of the cysteine residues is so sterically blocked that it is unable to form a disulfide bond. (3) The F3 molecules exhibiting the simpler oxidation behavior contain only one cysteine residue. The first possibility can be excluded by first reducing the F3 molecule and then assaying for the number of cysteine residues. We have observed no increase in cysteine residues nor any change in the oxidation behavior. The second possibility seems unlikely, especially since isolated F3 histone has little secondary structure at low pH and ionic strength and further since the oxidation behavior characteristic of two cysteine residues is not seen even in the presence of high concentrations of urea. We favor the third explanation and thus conclude that in all creatures with the simpler oxidative behavior (including rodents) there is a single cysteine residue in the F3 histone, whereas more advanced mammalian orders (lagomorphs, carnivores, ungulates, and primates) possess two such residues.

This is a surprising observation. Not only is the mutational introduction of an additional cysteine into a protein a rare event, but also the F3 histone has preserved one of the cysteine residues in all creatures examined so far. Since it has presumably preserved this residue because of the selective advantage it offers, it is curious that the introduction of another cysteine residue relatively close by should not dramatically affect the biological function of the molecule. It will be of interest to inquire whether the two cysteine residues behave in different ways toward sulfhydryl agents when they are part of the nucleoprotein complex. Such experiments are currently under way.

Acknowledgment

We thank David Bilek for his help both with experimentation and photography.

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Stability of Chromatographic Patterns of Aminoacyl Transfer Ribonucleic Acid from Individual Mouse Plasmacytomas and Variability among Different Immunoglobulin A Producing Plasmacytomas and Normal Organs*

J. Frederic Mushinski

ABSTRACT: The leucyl-, seryl-, and tyrosyl-tRNAs from IgA-producing plasmacytomas and normal tissues were compared using reversed-phase chromatography. The biological stability of these chromatographic patterns was established by comparing the patterns of these aminoacyl-tRNAs from different transplant generations of a single tumor line, which were found to be very similar. Confirmation of the concept that such patterns reflect a stable population of isoaccepting species of tRNA comes from the finding that the tRNAs from two different tumor lines which originated in the same primary mouse have the same patterns for the three aminoacyl-tRNAs examined. Four normal tissues examined in this way showed almost no variation in the chromatographic pattern of Tyr-tRNA, small differences in Leu-tRNA, and somewhat greater differences in Ser-tRNA patterns. Four plasma cell tumors which secrete IgA immunoglobulins with antibody activity showed pattern differences among themselves which were much more striking than the differences among the normal organs. Possible sources of this variable degree of pattern variation are discussed.

A previous report from this laboratory (Mushinski and Potter, 1969) described differences in the relative amounts of each of the five chromatographic peaks of Leu-tRNA when the tRNA from mouse liver and several mouse plasma cell tumors, all of which secreted κ-type immunoglobulin light chains, were compared by reversed-phase column chromatog-

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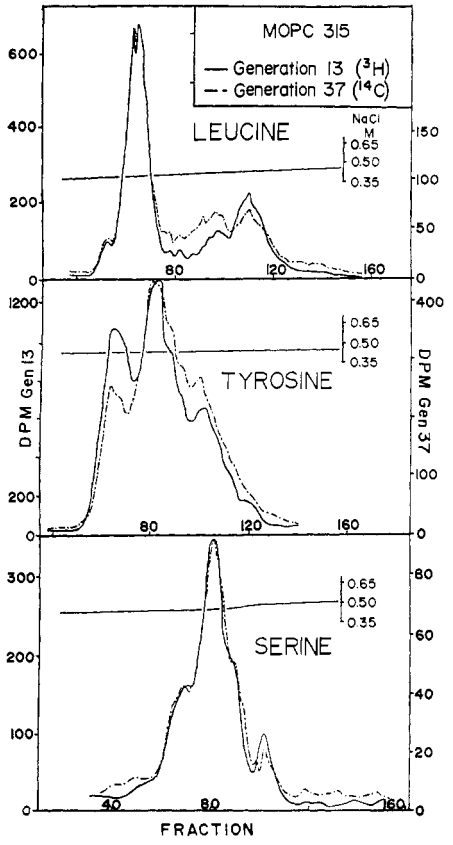

FIGURE 1: Aminoacyl-tRNA patterns comparing two transplant generations of MOPC 315. tRNA from generation 13 was prepared Jan 24, 1968, and generation 37 on July 2, 1969. The NaCl gradients used for elution are explained in the text and illustrated on each chromatogram.

