Comparison of Chromatographic Patterns of Low Molecular Weight RNA From Burkitt Lymphoma, Infectious Mononucleosis, EB-Virus Transformed, and Normal Human Lymphoblasts

By Seitoku Fujioka, Susan O'Hopp, Paul Gerber, and Robert C. Gallo

The chromatographic characteristics of uridine- and methyl-labeled low molecular weight RNA (4S and 5S) of human lymphoblasts from Burkitt lymphoma, infectious mononucleosis, and EB-virus transformed lymphocytes were compared with lymphoblasts from normal donors (cell line) and phytohemagglutinin (PHA)-transformed peripheral blood lymphocytes. No significant differences in these RNA profiles were found between the lymphoblast cell line from a normal donor and the lymphoblast cell line derived from a patient with infectious mononucleosis. The profiles of uridine-labeled RNA but not methyl-labeled RNA of Burkitt lymphoma and EB-virus transformed cells differed from the normal control cells, i.e., the lymphoblast cell line derived from a normal donor and PHA-transformed lymphocytes, respectively. The findings suggest that the elution pattern of low molecular weight nonmethylated RNA of Burkitt lymphoma and EB-virus transformed cells are similar but differ from normal and infectious mononucleosis lymphoblasts. These differences apparently reflect variations in the relative amount of certain species of nonmethylated low molecular weight RNA that probably represents 5S RNA and/or messenger RNA fragments.

CHROMATOGRAPHIC EVIDENCE has been accumulated that indicates that alterations in tRNA occur in cells after phage infection,[1,2] sporulation,[3] and during differentiation.[4,5] Differences in a few tRNA's of tumor cells compared to control tissues have also been found.[6,7] Previous reports have discussed how tRNA changes might participate in control of protein synthesis and how insufficient or altered tRNA's might result in aberrations of cellular differentiation, including neoplasia.[8-12] Due to great difficulties in obtaining adequate amounts of cells for some of the systems reported on here, it was not possible to properly conduct aminoacyl-tRNA profiles. The experiments were, therefore, limited to studying the chromatographic characteristics of labeled low molecular weight RNA [4S RNA (pre-tRNA + tRNA) and 5S

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RNA] isolated after the addition of labeled uridine or methionine to intact cells in tissue culture. The studies were aimed at surveying these RNA patterns in various "types" of human lymphoblasts, which may represent various stages of transition to the neoplastic state. These cells included lymphoblasts not established in tissue culture [phytohemagglutinin (PHA)-transformed lymphocytes] and the following tissue culture established human lymphoblasts: lymphoblasts derived from a normal donor; lymphoblasts from a patient with infectious mononucleosis; lymphoblasts obtained after transformation of normal human lymphocytes with EB virus; and lymphoblasts derived from lymph nodes of a patient with Burkitt lymphoma.

MATERIALS AND METHODS

Cells

Tissue culture normal human lymphoblasts (RPMI 1788) were established from normal peripheral blood lymphocytes. Karyotype was normal diploid (male); virology was free of Herpes-like EB virus; antigen specificity was HL-A_2, HL-A_6, HL-A_7; minimum doubling time was 20–24 hr.

Tissue culture Burkitt lymphoma cells (P_3J) were originated from Burkitt lymphoma (Jijoye) lymphocytes. Karyotype was abnormal; virology was positive for EB virus; minimum doubling time was 20–24 hr.

Tissue culture infectious mononucleosis cells (RPMI 4018) were established from peripheral blood of a patient with infectious mononucleosis. Minimum doubling time was approximately 24 hr.

These three cell lines were obtained from and cultured by Associated Biomedic Systems, Buffalo, N.Y., utilizing a continuous flow method.

PHA-transformed normal human lymphoblasts were derived from normal peripheral blood lymphocytes isolated and purified as previously described.[13,14] The cells were incubated for 24 hr at 37°C with phytohemagglutinin M (Bacto PHA M), with conditions the same as previously described.[13,14]

EB-virus transformed human lymphoblasts were obtained by isolation of lymphocytes from the same donor used for PHA transformation. The cells were than treated with EB virus as described elsewhere.[15]

Labeling Low Molecular Weight RNA with Radioactive Uridine or Methionine

Cells (10^6/ml) were incubated at 37°C for 16 hr in Eagle's MEM containing 30% fetal calf serum with either 15 μCi/ml of uridine-5-^{3}H (NET-174; 24.9 Ci/mmole), 2 μCi/ml uridine-2-^{14}C (CFA-315; 58 mCi/mmole), 12.5 μCi/ml L-methionine-methyl-^{3}H (NET-061H; 3.5 Ci/mmole), or 4 μCi/ml L-methionine-methyl-^{14}C (Amersham/Searle; 60 mCi/mmole), respectively. After the incubation, the labeled cells were washed with physiologic saline three times.

