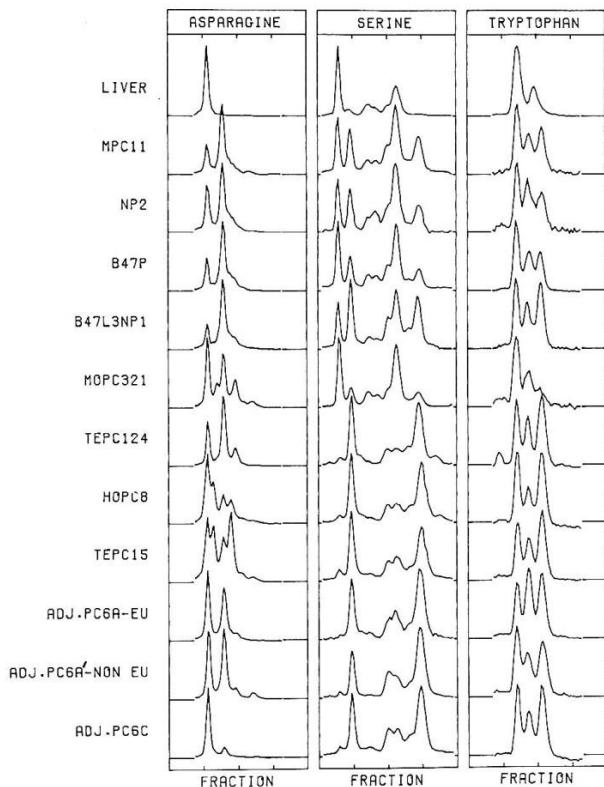


Fig. 3. Composites of RPC-5 chromatograms for three amino acids with peaks of aminoacyl-tRNA present in plasmacytomas but absent from liver profiles.

Tumor-associated aminoacyl-tRNAs

The remaining ten amino acids have considerable variability among the elution profiles observed in their aminoacyl-tRNAs from liver and the 11 plasmacytomas examined. There are three amino acids for which there appeared to exist an isoacceptor that was present in the aminoacyl-tRNAs of tumors but was absent from the liver profile. These include asparagine, serine and tryptophan. Their elution profiles are shown in Fig. 3.

Asparaginyl-tRNA

Liver asparaginyl-tRNA appears to have only one component which coelutes with the first of the multiple peaks generally found in tumors. All the plasmacytomas are similar in that they have more than one peak of asparaginyl-tRNA, but they differ greatly in the number of peaks and the relative amounts of each of the peaks. The two Adj.PC 6A lines and the four clones related to MPC 11 have a very prominent peak 3 besides peak 1. MOPC 321, TEPC 124, HOPC 8 and TEPC 15 have, in addition to peaks 1 and 3, prominent peaks 2 and 4. Adj.PC 6C, on the other hand, is more like liver with only a small peak 3 ($r(\text{Adj.PC } 6\text{C vs. liver}) = 0.99$).

Seryl-tRNA

Several late eluting peaks are observed in the elution profiles of seryl-tRNA of plasmacytomas, which are not found in liver. Only the latest eluting peak seems to be completely absent from the elution pattern of seryl-tRNA from normal liver, but the patterns of most tumors generally resemble the liver pattern, only shifted toward the right side of the graph, i.e. toward the higher salt concentration of the gradient. Examples of 'shifted' seryl-tRNA patterns are seen in the lowest six profiles in the seryl-tRNA composite. The MOPC 321 profile is very much like that of liver with the addition of a small final peak and increased amounts of the final peaks seen in liver. In MPC 11 and its related clones a mixture of the liver and tumor patterns can be discerned. The liver-like seryl-tRNA profile of MOPC 321 has a correlation coefficient, r , of 0.93 compared with liver but only 0.51 when compared with TEPC 124. The correlation coefficient, r , of the seryl-tRNA profiles of Adj.PC 6A and Adj.PC 6A', both which have typical tumor patterns is 0.91. However, the r value comparing the profiles of Adj.PC 6A and liver is 0.33.