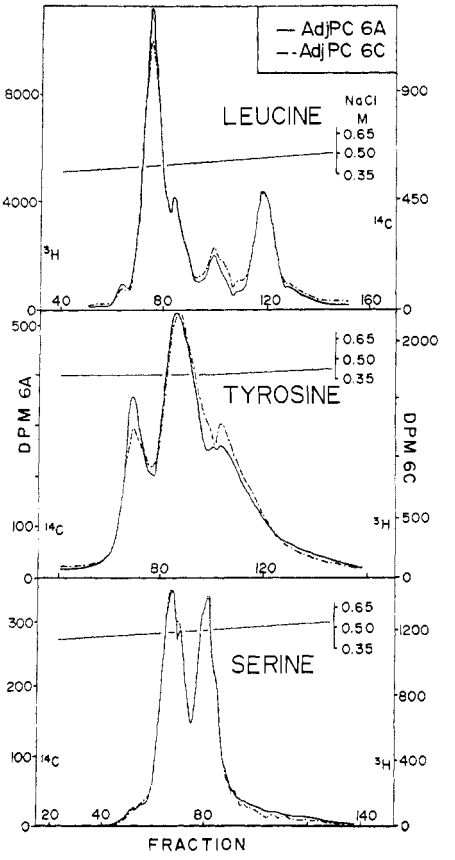

FIGURE 2: Aminoacyl-tRNA patterns comparing two sister lines of IgA-producing myelomas transplanted separately for more than 5 years and compared by double-label chromatography.

raphy. Recent work by Gallo and Pestka (1970) demonstrated chromatographic differences among aminoacyl-tRNAs prepared from normal and leukemic human lymphoblasts. Furthermore, an extensive body of literature has accumulated reporting changes in aminoacyl-tRNA chromatographic profiles in a variety of different conditions. (See Yang *et al.* (1969) and Gallo and Pestka (1970) for a representative list of these references.)

We have felt that these and further observations would have significance only if a degree of biological stability for these chromatographic profiles could be demonstrated. Here we report the similarity of patterns of tRNA isolated from different transplant generations of one tumor line, MOPC 315, and from "sister tumor lines," Adj. PC 6A and Adj. PC 6C, originating from separate nodules in an original host mouse, which were transplanted separately for many generations.

To extend our previous observation of differences in patterns of Leu-tRNA isolated from a series of biologically similar tumors we have examined the aminoacyl-tRNA chromatographic patterns from four IgA-secreting mouse myelomas, MOPC 167, McPC 603, MOPC 315, and MOPC 460. The large number of tRNA sources precluded examining the tRNAs for every amino acid, so those specific for leucine, serine, and tyrosine were chosen for this investigation. To put the tRNA patterns from the tumors in perspective, we also examined the same three aminoacyl-tRNAs from 4 normal BALB/c organs: liver, spleen, kidney, and testis.

The mouse plasma cell tumors were found to demonstrate tRNA pattern differences among themselves and in comparison to normal mouse tissues, the Ser-tRNA showing the most variations. The four normal tissues also showed some tRNA variations similar to, but less striking than, the variations seen among the tumor patterns.