Extraction of Low Molecular Weight Cytoplasmic RNA

The packed, labeled cells were resuspended in the equal volume of 0.01 *M* Tris HCl, pH 7, containing 0.15 *M* NaCl, 10 m*M* $MgCl_2$, 1 m*M* EDTA, and 1% bentonite. An equal volume of water-saturated phenol was added, and the mixture vigorously shaken for 20 min at room temperature. The aqueous layer was collected after centrifugation.[16] After a second phenol extraction, the 2.2 volume of cold ethanol was added to the combined aqueous layer and kept at −20°C overnight. The precipitate of nucleic acids was dissolved in 1 *M* NaCl solution containing 10m*M* $MgCl_2$, 1 m*M* EDTA, and shaken for 30 min at 37°C. After centrifugation the supernatant solution was subjected to cold 70% ethanol precipitation. The precipitate was dissolved in 0.25 *M* NaCl solution containing 10 m*M* $MgCl_2$, 1 m*M* EDTA, and put on a DEAE column (1 × 4 cm). The charged column was

washed with the same solution. Low molecular weight RNA was eluted with 0.7 *M* NaCl solution containing 10 m*M* $MgCl_2$ and 1 m*M* EDTA. The RNA solution was then incubated in 0.01 *M* Tris HCl pH 9, 10 m*M* $MgCl_2$, and 1 m*M* at 37°C for 30 min to deacylate amino acids from tRNA. The pH was then returned to 7, and the optical density at wave length of 260 mμ and radioactivity were determined.

Reverse Phase Partition Co-Chromatography

The appropriate amounts of [^{3}H]- and [^{14}C]-labeled RNA (0.1–0.4 OD_{260} U) from two different cell sources were co-chromatographed on a Freon reverse-phase partition column[17] as previously described.[7] The samples of [^{3}H]-RNA and [^{14}C]-RNA were co-chromatographed rather than applied individually in separate column runs because of the variabilities in elution of RNA that occur with minor temperature, pH, or salt conditions.[17] These factors can only be adequately controlled by simultaneous application of the samples under comparison. We have chosen [^{3}H]- vs. [^{14}C]-labeled precursors to distinguish the two. Ten-milliliter fractions were collected and precipitated with cold TCA. The precipitates were collected on Millipore filter (Ha 0.45 μ, 25 mm), dried, and counted. Samples were counted a minimum of two times for 10 min. The ^{3}H and ^{14}C radioactivity was expressed as a percentage of the total eluted ^{3}H and ^{14}C radioactivity, respectively. The data were also expressed as ratios of [^{14}C] dpm to [^{3}H] dpm after normalizing some portion of the curves to a value of 1. The ratios of portions of the curves with relatively low dpm are not shown because minor fluctuations of low counts would give spurious appearance of large differences.

RESULTS

Comparison of Elution Profiles of Uridine- and Methyl-Labeled Low Molecular Weight RNA From Burkitt Lymphoma Lymphoblasts and Lymphoblast Cell Line Isolated From Normal Donor

Figure 1 shows the co-chromatography of labeled low molecular weight RNA from the Burkitt lymphoma cell line and the cell line 1788. The ^{14}C-uridine-labeled RNA's ("normal" cell line) shows a flat peak (Tube No. 64–75), followed by a sharp peak (Tube No. 77–81), and a small shoulder (Tube No. 95–102). Burkitt lymphoma RNA (^{3}H-uridine-labeled) shows a smaller early peak that does not maximize at the same area as the first portion of the peak from the 1788 cells. In addition, a sharp peak appears in the Burkitt RNA (Tube No. 70–74) at the later portion of the flat peak 1 of the 1788 cells. The ratio of the per cent radioactivity of ^{3}H-uridine RNA to ^{14}C-uridine RNA, shown in the upper portion of Fig. 1, indicates that Burkitt lymphoma cells have relatively less low molecular weight RNA eluting at the lower concentrations of NaCl. For example, the actual dpm's used to calculate the data for fraction No. 65–66 (early eluting species) were approximately 7950 and 5400 for cell line 1788 and Burkitt cells, respectively. Conversely, the dpm's for tubes 84–85 (late eluting species) were approximately 4000 for cell line 1788 and 6000 for the Burkitt cells. As noted in the previous section, the ratios of [^{3}H] and [^{14}C] dpm are only plotted for those tube fractions that contained significant counts. The ratios of the very early and late eluting material, for instance, are not shown because an impression of large differences would appear from differences not statistically justified (e.g., 50 dpm vs. 20 dpm). The relative differences in the elution of these RNA species from these two cell lines are analogous to the differences found between PHA and EB-virus transformed peripheral blood lymphocytes, i.e., relatively greater amount of earlier