Tryptophanyl-tRNA

A still different situation is present for tryptophanyl-tRNA where all the tumor profiles examined have at least three peaks, whereas liver has only two. Some of the tumors have, in addition, a very early eluting peak. However, while liver peak 1 coelutes with tumor peak 1, liver peak 2 appears to elute somewhere between tumor peaks 2 and 3. Although the last two peaks of tryptophanyl-tRNA in profiles from tumors could not be completely resolved by RPC-5 chromatography it appeared on some double-label column runs that a peak eluting in the position of liver peak 2 could be seen as a shoulder or small peak in the trough between tumor peaks 2 and 3. Thus it is very likely that there may be a small amount of liver peak 2 in the tumor profiles and possibly some tumor peak 2 in liver, but there does not seem to be a significant amount

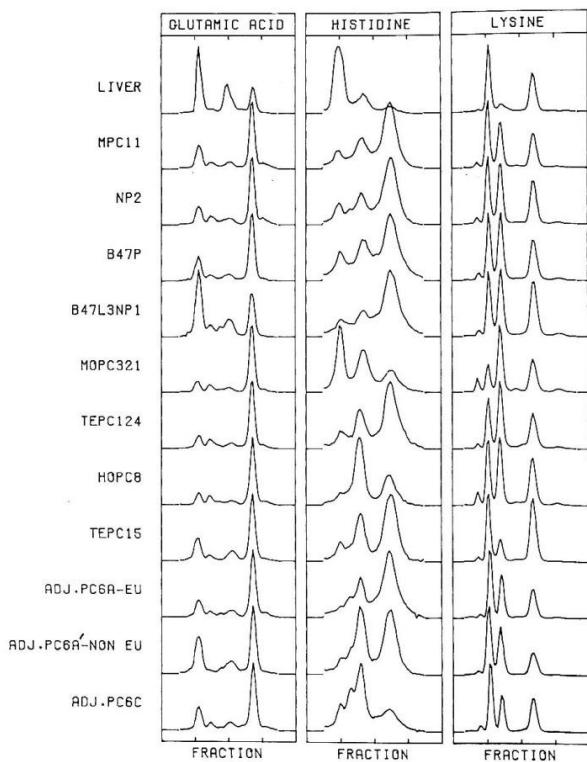


Fig. 4. Composites of RPC-5 chromatograms for three amino acids with aminoacyl-tRNA profiles characteristic of most of the plasmacytomas but different from the patterns seen in normal liver.

of tumor peak 3 in liver tryptophanyl-tRNA. Similar to the finding for seryl-tRNA, MOPC 321 has the most liver-like profile of tryptophanyl-tRNA among the plasmacytoma profiles examined. ($r(MOPC\ 321\ vs.\ liver) = 0.96$ while $r(MOPC\ 321\ vs.\ TEPC\ 124) = 0.82$ and $r(TEPC\ 124\ vs\ liver) = 0.75$).

Characteristic tumor profiles

Fig. 4 illustrates the aminoacyl-tRNA elution profiles for glutamic acid, histidine and lysine, the amino acids for which there exists a great variability in the relative amounts of the various isoaccepting tRNAs among plasmacytomas and liver. In almost all the tumors there is a characteristic, recognizable tumor profile, but there does not seem to be any particular peak of aminoacyl-tRNA found only in tumors or only in liver.

Glutamyl-tRNA

RPC-5 chromatography resolves glutamyl-tRNA into four or five peaks, only three of which are prominent in the liver profile and which diminish in amplitude from left to right. Although, as mentioned above, some variations in the profiles of glutamyl-tRNA have been observed among different tRNA preparations from the same plasmacytoma, it has been consistently observed that the

first three peaks are less abundant than peak 4 in most plasmacytomas. Of course, additional, unresolved isoacceptors may be present, especially in the area of peak 3, which in some column profiles appears heterogeneous.

Histidinyl-tRNA

With a few exceptions histidinyl-tRNA has a characteristic tumor profile where the relative amounts of the isoaccepting species are the reverse of the elution profile seen for liver. That is, the liver pattern consists of three peaks (a minimum estimate) which decrease in amplitude from left to right, but the four MPC 11 tumors, TEPC 124, and TEPC 15 profiles have the same peaks with increasing amplitude. The peaks may not be homogeneous, and the presence of minor histidinyl-tRNA species within the three major peaks becomes more obvious in HOPC 8 and the three Adj.PC 6 tumor profiles where peak 2 is particularly abundant. As noted previously for seryl-tRNA and tryptophanyl-tRNA, the histidinyl-tRNA of MOPC 321 has a liver-like pattern ($r(MOPC\ 321\ vs.\ liver) = 0.87$ while $r(MPC\ 11\ vs.\ liver) = 0.43$).