Materials and Methods

Plasma Cell Tumors. Plasma cell tumors which had been induced by intraperitoneal mineral oil (MOPC) or adjuvant (Adj. PC) injections and maintained by continous transplantation in BALB/c mice (Potter, 1967) were generously supplied by Drs. Michael Potter and K. Robert McIntire of the National Cancer Institute.

tRNA Preparation. Tumors and normal organs were removed immediately after killing the mice by cervical dislocation and the tissues were processed as described previously (Mushinski and Potter, 1969). In brief, the appropriate tissues were pooled, homogenized in hypotonic buffer in the presence of bentonite and phenol, reextracted with phenol two to three times, ethanol precipitated, extracted with 1 M NaCl–0.001 M $MgCl_2$, stripped of endogenously acylated amino acids in alkaline Tris buffer, and chromatographed in the cold on columns of Sephadex G-100. The tRNA-containing fractions were pooled and either dialyzed exhaustively against distilled water and lyophilyzed or concentrated by pressure dialysis and dialyzed against 0.001 M $MgCl_2$.

Synthetase Preparation and Aminoacylation of tRNA. A DEAE-cellulose column fraction of ribosome-free homogenate (Muench and Berg, 1966) prepared from each tumor was used for aminoacylation of its homologous tRNA. A similar

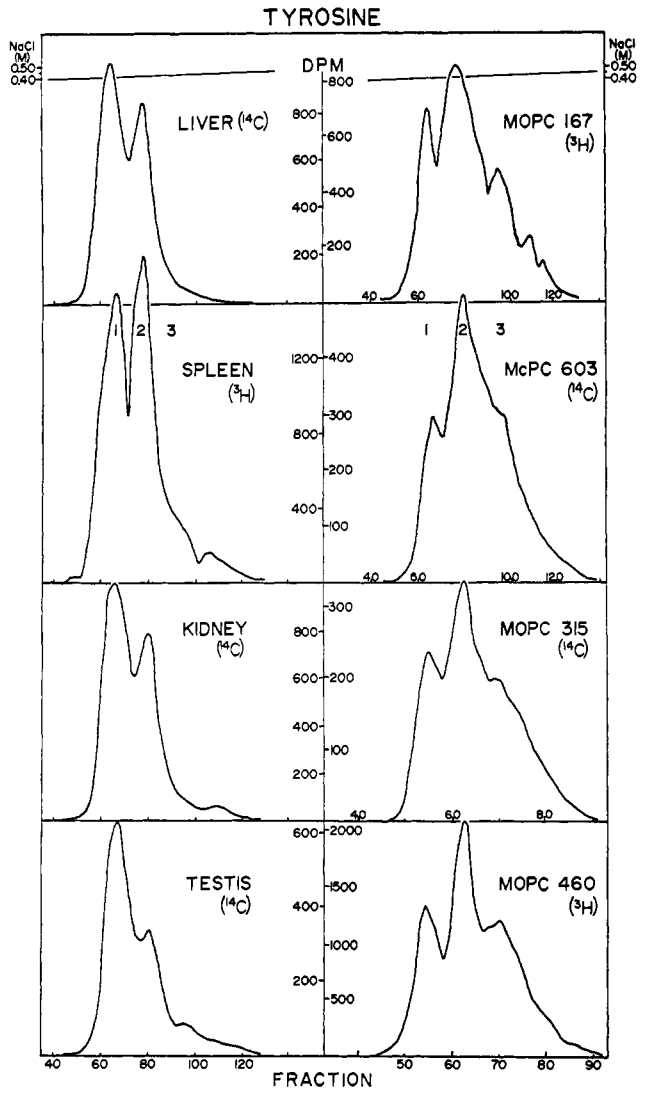

FIGURE 3: Tyr-tRNA patterns of normal tissues, on the left, charged with liver enzyme, and plasma cell tumors, on the right, charged with enzymes from the same tumor. The NaCl gradients are shown at the top of each column of graphs, and peaks are numbered as indicated beneath the top patterns. Note that the abscissa scale has been expanded in the two lowest patterns on the right in order to make these NaCl gradients and elution patterns more comparable to those of the other patterns.