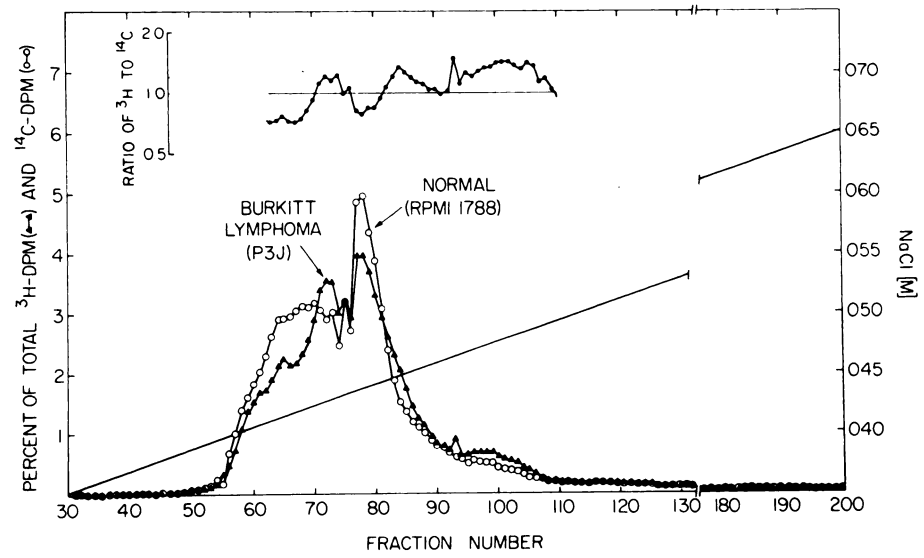

Fig. 1. Shows co-chromatographic profile of ^{14}C-uridine-labeled 4S (5S) RNA (2.48 × 10^5 cpm) of tissue culture "normal" human lymphoblasts (RPMI 1788) (open circles) and ^{3}H-uridine-labeled 4S (5S) RNA (5.04 × 10^5 cpm) of tissue culture Burkitt lymphoma cells (P^{3}J) (black triangles) by Freon reverse-phase partition co-chromatography. Fractionation was performed as described in Methods. Inserted figure (black circles) indicates ratios of ^{3}H dpm to ^{14}C dpm.

elution RNA species for the PHA-transformed lymphocytes while EB-virus transformed cells show a relatively greater labeling of late eluting species (Fig. 3).

Most low molecular weight RNA utilized in this study is, of course, tRNA. It is of interest to note that tRNA profiles on reverse-phase partition chromatography have shown that fractions eluting at low concentration of NaCl include alanyl-, asparagyl-, asparaginyl-, glutamyl-, glutaminyl-, isoleucyl-, lysyl-, methionyl-, and valyl-isoaccepting species of tRNA.[7,18] The methylated RNA isolated in these experiments is chiefly, if not solely, tRNA. It has been shown by several investigators that the capacities of the enzymes catalyzing tRNA methylation (tRNA methylases) from DNA virus-transformed cells were higher than normal cells.[19-22] The differences in the labeled RNA profiles between normal and Burkitt lymphoma could be secondary to these differences in capacities of tRNA methylases. However, in contrast to the differences in the profiles of uridine-labeled 4S (5S) RNA between the Burkitt lymphoma and "normal" lymphoblasts, co-chromatography of ^{3}H-(methyl)-methionine labeled Burkitt lymphoma and ^{14}C-(methyl)-methionine labeled "normal' lymphoblasts low molecular weight RNA showed only very slight differences.

Comparison of Elution Profiles of Uridine- and Methyl-Labeled Low Molecular Weight RNA From Infectious Mononucleosis Lymphoblasts and Lymphoblast Cell Line Isolated From Normal Donor