Lysyl-tRNA

The characteristic tumor pattern for this family of isoaccepting tRNAs is associated with a prominent peak 3 which is present in only minimal amounts in liver. In many of the tumor profiles the initial minor peak is more abundant than it is in liver. Once again MOPC 321 is unique among tumor patterns of lysyl-tRNA with a particularly prominent peak 1 and an unusually small peak 2, but unlike seryl-tRNA, tryptophanyl-tRNA, and histidinyl-tRNA, the pattern of lysyl-tRNA from MOPC 321 does not seem particularly liver like. Instead, the TEPC 15 profile of lysyl-tRNA more closely resembles the liver profile with an unusually small peak 3 for a tumor profile ($r(MOPC\ 321\ vs.\ liver) = 0.61$ and $r(TEPC\ 15\ vs.\ liver) = 0.92$).

Marked variability among tumors and liver

The chromatographic profiles of isoleucyl-, prolyl-, threonyl-, and tyrosinyl-tRNA show considerable variability that could be recognized as characteristic of all tumors. These are illustrated in Fig. 5.

Isoleucyl-tRNA

The isoleucyl-tRNA profiles from all the tumors and liver are very similar with two exceptions, HOPC 8 and TEPC 15. The profiles of these two tumors are characterized by a very high peak 4, making them quite different from that of liver and the other tumors ($r(HOPC\ 8\ vs.\ TEPC\ 15) = 0.99$ while $r(HOPC\ 8\ vs.\ liver) = 0.72$ and $r(MOPC\ 321\ vs.\ liver) = 0.98$).

Prolyl-tRNA

The three peaks apparent in chromatograms of liver prolyl-tRNA are also present in the tumor profiles examined, but they are distributed differently. Peak 1 is small in the profiles of HOPC 8, Adj.PC 6A' and Adj.PC 6C; TEPC 15 and Adj.PC 6A' are the only tumors having prolyl-tRNA profiles with peak 3 lower than peak 2.

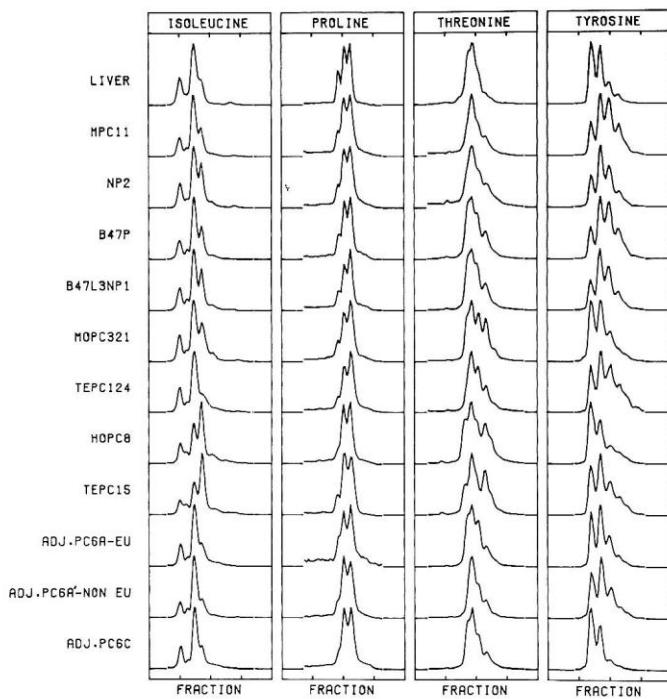


Fig. 5. Composites of RPC-5 chromatograms with a variability of aminoacyl-tRNA profiles limited to only a few tumors.

Threonyl-tRNA

These isoacceptors were not well resolved on RPC-5 column chromatography, but the liver profile may be described as three major peaks preceded by and followed by two very small peaks. The first major peak is virtually absent in the profiles of TEPC 124 and Adj.PC 6A', while the other two major peaks appear in all tumors in varying amounts. The two small peaks that follow the three major peaks in liver show the most variability in the profiles of threonyl-tRNA. These two isoacceptors are quite prominent in most tumors and make up as much as 30% of the total threonyl-tRNA in MOPC 321, HOPC 8, and TEPC 15.