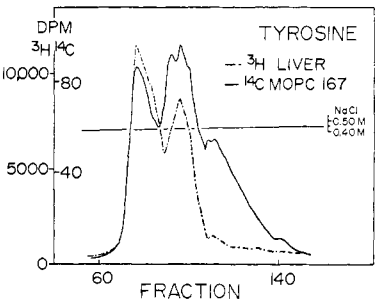

FIGURE 4: Tyr-tRNA patterns for liver and MOPC 167 chromatographed simultaneously on a Freon column as described in the text.

preparation from BALB/c liver was used for aminoacylation of tRNA from normal mouse tissues. These enzymes were concentrated by pressure dialysis and stored in 50% glycerol at −15°.

The conditions for all the aminoacylation reactions were the same as described previously (Mushinski and Potter, 1969). L-[U-^{14}C]Leucine, L-[4,5-^{3}H]leucine, L-[G-^{3}H]serine, and L-[G-^{3}H]tyrosine were obtained from New England Nuclear and L-[U-^{14}C]serine, and L-[U-^{14}C]tyrosine from Schwarz BioResearch. At various enzyme and tRNA concentrations the reactions reached a plateau by 20 min at which time the reactions were routinely stopped by adding a pinch of bentonite and an equal volume of water-saturated phenol and stirring vigorously. The phases were separated and two more phenol extractions of the aqueous layer made. Then one-tenth volume of 20% potassium acetate (pH 5.5) was added to the aqueous phase followed by three volumes of cold absolute ethanol. The resulting precipitation proceeded overnight, was collected by centrifugation, was drained in the cold, and dissolved in 0.25 ml of initial chromatography buffer and stored frozen. Maximum charging observed was 172 pmoles of tyrosine, 602 pmoles of leucine, and 960 pmoles of serine per mg of tRNA.

Reversed-Phase Chromatography of Aminoacyl-tRNA. To separate the isoaccepting species of tRNA, the Freon-Aliquat column chromatographic system, RPC 2, described by Weiss and Kelmers (1967) was used. The columns were 120 × 1 cm, water jacketed, and maintained at 15°. Between 4000 and 150,000 cpm [^{3}H]aminoacyl-tRNA from one preparation was mixed with a similar number of counts of [^{14}C]aminoacyl-tRNA from another source and applied to the column in 1–2 ml of starting buffer. Since similar profiles were found even when very different amounts of RNA and radioactivity were applied, no attempt was made to use any particular minimum amount of radioactivity and no carrier RNA was used. The column was then eluted with a 2-l. linear NaCl gradient in a buffer containing 0.01 M sodium acetate and 0.01 M magnesium acetate at pH 4.5. The elution rate was 1.5 ml/min, and fractions of 9–10 ml were collected. The best gradient conditions varied with the aminoacyl-tRNA being separated; the concentrations of NaCl in the mixing chamber and reservoir of the two-part gradient mixer were as follows: leucine, 0.35–0.55 M; serine, 0.40–0.65 M; and tyrosine, 0.40–0.467 M. It has been reported (Yang and Novelli, 1968) that the addition of a reducing agent to the eluting buffers improved recovery and resolution in some cases. We did not find this significantly helpful and did not use it in these experiments. Mean recovery of trichloroacetic acid precipitable counts from the illustrated chromatograms was 77.7% for Tyr-tRNA, 61.6% for Leu-tRNA, and 69.7% for Ser-tRNA with standard deviations of 16.9, 13.2, and 15.2%, respectively. Each fraction was treated with 1.0 ml of 50% trichloroacetic acid after adding a drop of DNA–albumin carrier. After a minimum of 1 hr at 4° the precipitates were collected on Millipore (HAWP) filters under suction. The filters were washed with about 5 ml of cold 5% trichloroacetic acid, dried, hydrolyzed for 16 min with 0.2 ml of 1 N HCl at 100° as previously described (Mushinski and Potter, 1969), and counted in a liquid scintillation counter in a solution of 3 ml of absolute ethanol and 10 ml of a toluene solution containing 0.6% 2,5-diphenyloxazole and 0.006% 1,4-bis[2-(5-phenyloxazolyl)]benzene. Three replicate counts were averaged, and disintegrations per minute for ^{3}H + ^{14}C were computed in an IBM 1620 computer using a standard quench curve for this solvent system and automatic external standardization. Every fraction in the radioactive portion of the chromatogram was counted and plotted with the aid of a