The chromatographic pattern of low molecular weight RNA from infec-

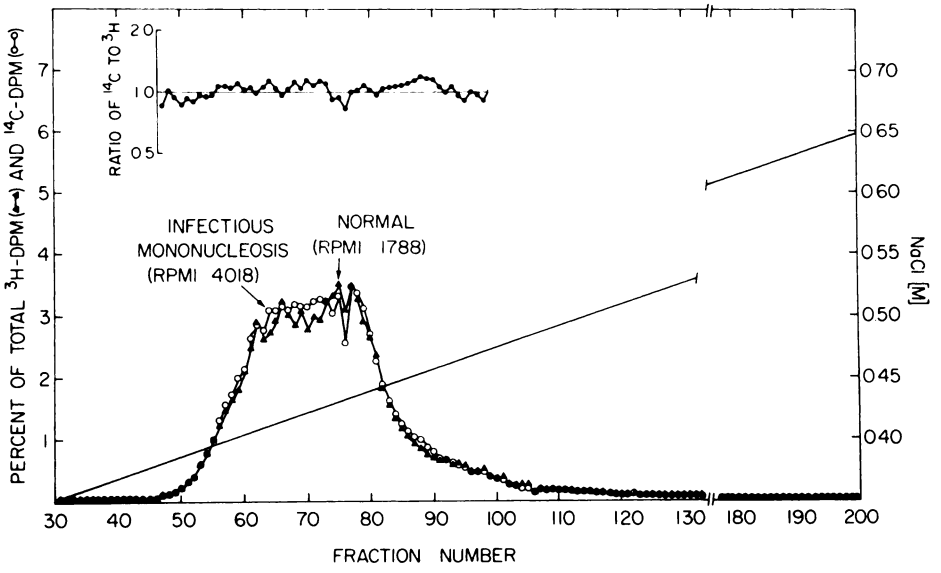

Fig. 2. Shows co-chromatographic profile of ^{3}H-uridine-labeled 4S (5S) RNA of tissue culture "normal" human lymphoblasts (RPMI 1788) (black triangles) and ^{14}C-uridine-labeled 4S (5S) RNA of tissue culture infectious mononucleosis cells (RPMI 4018) (open circles) by Freon reverse-phase partition co-chromatography. Applied radioactivities were approximately 2.09 × 10^{5} cpm (^{3}H) and 1.28 × 10^{5} cpm (^{14}C), respectively. Inserted figure (black circles) indicates ratios of ^{14}C dpm to ^{3}H dpm.

tious mononucleosis lymphoblasts and the lymphoblast cell line derived from a normal donor (1788) are similar, as shown in Fig. 2 (uridine-labeled). The ratios of ^{14}C to ^{3}H label, shown in the upper portion are relatively constant in contrast to the fluctuations illustrated in Fig. 1 for the comparison of Burkitt lymphoma and the 1788 cells. These results indicate that the relative amounts and types of heterogeneous tRNA's and other low molecular weight RNA of "normal" and infectious mononucleosis lymphoblasts are quite similar, if not identical. In addition, the methyl-labeled RNA profiles were virtually identical (not shown), indicating there are no apparent large differences in the magnitude of methylation of any particular tRNA species between the two.

Comparison of Elution Profiles of Uridine-Labeled Low Molecular Weight RNA From Normal Peripheral Blood Lymphocytes Transformed by PHA and Lymphoblast Cell Line Isolated From Normal Donor

For comparisons of RNA profiles of normal blood lymphocytes transformed to proliferative lymphoblasts by EB virus, we felt that the "normal" cell line 1788 was not sufficient. Instead, we compared the RNA profile of the EB-virus cells to normal blood lymphocytes transformed to lymphoblasts by PHA. In preliminary experiments we found no significant differences in the uridine-labeled RNA profiles between the PHA normal lymphoblasts and the 1788 lymphoblasts.

Comparison of Elution Profiles of Uridine- and Methyl-Labeled Low Molecular Weight RNA Between Normal Peripheral Blood Lymphocytes Transformed by EB Virus and by PHA

The low molecular weight RNA from PHA-transformed normal blood lymphocytes showed a higher relative incorporation of ^{3}H-uridine into the RNA species that elute early (lower salt concentration) and a lower labeling of late eluting fractions compared to the RNA of the EB-virus transformed blood lymphocytes (Fig. 3). The known low molecular weight human lymphoblasts RNA's that elute late on Freon columns consist of some species of leucyl-, seryl-, tyrosyl-, and phenylalanyl-tRNA.[7] Only minor differences were found between the methylmethionine-labeled 4S RNA between these cells (not shown). These results are similar to the patterns found with the Burkitt lymphoma lymphoblast cell line and the lymphoblast cell line isolated from a normal donor (1788), i.e., the type of differences found between Burkitt lymphoma lymphoblasts and the lymphoblasts isolated from a normal donor is mimicked by the differences between the EB-virus transformed and PHA-transformed lymphoblasts. The similarities in the elution profiles between the Burkitt lymphoma and EB-virus transformed lymphoblasts 4S (5S) RNA are emphasized by comparing the ^{3}H-^{14}C ratios shown in the top portions of the figures. In both cases there is a relative increase in the late eluting RNA species. In an attempt to increase the resolution, a 3 liter gradient was used in the chromatograhic run shown in Fig. 3 instead of the usual 2 liter gradient. This accounts for the appearance of broader and lower peaks in Fig. 3A compared with the other figures.

DISCUSSION

Variation in the amount or types of RNA species might account for differences in growth and/or differentiation between one tissue and another.