Tyrosinyl-tRNA

The tyrosinyl-tRNA isoacceptors also show a variable distribution of peaks in the chromatographic profiles examined here. Peak 1 is low in the four MPC 11 derived plasmacytomas, while peak 3 is characteristically high. In TEPC 124 the peaks following peak 3 are particularly abundant. The profiles of HOPC 8 and Adj.PC 6C were most like that of liver in the scarcity of peak 3 and the peaks following it.

Brain tRNAs

The isoaccepting tRNAs which had displayed the most variability in the chromatographic profiles of liver and plasmacytomas were also studied in

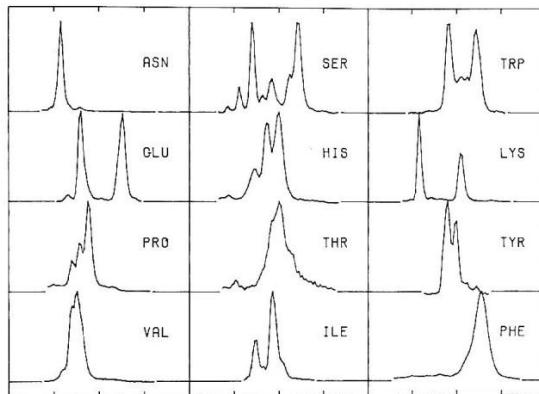


Fig. 6. RPC-5 profiles of mouse brain aminoacyl-tRNAs for 12 amino acids as indicated. The profiles are arranged for comparison with the composites of other tissues' profiles shown in Figs. 1—5.

mouse brain. Brain was chosen as another example of a normal, differentiated tissue. Fig. 6 illustrates the aminoacyl-tRNA profiles obtained after aminoacylating tRNA from pooled adult mouse brains. By comparison with the appropriate preceding figure, the brain profiles of each aminoacyl-tRNA can be classified as being similar to liver or similar to those patterns previously characterized as tumor like. These are summarized in Table IV. Since asparaginyl-tRNA of brain lacked the peak 2 characteristic of all plasmacytomas it is classified as resembling liver. Lysyl-, prolyl-, threonyl-, tyrosinyl-, isoleucyl-, and phenylalanyl-tRNA profiles also resembled the chromatographic profiles of liver more than those patterns common to most plasmacytoma profiles. The presence of some, but not all, 'shifted' peaks of seryl-tRNA in brain and the presence in brain of patterns of peaks characteristic of the chromatographic profiles of most plasmacytomas' tryptophanyl-tRNA, and histidinyl-tRNA placed these in the tumor-like category. Brain valyl-tRNA does not have the large initial peak found in liver profiles, so it was called tumor like despite a correlation coeffi-

TABLE IV
CHARACTERISTICS OF BRAIN PROFILES OF TWELVE AMINOACYL-tRNAs

Brain resembles liver		Brain resembles tumors		Brain resembles neither	
Aminoacyl-tRNA	r(liver) *	Aminoacyl-tRNA	r(liver)	Aminoacyl-tRNA	r(liver)
Asparaginyl-tRNA	0.97	Seryl-tRNA	0.75	Glutamyl-tRNA	0.95
Lysyl-tRNA	0.99	Tryptophanyl-tRNA	0.83		
Prolyl-tRNA	0.99	Histidinyl-tRNA	0.55		
Threonyl-tRNA	0.98	Valyl-tRNA	0.98		
Tyrosinyl-tRNA	0.97				
Isoleucyl-tRNA	0.99				
Phenylalanyl-tRNA	1.00				

* r(liver, the correlation coefficient obtained when the brain profile for the aminoacyl-tRNA in question was compared with the liver profile for the same amino acid. See Materials and Methods and footnote on page 259 in the text for details of calculation of r .

cient of 0.98 when compared with the liver profile. The difficulties in obtaining reproducible patterns of glutamyl-tRNA for liver and tumors also affected the brain profiles of glutamyl-tRNA, so we are unable to classify it as liver like or tumor like.

