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Aminoacyl Transfer RNA Profiles in Human Myeloma Cells

By Seitoku Fujioka and Robert C. Gallo

Comparative studies of aspartyl-, leucyl-, seryl- and tyrosyl-tRNA by a reverse phase column co-chromatography have been performed between a human normal lymphoblast cell line (RPMI 1788) and a human myeloma cell line (RPMI 8226). The cell lines were derived from normal peripheral blood and peripheral blood of a patient with multiple myeloma and produce IgM and lambda-type light chain (Bence Jones protein), respectively. Similar chromatographic patterns were obtained for leucyl- and seryl-tRNA. Minor differences in aspartyl-tRNA and larger differences in tyrosyl-tRNA were observed.

THERE ARE SEVERAL POSSIBLE EXPLANATIONS for the aberrant protein synthesis in myeloma cells. One possibility involves an abnormality of translational control, which was suggested by the findings of Yang and Novelli, of different heterogeneities of certain amino acid-specific tRNAs (seryl-tRNA) in mouse plasma cell tumors synthesizing different immunoglobulins.[1] Chromatographic differences of certain aminoacyl-tRNA (tyrosyl-tRNA) have also been demonstrated between human normal and leukemic cells,[2,3] and it was previously suggested that the apparent defect in leukocyte differentiation in leukemia might be due to an abnormality in translational control.[3,4] In the present study, chromatographic profiles of aspartyl-, leucyl-, seryl- and tyrosyl-tRNA of a human myeloma cell line were compared to a normal cell line by a reverse phase partition chromatography.

Materials and Methods

Cells

Human "normal" lymphoblast line (RPMI 1788) was established from human normal lymphocytes; karyotype: diploid (male); virology: free of Herpes-like EB virus; antigen specificity: HL-A 2, HL-A 6, and HL-A 7; minimum doubling time: 20 to 24 hr. Human myeloma cell line (RPMI 8226) originated from the peripheral blood of a patient with multiple myeloma; virology: free of EB virus; minimum doubling time: approximately 30 hr. The "normal" cell line secretes IgM while the myeloma cell line produces lambda light chain protein. In addition to the synthesis and excretion of lambda light chains, the myeloma line has other properties of a neoplastic cell, including high macroscoping cloning efficiency,[5] ability to grow in heterologous host, and ability to grow in culture without serum.[6] On injection into radiated animals the myeloma cells can produce tumors. Both cell

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lines were obtained from the Associated Biomedic Systems, Buffalo, N.Y., where they were maintained in culture by a continuous flow method. The myeloma line originated in the laboratory of G. E. Moore.[5,6]