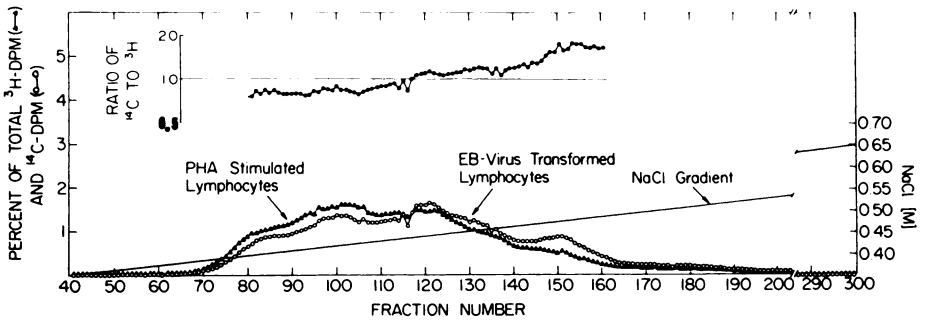

Fig. 3. Shows co-chromatographic profile of ^{3}H-uridine-labeled 4S (5S) RNA of PHA-stimulated normal human lymphoblasts (black triangles) and ^{14}C-uridine-labeled 4S (5S) RNA of EB-virus transformed human lymphocytes (open circles) by Freon reverse-phase partition co-chromatography. Applied radioactivities were approximately 2.70 × 10^{5} cpm (^{3}H) and 1.80 × 10^{5} cpm (^{14}C), respectively. Chromatography was performed with 3 liters of NaCl linear gradient instead of 2 liters as used in other studies. Inserted figure (black circles) indicates ratios of ^{14}C dpm to ^{3}H dpm.

Categorizing RNA Differences

A difference in RNA's between tissues can be put into two main categories: The first are the qualitative differences in a given species of RNA, e.g., a new or absent species or a difference in the primary or secondary structure (either due to primary nucleotide base sequence differences or differences in modification of nucleotides, as, for example, methylation of tRNA). Evidence for qualitative differences of this type have been described between normal and neoplastic cells. For example, Turkington and Self[23] and Neiman and Henry[24] reported, respectively, that breast cancer and human chronic lymphocytic leukemia leukocytes contain RNA species not found in appropriate normal control tissues. These conclusions were arrived at from molecular DNA-RNA hybridization studies and presumably reflect differences in messenger RNA between the normal and neoplastic cells. Qualitative differences in tRNA's between normal and neoplastic cells have also been described.[6,7,25-29] These have consisted of the presence of an extra species of tRNA for aspartic acid in human myeloma cells compared to the lymphoblast cell line 1788,[25] and the same extra species of tRNA for aspartic acid in cells transformed by each of the major oncogenic DNA viruses (SV-40, polyoma, and adenovirus) compared to the normal control tissue culture cells prior to transformation[26] (unpublished results—R. Gallagher, R. Ting, and R. Gallo; personal communication—W. K. Yang). An additional example is the report by Gonano and Chiarugi[27] showing an extra species of tRNA for phenylalanine in Morris hepatoma not present in normal liver.

Other reports have shown differences that are not "all or none" but appear to be qualitatively different in either primary base sequence or in side group modification of the nucleotides. These studies include especially elution profile comparisons of specific aminoacyl tRNA's. For example, elution of a species of tyrosyl-tRNA from tumor cells occured at a higher salt concentration than that of control cells. This has been noted in several reports.[6,7,28,29] Elution from these columns at higher salt concentrations is probably chiefly due to the binding of hydrophobic side groups.[17] Thus, the interpretation of these findings has been that the primary nucleotide sequence of a species of tyrosyl-tRNA of the tumor cells may contain more modifications, i.e., more hydrophobic side groups such as methyl groups.[6,7] This possibility was initially speculated on by Srinivasan and Borek[30] from their tRNA methylase experiments.

Second, quantitative differences between normal and neoplastic cells in a given type of RNA have also been reported. For example, in most reports showing apparent qualitative differences in a tRNA species, additional quantitative changes have also been noted, and in at least one report only quantitative differences were described.[31]

Origin of RNA Differences Between Cells

The finding of RNA differences (qualitative or quantitative) between normal and neoplastic cells does not tell us the nature of the source of these differences. This is particularly important to emphasize in view of the reported differences