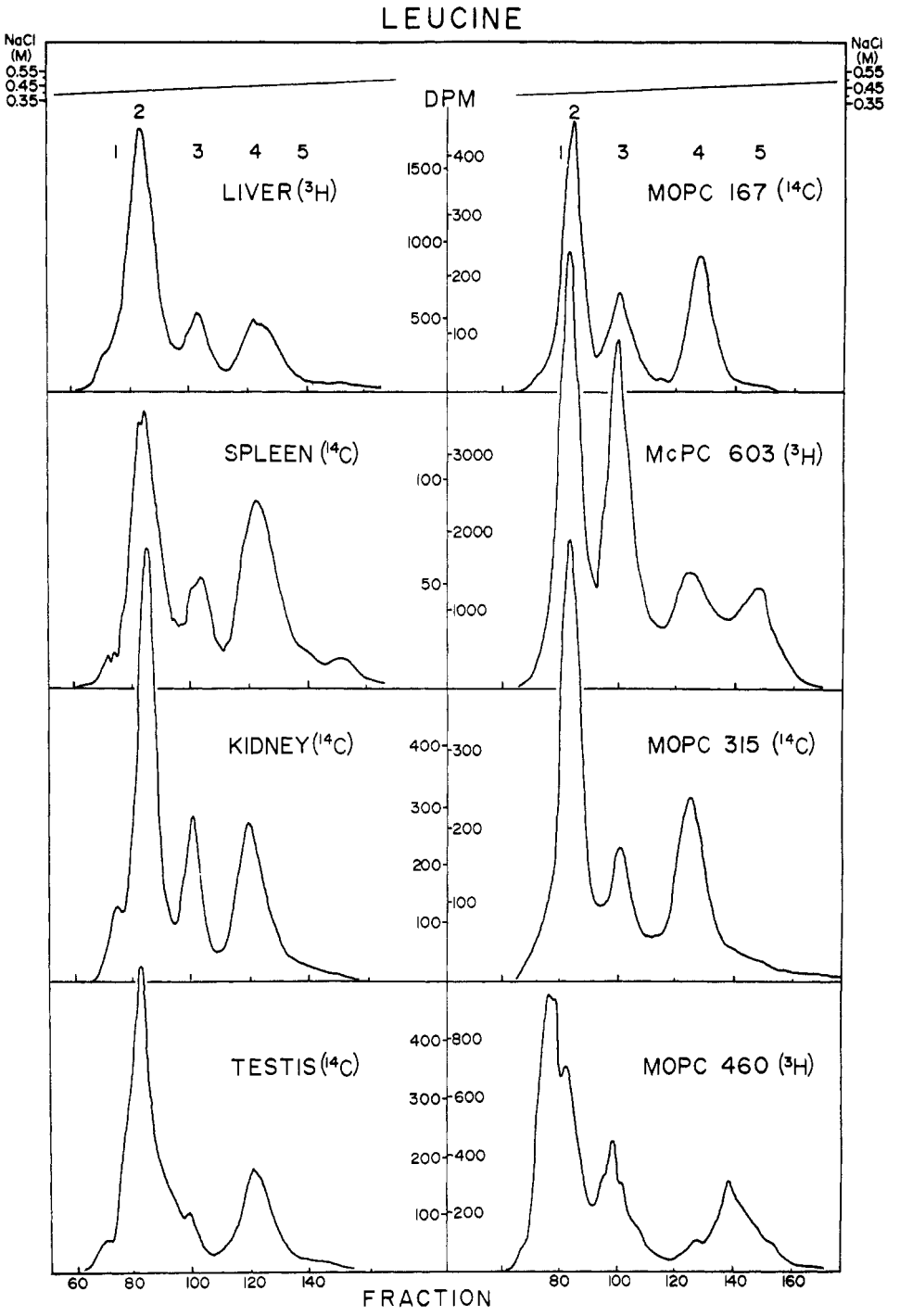

FIGURE 5: Leu-tRNA patterns of normal tissues, on the left, charged with liver enzyme, and plasma cell tumors, on the right, charged with enzymes from the same tumor. The linear NaCl gradients are illustrated at the top of each column of graphs, and the peaks are numbered as shown just above the top patterns.

Moseley X-Y recorder. More than one-hundred points are included in each pattern and presented here as a continuous line through all the points.

Results

tRNA Patterns Isolated from Tumors at Different Transplant Generations. To investigate the biological stability of tRNA patterns, tRNA was isolated from MOPC 315 tumors at three different times during normal maintenance of the tumor by continuous passage in BALB/c mice. tRNA patterns for the earliest and latest preparations were compared by double-label chromatography as seen in Figure 1. Eighteen months and twenty-four serial transplantations separated the two tumor pools from which tRNA was prepared. Both preparations were stripped and charged on the same day with the same enzyme. The Ser-tRNA pattern seems to have changed least between preparations.[1] Tyr-tRNA seems to have under-

[1] The pattern for Ser-tRNA from MOPC 315 illustrated in Figure 1 is rather atypical due to an irregularity in the generation of the gradient and prolonged use of the chromosorb packing. The patterns shown in

gone a slight reciprocal change in amounts of peaks 1 and 3, while Leu-tRNA shows a flattening in the area of peaks 3–5. The general outlines of the patterns are remarkably stable over this long period of time.

tRNA Patterns from "Sister Line" Tumors. Another comparison, designed to investigate the stability of tRNA patterns, studied tumors Adj. PC 6A and Adj. PC 6C which originated simultaneously as different granulomatous nodules in a single BALB/c mouse. The IgA myeloma protein products of these sister line tumors have remarkable antigenic similarity but a distinctive size difference (Potter and Kuff, 1964; Lieberman *et al.