Discussion

The extensive chromatographic study reported here is the first one to include the aminoacyl-tRNA patterns of all 20 amino acids and examine tRNA from multiple plasmacytomas in groups selected because they are closely related in origin or secrete myeloma proteins with similar amino acid sequences. The aminoacyl-tRNA profiles presented here can be grouped into two classes, constant and variable, determined by how much difference exists among the profiles of the 12 tissues examined. The amino acids with the most variable aminoacyl-tRNA profiles are not exclusively those that vary most widely among myeloma proteins nor predominantly those with a large number of codewords.

Further, the plasmacytoma aminoacyl-tRNA profiles do not have a consistent correlation with the structure of each tumor's immunoglobulin. Firstly, no peak or pattern of peaks of isoaccepting tRNA is clearly associated with immunoglobulin production and missing after production ceases, e.g. in non-producing (NP) lines. In addition, the tumors that produce heavy chain (MPC 11, B47P, TEPC 15, HOPC 8, Adj.PC 6A, Adj.PC 6A' and Adj.PC 6C) do not display any common element in their patterns different from the profiles common to tumors not producing heavy chain (NP2, B47L3NP1, MOPC 321, and TEPC 124). IgA secretors (HOPC 8, TEPC 15, Adj.PC 6A, Adj.PC 6A' and Adj.PC 6C) do not share a profile similarity distinct from that of the IgG secretors (MPC 11 and B47P) with the possible exception of seryl-tRNA profiles. The five IgA producers and the kappa chain producer, TEPC 124, have very similar profiles of seryl-tRNA which resemble the liver profile of seryl-tRNA, only shifted to the right. The two IgG producers and the kappa chain producer, MOPC 321, have similar seryl-tRNA profiles that suggest a combination of the liver (unshifted) profile and the shifted profile. RPC-2 chromatographic profiles of seryl-tRNAs from an IgA producing plasmacytoma, MPC 62, and an IgG producer, MPC 47 [16] showed that the MPC 62 (IgA) profile appears shifted to the right when compared with MPC 47 (IgG), very similar to the findings of this report. However, another publication of RPC-2 profiles of seryl-tRNAs from four IgA-secreting plasmacytomas [18] shows one tumor, McPC 603, with an unshifted pattern, two tumors, MOPC 167 and MOPC 315, with shifted patterns, and one, MOPC 460, with a combination of shifted plus unshifted peaks. Thus the shifted appearance of seryl-tRNA profiles does not correlate with IgA production.

Another comparison of RPC-5 column profiles of seryl-tRNA from plasmacytomas [20] shows a shifted pattern from a tumor secreting only lambda light chains and a non-shifted (liver-like) pattern from one secreting only kappa chains. This difference in profiles was felt to be correlated with the different contents of serine in the lambda and kappa chains being secreted, and the authors hypothesized that a seryl-tRNA could function as a regulator of

immunoglobulin synthesis. Both these possibilities seem unlikely since TEPC 124 and MOPC 321, which secrete kappa chains with no difference in the serine content, have shifted and non-shifted seryl-tRNA profiles, respectively. Furthermore, when the IgG producers, MPC 11 and B47P, became non-producers, NP2 and B47L3NPL, there was no change in the profile of their seryl-tRNAs.

The possibility of detecting tRNA correlations with the hypervariable region structure of immunoglobulins is considered in the analysis of tRNA profiles from plasmacytomas with only three differences in the amino acid sequence of their light chain products. TEPC 124 has a tryptophan in position 27D, where MOPC 321 has a threonine. In the aminoacyl-tRNA profiles TEPC 124 has a bigger peak 3 for tryptophanyl-tRNA than MOPC 321, and TEPC 124 lacks the peak 1 of threonyl-tRNA which is present in MOPC 321. At position 94 of TEPC 124 alanine takes the place of Asx (asparagine or aspartic acid) in MOPC 321. The tRNA profile analysis suggests that TEPC 124 has one alanyl-tRNA isoacceptor more and one asparaginyl-tRNA isoacceptor less than MOPC 321. These results, suggestive of correlation, fail to hold up as a proof of sequence-related tRNA profiles when the third amino acid interchange is considered. At position 27, TEPC 124 has glutamine and MOPC 321 lysine, and TEPC 124 has both a bigger glutaminyl-tRNA peak 2 and a bigger lysyl-tRNA peak 2 than MOPC 321. This difficulty in correlating sequence and tRNA profiles is reinforced by the many differences in profiles of aminoacyl-tRNAs that do not correspond to any differences in the primary structure of the kappa light chains being secreted (cf. MOPC 321 and TEPC 124 profiles of arginyl-, isoleucyl-, seryl-, histidinyl-, and tyrosinyl-tRNAs in Fig. 1B and 3-5).