in RNA species between the same tissue at two different states of differentiation. Presumably these differences might be mechanistically involved in determining the cell's state of differentiation. For instance, it is known that major alterations in tRNA occur with hormone-induced biological changes. This includes (at minimum) thyroxine-,[32] cortisone-,[33] estrogen-,[34,35] and growth hormone[36]-induced changes. Most of these changes are quantitative,[32-34] but qualitative tRNA differences following hormonal influences have been described.[35,36] Differences have been described between organs of the same animal[37] and between the same tissues at different levels of differentiation.[4,5,38-40] Finally, there are several reports showing alterations in tRNA's, quantitative[41] but also qualitative,[26,42] that occur after viral infection. (In no case to our knowledge has this been shown to be due to coding of a new species by the virus. It is likely that the viral-induced changes reflect a depression of host cell genes.) Thus, the possibilities to consider for the origin of RNA differences between normal and tumor cells must include at least the following: a carcinogen-induced mutation resulting in a new species of RNA (primary base sequence change), or a modification of RNA through the activity of an altered modifying enzyme, e.g., RNA methylase; a viral-coded RNA, i.e., an RNA transcribed directly from newly inserted viral DNA; and what we believe is the most likely explanation for most or all of the reported differences is a variation in host genome expression induced by virus or any other inciting agent that changes the state of differentiation and/or growth of the cell.

With this background in mind we have carried out a preliminary survey of the chromatographic elution profiles of the low molecular weight RNA's (presumably all 55–60 species of tRNA and 5S RNA) in cellular systems that we believe offer the best controlled human system for evaluating several questions of interest. Five different sources of human lymphoblasts were studied: normal peripheral blood lymphoblasts (not tissue culture established); lymphoblasts derived from a normal donor but established in tissue culture; infectious mononucleosis lymphoblasts; EB-transformed lymphoblasts; and Burkitt lymphoma lymphoblasts. If relatively large RNA differences were solely and consistently produced by changes induced by tissue culture, then all systems except the PHA cells should be similar or identical. If relatively large changes were induced simply by exposure to EB virus (either by direct coding or by inducing gene depression), then one might expect that the infectious mononucleosis cells, EB-virus transformed cells, and Burkitt lymphoma cells should be the same but different from the PHA-transformed cells, and possibly also from the "normal" cell line 1788 (the lymphoblast cell line 1788, derived from a normal donor, may also contain the EB virus genome). Finally, if changes in RNA species were associated with frank neoplastic conversion one might anticipate that the EB-virus transformed lymphoblasts and Burkitt lymphoma lymphoblasts to be similar but differ from the infectious mononucleosis, the PHA-transformed lymphocytes, and the "normal" cell line 1788. We are, of course, aware that the so-called "normal" 1788 cell line and the infectious mononucleosis cell line, as established tissue culture cells, might also be neoplastic, and there is no known method of obtaining an unambigous answer to this problem. However, part of the purpose of biochemical char-

acterizations of this type are, in fact, designed to help determine which cells appear to be more closely related.

The RNA profiles of these lymphoblasts show one interesting general pattern. The uridine-labeled RNA from PHA-transformed normal peripheral blood lymphoblasts, infectious mononucleosis lymphoblasts, and the "normal" lymphoblast cell line 1788 show relatively greater amounts of early eluting species and relatively less of the late eluting RNA's, compared to the RNA's of EB-virus transformed cells and the Burkitt cells (which tend to show relatively more of the late eluting and less of the early eluting species). This is particularly evident from Fig. 3 where uridine-labeled RNA of EB virus and PHA-transformed lymphoblasts are directly compared. The ratio of [^{14}C]-uridine-labeled RNA (EB-virus transformed cells) to [^{3}H]-uridine-labeled RNA (PHA-transformed blood lymphocytes) is approximately 0.75: 0.85 for the species eluting early from the column (fraction No. 60–110) and increases to approximately 2.0 for the species eluting late (e.g., fraction No. 150–160). Similarly, the ratio of [^{3}H]-uridine-labeled RNA (Burkitt lymphoma) to [^{14}C]-uridine-labeled RNA ("normal" cell line 1788) shown in Fig. 1 is approximately 0.70:0.85 for fraction No. 60–68, but the ratio increases to about 1.3:1.5 for fractions 95–105. The column run shown in Fig. 3 was with a 3 liter gradient. Therefore, it appears spread out in comparison to all other column runs that were performed with 2 liter gradients.

From these observations it cannot be concluded that the differences include qualitatively different species. The variations could be due to the relative amounts of specific RNA species.

Interpretation of the Species of RNA Showing Differences

No conclusive statement can be made from these experiments that would indicate the exact types of RNA species showing relative differences. However, the pattern of relative differences was not found when the RNA's were labeled with [^{14}C] and [^{3}H]-(methyl)-methionine. Methyl labeling of low molecular weight RNA is chiefly base modification of tRNA. The other known significant low molecular weight RNA, 5S RNA, is not methylated. Therefore, the findings are in keeping with the idea that the relative differences in these elution profiles were in this species of RNA. However, other interpretations are also plausible. For example, although the procedure used to isolate RNA was designed to include only low molecular weight 4S-5S RNA, it is possible that fragments of messenger RNA, which like 5S RNA are not methylated species, were included.