*, 1968; Mushinski, 1971). These lines have been separately maintained by serial BALB/c transplantation over nearly 10 years and more than 100 transplant generations. tRNA was prepared from these tumors, charged with homologous enzymes, and chromatographically compared as seen in Figure 2. The remarkable similarity of these patterns reinforces the conclusion that these tRNA patterns are stable over long periods of time and may reflect a biochemical situation established in a clone of cells at the time of their differentiation into a cell specifically determined to synthesize a unique protein product.

Chromatographic Patterns of Tyr-tRNA. Figure 3 contains a composite of the typical Tyr-tRNA elution patterns for the four normal organs and the four IgA-producing plasma cell tumors chosen for study. Each pattern originally was one part of a double-label chromatography run which permits orientation of the resulting peaks in the pattern of one preparation with those of the others. This shallowest of all gradients resulted in a partial separation of what appears to be three major components, as identified in the figure.

All four normal organs have the same basic pattern, and the patterns of the four tumors also look alike. However, all of the tumor patterns have a prominent third peak not seen in any normal tissue. This point is particularly clear in Figure 4 where a double-label experiment comparing the Tyr-tRNA from liver and from MOPC 167 on a single column is illustrated. In the normal tissues the first peak is larger than the second except for the spleen pattern where the second peak assumes prominence, and the third peak is all but absent. In all four tumors the second peak is predominant and the third peak has achieved nearly the same height as the first peak.