Transfer RNA Isolation

The cells were washed twice with 0.15 *M* NaCl and suspended in an equal volume of 0.01 *M* Tris-HCl buffer, pH 7, containing 0.15 *M* NaCl, 1 m*M* EDTA, 1 m*M* $MgCl_2$, and 1% bentonite. The procedure used for tRNA isolation involves slight modifications of standard techniques and has been described previously[3] except for the introduction of the DEAE-cellulose column chromatography, which was adapted from Yang and Novelli.[7] An equal volume of phenol saturated with the same buffer was added to the cell suspension and vigorously shaken for 20 min at room temperature.[8] Aqueous and phenol layers were separated by centrifugation at 9000 rpm for 10 min (SS-34 Sorvall centrifuge). The phenol extraction was performed twice. The combined aqueous layer from the phenol extractions was then treated with an equal volume of chloroform, and the aqueous and chloroform layers were separated by centrifugation. Nucleic acids in the aqueous layer were precipitated by adding 2.2 volumes of cold ethanol containing 0.1 volume of 20% KAc and allowing it to set at −20°C overnight. The precipitate was dissolved in a 1 *M* NaCl solution containing 1 m*M* EDTA, 10 m*M* $MgCl_2$ and 0.2% bentonite, vigorously shaken for 30 min at 37°C and then centrifuged. The insoluble material was extracted a total of three times with the salt solution, and then the combined supernatants were subjected to cold ethanol precipitation. The precipitate was again dissolved in 1 *M* NaCl containing 1 m*M* EDTA and 10 m*M* $MgCl_2$. After cold ethanol precipitation, the pellet was dissolved in 0.1 *M* Tris-HCl buffer, pH 7, containing 10 m*M* $MgCl_2$ and 2 mg/100 ml bentonite; and pancreatic deoxyribonuclease (ribonuclease "free") was added to a final concentration of 5 μg/ml and incubated at 37°C for 15 min. After cold ethanol precipitation, the pellet was dissolved in 0.15 *M* NaCl containing 1 m*M* EDTA and 10 m*M* $MgCl_2$ and applied to a DEAE column (1 cm × 3 cm). The column was washed with 0.25 *M* NaCl and the tRNAs were eluted with 0.7 *M* NaCl containing 1 m*M* EDTA and 10 m*M* $MgCl_2$. The tRNA was precipitated with ethanol; dissolved in 0.1 *M* Tris-HCl buffer, pH 9, containing 1 m*M* EDTA and 10 m*M* $MgCl_2$ and incubated at 37°C for 30 min for deacylation. The pH was then adjusted to pH 7 and 2.2 volumes of cold ethanol with 0.1 volume of 20% KAc were added for final precipitation. The precipitate was dissolved in 0.1 *M* Tris-HCl buffer, pH 7, containing 1 m*M* EDTA and 10 m*M* $MgCl_2$, and stored in a liquid nitrogen refrigerator.

Aminoacyl-tRNA Synthetases

The washed cells were homogenized in the equal volume of 0.05 *M* KCl, pH 6.1, with a Potter-Elvehjem Teflon homogenizer. The homogenate was centrifuged at 105,000 *g* for 60 min. The supernatant was obtained and its pH was decreased to pH 5.2 by 0.1 *N* HCl with constant vigorous mixing.[9] The precipitate at pH 5.2 was resolved in 0.01 *M* Tris-HCl buffer, pH 7.6, and subjected to Sephadex G-100 column (1.8 cm × 30 cm, flow rate 0.1 ml/min). The first peak and the front half of the second peak were collected and used for aminoacylation reaction.[7]

Preparation of Aminoacyl-tRNA

For assaying amino acid acceptor activity, reaction mixtures in a final volume of 0.1 ml. contained: 5 μmoles Tris-HCl, pH 7.5; 5 μmoles KCl; 0.2 μmole dithiothreitol; 0.4 μmole ATP; 0.3 to 2 μmoles MgAc; 2 mμmoles ^{14}C- or ^{3}H-labeled amino acid; 2 mμmoles each of the other 19 unlabeled amino acids; 0.2 A_{260} units of tRNA and a concentration of protein which produced optimum charging. The mixture was incubated at 37°C for 15 min and terminated by the addition of cold 10% TCA. The samples were collected on type HA Millipore filters (0.45 μ pore size) and counted on a liquid scintillation spectrometer. The syntheses of ^{14}C- or ^{3}H-labeled aminoacyl-tRNA for column cochromatography were performed in a 0.5 ml to 1.5 ml reaction mixture. The components were increased

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proportionately. In each case the concentration of tRNA was shown to be limiting in preliminary experiments. After the reaction was carried out at 37°C for 15 min, the mixture was cooled and put on a DEAE column (1 cm × 3 cm) which was previously equilibrated with 0.25 *M* NaCl containing 10 m*M* $MgCl_2$ and 1 m*M* EDTA-Na_2. The charged column was washed with the same solution and the tRNA was eluted with 0.7 *M* NaCl containing 10 m*M* $MgCl_2$ and 1 m*M* EDTA-Na_2. In every case the experiments were repeated with the labeled amino acids reversed.