It is unlikely that the relative differences in low molecular weight RNA species between normal lymphoblasts (PHA-transformed blood lymphocytes) and lymphoblasts transformed by EB virus result from RNA species directly transcribed from EB-virus DNA. The extent of the relative differences suggests instead differential genome expression presumably related to infection by the virus. It is of interest in this regard that these differences were not found in lymphoblasts cultured from a patient with infectious mononucleosis, which leads us to consider that the cell line established from the patient with infectious mononucleosis may be derived from a normal lymphocyte. Alter-

natively, it is possible that the differences are related to the neoplastic state, without involving a requirement for the virus.

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REFERENCES

1. Sueoka, N., and Kano-Sueoka, T.: A specific modification of leucyl-tRNA of *Escherichia coli* after phage T_2 infection. Proc. Nat. Acad. Sci. USA 52:1535, 1964.

2. Waters, L. C., and Novelli, G. D.: A new change in leucine transfer RNA observed in *Escherichia coli* infected with bacteriophage T_2. Proc. Nat. Acad. Sci. USA 57:979, 1967.

3. Kaneko, I., and Doi, R. H.: Alteration of valyl-sRNA during sporulation of *Bacillus subtilis*. Proc. Nat. Acad. Sci. USA 55: 564, 1966.

4. Lee, J. C., and Ingram, V. M.: Erythrocyte transfer RNA: Change during chick development. Science 158:1130, 1967.

5. Vold, B. S., and Sypherd, P. S.: Modification in transfer RNA during the differentiation of wheat seedlings. Proc. Nat. Acad. Sci. USA 59:453, 1968.

6. Baliga, B. S., Borek, E., Weinstein, I. B., and Srinivasan, P. R.: Differences in the transfer RNA's of normal liver and Novikoff hepatoma. Proc. Nat. Acad. Sci. USA 62:899, 1969.

7. Gallo, R. C., and Pestka, S.: Transfer RNA species in normal and leukemic human lymphoblasts. J. Molec. Biol. 52:195, 1970.

8. —: Biochemical considerations. From Perry, S. and Gallo, R. C.: Physiology of human leukemic leukocytes: Kinetic and biochemical considerations. *In* Gordon, A. S. (Ed.): Regulation of Hematopoiesis, Vol. II. New York, Appleton-Century-Crofts, 1971.

9. —: Transfer RNA and transfer RNA methylation in growing and "resting" adult and embryonic tissues and in various oncogenic systems. Cancer Res. 31:621, 1971.

10. —, and Whang-Peng, J.: Observations on the regulatory effects of the transfer RNA minor base, N^6-Δ^2-isopentenyladenosine, on human lymphocytes. *In* Beers, R. F., Jr., and W. Braun (Eds.): Fourth International Miles Symposium on Molecular Biology: Biological Effects of Polynucleotides. New York, Springer-Verlag, pp. 303-334, 1971.

11. Herrera, F., Adamson, R. H., and Gallo, R. C.: Transfer RNA as an informative molecule and its uptake by normal and leukemic human and murine leukocytes. *In* Ledoux, L.G.H., (Ed.): NATO Symposium: Uptake of Informative Molecules by Living Cells. Amsterdam, North Holland, pp. 377-397, 1971.

12. Weinstein, I. E.: A possible role of transfer RNA in the mechanism of carcinogenesis. Cancer Res. 38:1871, 1968.

13. Gallo, R. C., Whang-Peng, J., and Perry, S.: Isopentenyladenosine stimulates and inhibits human lymphatic transformation and mitosis. Science 165:400, 1969.

14. Riddick, D. H., and Gallo, R. C.: Correlation of transfer RNA methylase activity with growth and differentiation in normal and neoplastic tissues. Cancer Res. 30:2484, 1970.

15. Gerber, P., Whang-Peng, J., and Monroe, J. H.: Transformation and chromosome changes induced by Epstein-Barr virus in normal human leukocyte cultures. Proc. Nat. Acad. Sci. USA 63:740, 1969.

16. Kirby, K. S.: A new method for the isolation of ribonucleic acids from mammalian tissues. Biochem. J. 64:405, 1956.

17. Weiss, J. F., and Kelmers, A. D.: A new chromatographic system for increased resolution of transfer ribonucleic acids. Biochemistry (Wash.) 6:2507, 1967.

18. Yang, W.-K., and Novelli, G. D.: Multiple isoaccepting transfer RNA's in a mouse plasma cell tumor. Proc. Nat. Acad. Sci. USA 59:208, 1968.

19. Mittelman, A., Hall, R. H., Yohn, D. S., and Grace, J. T., Jr.: The in vitro soluble RNA methylase activity of SV40-induced hamster tumors. Cancer Res. 27:1409, 1967.