Chromatographic Patterns of Leu-tRNA. The composite of representative Leu-tRNA patterns for normal organs and tumors is found in Figure 5. The five tRNA peaks described in our previous communication (Mushinski and Potter, 1969) are numbered at the top. All four normal organ systems have Leu-tRNA patterns where the peaks in decreasing magnitude are 2, 4, 3, 1, and 5. The similarity of these patterns is notable except for testis where peak 3 is very small.

In all the tumor patterns, peak 2 also predominates, but peak 3 varies from a huge peak in McPC 603 to a small peak in MOPC 315. Peaks 4 and 5 are also variable in size. In McPC 603 and MOPC 460, peak 5 is usually prominent while in MOPC 460, peak 4 has all but disappeared. This sort of variation is shown in detail in Figure 6 where peak 5 of McPC 603 is quite prominent, but in the corresponding position of the double-label chromatogram MOPC 167 has practically no Leu-tRNA.

Chromatographic Patterns of Ser-tRNA. The patterns of Ser-tRNA from organs and tumors are collected in Figure 7.

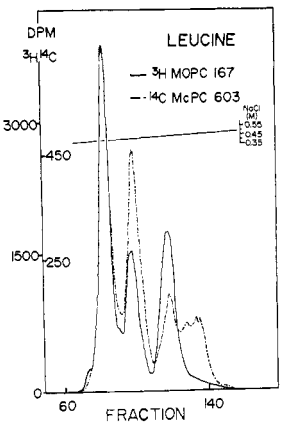

FIGURE 6: Leu-tRNA patterns for MOPC 167 and McPC 603 chromatographed simultaneously on a reversed-phase column as described in the text.

The patterns of peaks are much more complicated than for Tyr-tRNA and Leu-tRNA, although remarkably reproducible from day to day, and it is difficult to determine the number of individual peaks represented in the eight tissues. Based on many double-label chromatograms, three of which are shown in Figure 8, the most reasonable numbering system for eight peaks was derived.

Differences can be noticed among the normal organs although a basic similarity among them seems more evident than among the tumors compared to their right. A predominance of peaks 1 and 3 plus the presence of a low, broad peak eluting later is apparent in the four organs. The individual variations include the presence of a substantial peak 2 only in liver and a peak 5 prominent in testis but also present in liver.

The still greater variability among the Ser-tRNA patterns of the tumors leaves a prominent peak 3 as the only common element shared by all four. Perhaps the most evident feature of variation is the prominent peak 4 in the patterns of MOPC 167 and MOPC 315 with only a small representation in the other two tumors. This peak is seen in none of the four normal organs. Other special characteristics include a prominent peak 8 in MOPC 167, peak 7 in MOPC 460, peak 5 in MOPC 460, and peak 2 in McPC 603 and MOPC 460. Notably diminished are peak 1 in MOPC 167, and MOPC 315 and peak 3 in MOPC 460. Most of these differences are illustrated in the double-labeled chromatograms in Figure 8.

Certain trivial causes of the pattern differences have been ruled out such as isotopic contamination of the [^{3}H]- or [^{14}C]amino acid which could introduce spurious aminoacyl-tRNA peaks. This is unlikely in the light of the identical patterns shown here for Adj. PC 6A and Adj. PC 6C, and was ruled out in experiments in which the isotopes were reversed. Effects due to variations in aminoacyl-tRNA synthetase enzymes, rather than different tRNA populations, were ruled out in previous studies (Yang and Novelli, 1968; Mushinski and Potter, 1969) and in several cases in the present studies by the finding that charging with heterologous enzymes induced no significant changes in tRNA elution patterns. Figure 9 shows a double-label elution pattern for two preparations of Leu-tRNA from Adj. PC 6A which had been charged a year apart. Although much isotopic loss by deacylation is evident in the different ordinate scales for the two isotopes, the basic patterns have not changed. This demonstrates that differences in deacylation rate for different isoaccepting

Figures 7 and 8 are representative of seven very similar chromatograms obtained earlier in the life of this packing.