Difficulty in tRNA-sequence correlation is also observed when aminoacyl-tRNAs from the pair of tumors that secrete IgA with nearly identical amino acid sequences are compared. In this case HOPC 8 has asparagine where TEPC 15 has serine, in the third hypervariable region of the heavy chain. Otherwise these proteins appear to be identical in both light and heavy chains (Hood, L., personal communication). In general these two tumors have unusually similar aminoacyl-tRNA profiles for two tumors that arose completely independently. Considering specifically the two amino acids involved in this interchange, seryl-tRNAs are identical in profile, but asparaginyl-tRNAs differ widely. With identical profiles of seryl-tRNA, it is difficult to ascribe significance to the very different profiles of asparaginyl-tRNA, which are quite complex, especially since there are equally significant differences in the tRNAs of some amino acids that are the same in the protein sequences as we know them (cf. HOPC 8 and TEPC 15 profiles of glutaminyl-, arginyl-, glutamyl-, histidinyl-, lysyl-, prolyl-, and tyrosinyl-tRNAs in Figs. 1A, 1B, 4 and 5).

The finding of a peak of valyl-tRNA in liver but not in plasmacytomas and peaks of asparaginyl-, seryl-, and tryptophanyl-tRNAs in plasmacytomas but not in liver suggests that these peaks may be models of tRNA gene regulation influencing or influenced by cancer. However, in some instances (see Table IV) a normal tissue, brain, displays some of the peaks or profiles characteristic of tumors. Furthermore the tumor-associated peaks are frequently multiple. The most likely explanation for the multiple peak differences is excessive or incomplete posttranscriptional modification of several tRNA isoacceptors, simultane-

ously. The 'shifting' of seryl-tRNA isoacceptors in all plasmacytomas, completely in some and incompletely, i.e. mixed with unshifted liver-like isoacceptors, in others, suggests a single common modification in all the shifted molecules. Such a modification has been established by the finding that two shifted seryl-tRNAs from rat brain lack 2'-O-methylation of guanosine at position 17, which is present in the two corresponding seryl-tRNAs in rat liver [30]. There is an enormous volume of evidence that will not be reviewed here which shows that other changes in posttranscriptional RNA modifications occur in cancer and certain other physiological and pathological conditions. Such tRNA modifications may be an indirect effect of enzymatic alterations inherent in the pathological state of the neoplastic cell's metabolism.

The possibility must be entertained that the differences in aminoacyl-tRNA profiles in plasmacytomas may to some extent be due to the expression of an as yet undetected viral infection. Histidinyl-tRNA in avian myeloblastosis virus-infected myeloblasts [31] showed a pattern that resembles the tumor profiles shown here in Fig. 4. The lysyl-tRNA of polyoma-infected cultures [32] and avian myeloblastosis virus [33] showed an abundance of peak 3 characteristic of the tumor patterns illustrated in Fig. 4. These variations were ascribed by the authors to varying degrees of base methylation, and avian myeloblastosis virus is known to contain a guanine methylase [34].

It should also be remembered that the presence or absence of base modifications in tRNA may also be reflected in other functions that have been attributed to modified nucleotides, e.g. cytokinin activity [35,36] and growth inhibition [37,38]. Furthermore, tRNAs have other functions beside their adaptor role in protein synthesis, e.g. initiation of viral reverse transcriptase [39,40], amino acid donation to lipids and peptidoglycans [41], and selective stimulation of mRNA translation after interferon treatment [13].

This careful analysis of aminoacyl-tRNA profiles in many plasmacytomas selected for minimal differences in secreted proteins shows no consistent evidence that tRNA populations are adapted to the secreted myeloma protein. It is possible that the effects of malignancy on tRNA obscure protein-related tRNA differences. At present it appears that the many other influences on tRNA synthesis, modification, and utilization are more important than protein structure in determining the tRNA populations in these complex biological systems. Although this does not conclusively rule out tRNA as a modulator of protein synthesis in plasmacytomas it makes it rather unlikely. Future studies of this mechanism will have to approach the question directly using purified components of translation.

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