Fractionation of Aminoacyl-tRNA

^{14}C-labeled and ^{3}H-labeled aspartyl-, leucyl-, seryl-, or tyrosyl-tRNA samples from myeloma cells were cochromatographed on a reverse-phase partition chromatographic column.[10] The preparation of the column was described earlier.[3] Approximately 30,000 dpm of ^{14}C- and 100,000 dpm of ^{3}H-labeled aminoacyl-tRNA were combined, applied to the column and eluted with a 2-liter linear gradient of NaCl (from 0.30 *M* to 0.65 *M*). Ten-milliliter fractions were collected and subjected to cold TCA precipitation and counted on a liquid scintillation spectrometer as described earlier.[3] Radioactive chemicals were purchased from New England Nuclear or Amersham-Searle. Amino acids and other compounds were obtained from General Biochemicals or Sigma.

Results

Aspartyl-tRNA

Profiles of aspartyl-tRNAs of myeloma and normal cells are shown in Fig. 1. Both cell lines have an early eluting small peak I, a second very tiny peak II, and a major peak III. Myeloma cells have an additional late eluting fourth peak which was not found in normal cells. These findings were confirmed with the labeled amino acids reversed and were due to tRNA differences rather than differences in aminoacyl-tRNA syntheses since the same findings were obtained when charging was carried out with the heterologous enzyme. The aspartyl-tRNA profile of the myeloma cells appears identical to that reported by Yang et al. for mouse L-M tumors.[11]

Leucyl-tRNA

Two major peaks of leucyl-tRNA and suggestions of other smaller peaks

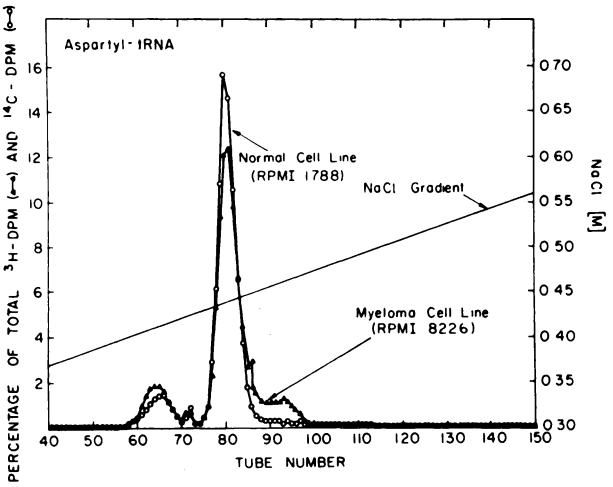

Fig. 1.—^{3}H-labeled aspartyl-tRNA (7.3×10^4 dpm) from myeloma cell line (RPMI 8226) and ^{14}C-labeled aspartyl-tRNA (9.5×10^3 dpm) from normal cell line (RPMI 1788) were fractionated by a reverse phase partition chromatographic column. The closed triangles and open circles connected by lines represent ^{3}H and ^{14}C dpm precipitated in each 10-ml fraction expressed as the percentage of total ^{3}H and ^{14}C dpm, respectively.

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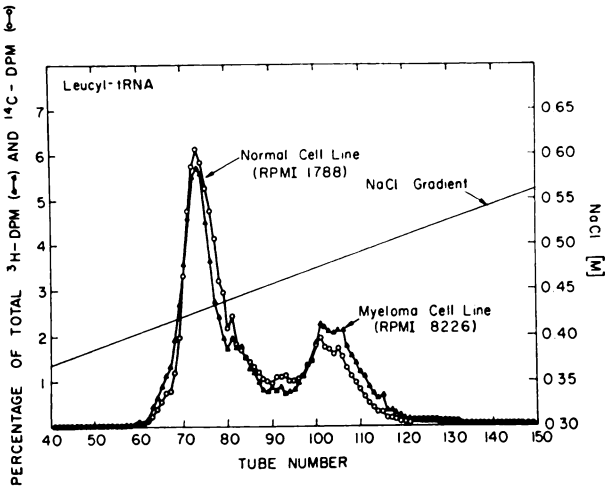