20. Kit, S., Nakajima, K., and Dubbs, D. R.: Transfer RNA methylase activities of SV-40-transformed cells and cells infected with animal viruses. Cancer Res. 30:528, 1970.

21. Fujioka, S., Ting, R. C., and Gallo, R. C.: Transfer RNA methylases of normal cells, virus transformed cells, and tumors derived from transformed cells. Cancer Res. 31:451, 1971.

22. Gallagher, R. E., Ting, R. C. Y., and Gallo, R. C.: Transfer RNA methylase alterations in polyoma transformed rat embryo culture cells. Proc. Soc. Exp. Biol. Med. 136:819, 1971.

23. Turkington, R. W., and Self, D. J.: New species of hybridizable nuclear RNA in breast cancer cells. Cancer Res. 30:1833, 1970.

24. Neiman, P. E., and Henry, P. H.: RNA-DNA hybridization and hybridization competition studies of the rapidly labeled RNA from normal and chronic lymphocytic leukemia lymphocytes. Biochem. J. 8:275, 1969.

25. Fujioka, S., and Gallo, R. C.: Aminoacyl transfer RNA profiles in human myeloma cells. Blood 38:246, 1971.

26. Gallagher, R. E., Ting, R. C. Y., and Gallo, R. C.: Comparison of transfer RNA (tRNA) species from control (early passage) and polyoma transformed rat embryo culture cells. Presented at the 1971 meeting of the American Society of Biological Chemists, San Francisco, June 1971.

27. Gonano, F., and Chiarugi, V. P.: Changes of phenylalanyl-tRNA of Morris hepatoma 5123 revealed by reverse phase chromatography. Exp. Molec. Path. 10:99, 1969.

28. Yang, W.-K., Hellman, A., Martin, D. H., Hellman, K. B., and Novelli, G. D.: Isoaccepting transfer RNA's of L-M cells in culture and after tumor induction in C3H mice. Proc. Nat. Acad. Sci. USA 64:1411, 1969.

29. Taylor, M. W., Granger, G. A., Buck, C. A., and Holland, J. J.: Similarities and differences among specific tRNA's in mammalian tissues. Proc. Nat. Acad. Sci. USA 57:1712, 1967.

30. Srinivasan, P. R., and Borek, E.: Enzymatic alteration of nucleic acid structure. Science 145:548, 1964.

31. Taylor, M. W.: Benzoylated DEAE cellulose chromatography of tumor and nontumor transfer RNA. Cancer Res. 30: 2463, 1970.

32. Yang, S. S., and Sanadi, D. R.: Changes in the distribution of transfer RNA species specifically induced by thyroxine. J. Biol. Chem. 244:5081, 1969.

33. Agarwal, M. K., Hanoune, J., Yu, F. L., Weinstein, I. B., and Feigelson, P.: Studies on the effect of cortisone on rat liver transfer RNA. Biochemistry (Wash.) 8:4806, 1969.

34. O'Malley, B. W., Aronow, A., Peacock, A. C., and Dingman, C. W.: Estrogen dependent increase in transfer RNA during differentiation of the chick oviduct. Science 162:567, 1968.

35. Sharma, O. K., and Borek, E.: Hormonal effect on transfer RNA methylases and on servine transfer RNA. Biochemistry (Wash.) 9:2507, 1970.

36. Jackson, C. D., Irving, C. C., and Sells, B. H.: Changes in rat liver transfer RNA following growth hormone administration and in regenerating liver. Biochim. Biophys. Acta 217:64, 1970.

37. Hatfield, D., and Portugal, F. H.: Seryl-tRNA in mammalian tissues: Chromatographic differences in brain and liver and a specific response to the codon, UGA. Proc. Nat. Acad Sci. USA 67:1200, 1970.

38. Lee, J. C., and Ingram, V. M.: Erythrocyte transfer RNA: Change during chick development. Science 158:1330, 1967.

39. Zeikus, J. G., Taylor, M. W., and Buck, C. A.: Transfer RNA changes associated with early development and differentiation of the sea urchin, *Stronglyocentrotus purpuratus*. Exp. Cell Res. 57:74, 1969.

40. Tonoue, T., Eaton, J., and Frieden, E.: Changes in leucyl-tRNA during spontaneous and induced matamorphosis of bullfrog tadpoles. Biochem. Biophys. Res. Commun. 37:81, 1969.

41. Raska, K., Frohwirth, D. H., and Schlesinger, R. W.: Transfer RNA in KB cells infected with adenovirus type 2. J. Virol. 5:464, 1970.

42. Kano-Sueoka, T., and Sueoka, N.: Leucine-tRNA and cessation of protein synthesis upon phage T_2 infection. Proc. Nat. Acad. Sci. USA 4:1229, 1969.