Fig. 2.—^{3}H-labeled leucyl-tRNA (1.8×10^{5} dpm) from myeloma cell line (RPMI 8226) and ^{14}C-labeled leucyl-tRNA (2.1×10^{4} dpm) from normal cell line (RPMI 1788) were fractionated by a reverse phase partition chromatographic column. The closed triangles and open circles connected by lines represent ^{3}H and ^{14}C dpm precipitated in each 10-ml fraction expressed as the percentage of total ^{3}H and ^{14}C dpm, respectively.

were found with both "normal" and myeloma cells. For some, as yet undetermined reason, the resolution of leucyl-tRNA in these experiments was poor compared to previous chromatographic experience with mammalian cell leucyl-tRNA from this and other laboratories.[3,7] At least five species have previously been fractionated from a "normal" and leukemic lymphoblast cell line.[3] The apparent small relative quantitative differences between the myeloma and "normal" cell lines shown in Fig. 2 were not reproducible.

Seryl-tRNA

Five distinct isoaccepting species of seryl-tRNA were fractionated from both normal and myeloma cells, minor peaks I and V, major peaks II and IV and an intermediate sized peak III (Fig. 3). No significant differences were found in the three major peaks, and the small *relative* quantitative difference in the early eluting peak I was not a consistent difference.

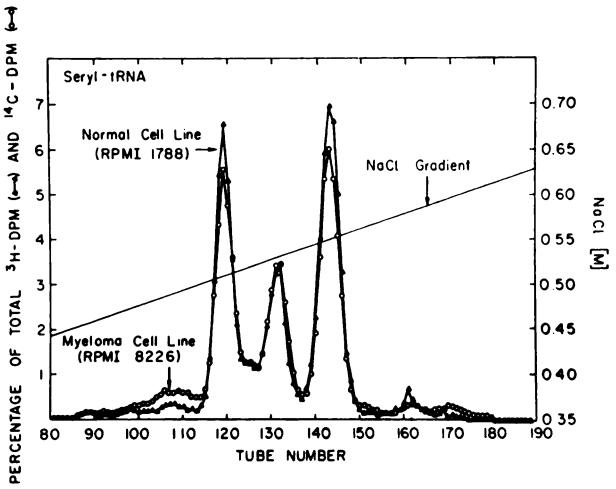

Fig. 3.—^{3}H-labeled seryl-tRNA (4.1×10^{5} dpm) from normal cell line (RPMI 1788) and ^{14}C-labeled seryl-tRNA (6.8×10^{4} dpm) from myeloma cell line (RPMI 8226) were fractionated by a reverse phase partition chromatographic column. The closed triangles and open circles connected by lines represent ^{3}H and ^{14}C dpm precipitated in each 10-ml fraction expressed as the percentage of the total ^{3}H and ^{14}C, respectively.

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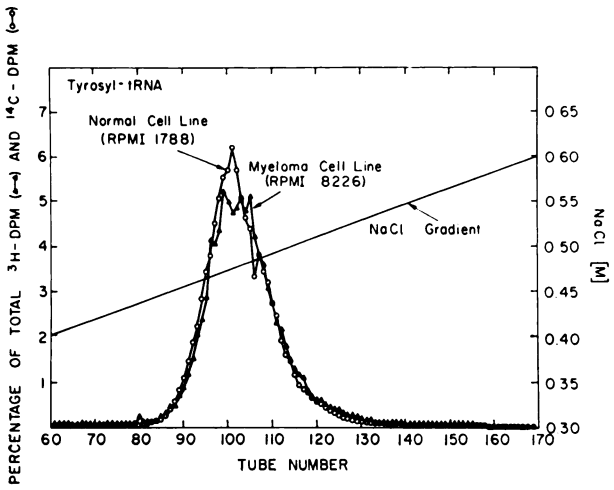

Fig. 4.—^{3}H-labeled tyrosyl-tRNA (5.8×10^{4} dpm) from myeloma cell line (RPMI 8226) and ^{14}C-labeled tyrosyl-tRNA (2.5×10^{4} dpm) from normal cell line (RPMI 1788) were fractionated by a reverse phase partition chromatographic column. The closed triangles and open circles connected by lines represent ^{3}H and ^{14}C dpm precipitated in each 10-ml fraction expressed as the percentage of the total ^{3}H and ^{14}C dpm, respectively.

Tyrosyl-tRNA

Tyrosyl-tRNA is not resolved well by any of the available columns used for tRNA fractionation. However, in some respects it is one of the most interesting tRNAs to examine since tyrosyl-tRNA differences have now been found between tumor cells and normal cells in a number of systems.[3,11-13] The profiles presented in Fig. 4 suggest that the myeloma cells contain at least four isoaccepting species of tyrosyl-tRNA and probably a fifth. Recent experiments with a new and markedly improved column procedure indicate that there are at least five isoaccepting species of tyrosyl-tRNA in animal cells (W.-K. Yang, personal communications). The peaks of tyrosyl-tRNA from normal cells do not correspond with any of the peaks of myeloma cell tyrosyl-tRNA, suggesting qualitative differences in these tRNAs between the two types of cells. These differences are probably related to differences in enzymatic base modification of the tRNA.

Discussion

Protein synthesis, of course, requires all 20 amino acids and tRNAs specific for these amino acids.[14] Since the existence of heterogeneous tRNAs for a single amino acid was discovered,[15] techniques of separation of heterogeneous tRNAs have substantially improved, and differences between these isoaccepting species have been found among tissues and cells in a variety of biodynamic states—for example, between normal and malignant cells;[2,3,11-13] mouse plasma cell tumors producing different proteins;[1] differentiating tissues;[16] cells exposed to environmental changes;[17,18] and even between different organs of the same animal.[19] Chromatographic differences in tyrosyl-tRNA between normal and tumor cells were first observed by using MAK columns.[12] In addition, it was reported in detail that both quantitative and qualitative differences between normal and leukemic tyrosyl-tRNA could be detected with Freon reverse phase partition columns.[3] In this case, the first major peak of leukemic tyrosyl-tRNA eluted later than the normal cell tyrosyl-tRNA. No differences in the

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profile of aspartyl-tRNA* and very minor differences in leucyl- and seryl-tRNA were found between normal and leukemic cells.[3] It appears that the chromatographic similarities and differences reported between normal and myeloma cell tRNAs are quite similar to those reported between normal and leukemic cells.

Yang and Novelli have found that mouse plasma cell tumors synthesizing and secreting IgA have remarkably different seryl-tRNA profiles compared to plasma cell tumors producing IgG.[1] IgA-producing tumor cells contain a small peak I and large peaks II and IV, while the IgG producer showed a marked peak I and a nearly absent peak IV.[1] If these differences were involved in the regulation of the amount and types of antibody synthesized (IgG and IgA), they cannot account for the variation in immunoglobulin synthesis between the normal human lymphocyte cell line 1788 producing IgM and the human myeloma cell line 8226 producing lambda chains since we found no differences in seryl-tRNA between these human cell lines. In both cases the patterns were similar to the IgA-producing plasma cell tumors of mice, showing prominent peaks II and IV and minor peaks I and V.

The biological importance, if any, in the differences in aspartyl-tRNA and tyrosyl-tRNA between the "normal" and myeloma cells reported in this communication and between "normal" and leukemic cells previously reported[3] remains to be determined. However, it is of obvious interest to pursue this question further, particularly in view of the number of reports in tumor systems showing a difference in tyrosyl-tRNA.[3,11-13] In *all* cases the tyrosyl-tRNA of the more neoplastic cells elutes at a higher salt concentration, suggesting the presence of more hydrophobic groups such as methyl groups in the tRNA of the neoplastic cells. We have previously presented evidence that indicates that under certain conditions tRNAs may be taken up by mammalian cells.[20,21] In an attempt to evaluate the functional significance of tRNA changes in tumor cells, a study of the effect of specific tumor cell tRNAs added directly to normal cells is now in progress.

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*Note added in proof: The late eluting aspartyl-tRNA peak found in the myeloma cells has now been found in both SV-40 and polyoma transformed neoplastic cells but not in the normal control cells prior to transformation (Gallagher, R., and Gallo, R.: Unpublished).

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