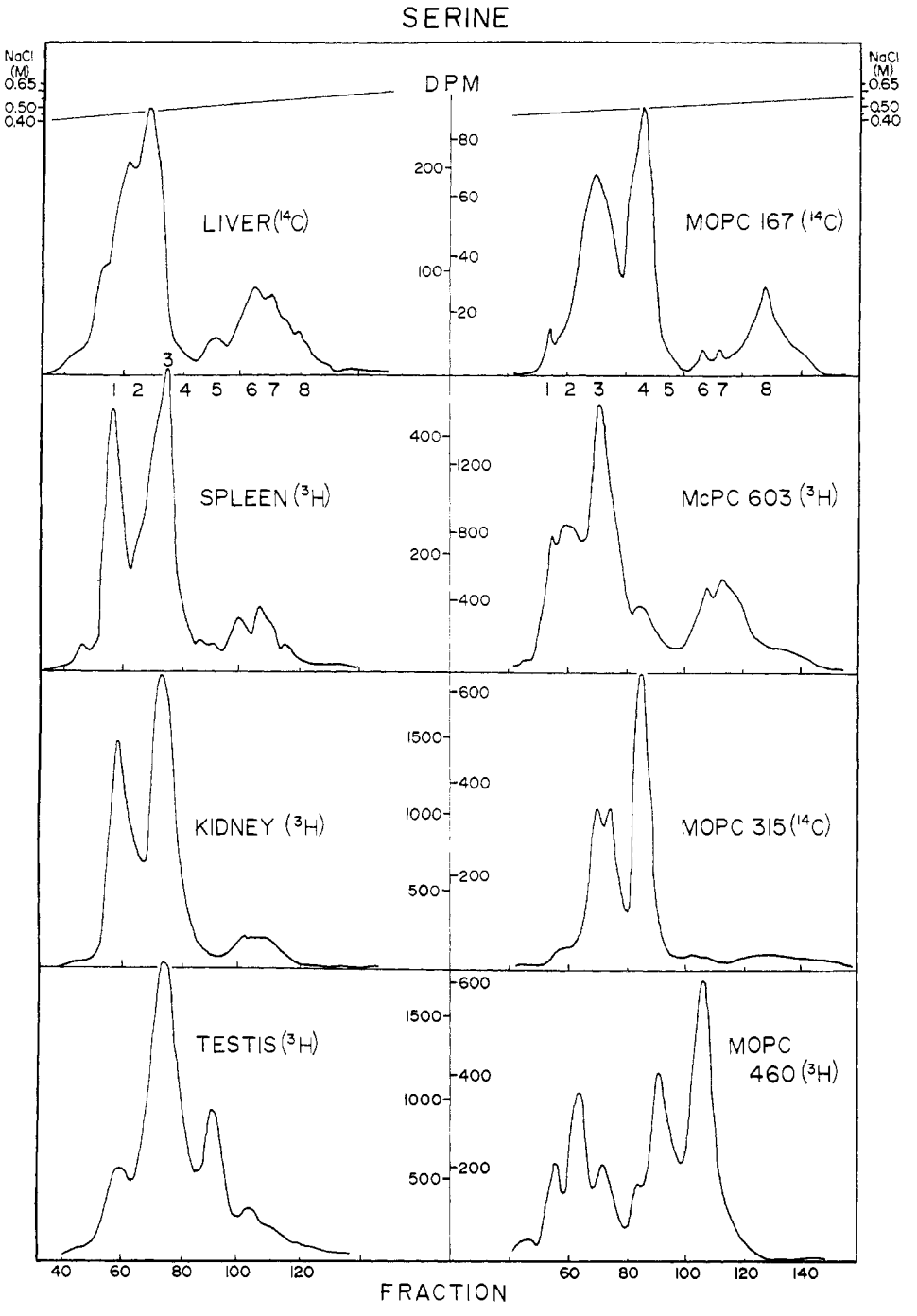

FIGURE 7: Ser-tRNA patterns of normal tissues, on the left, charged with liver enzyme, and plasma cell tumors, on the right, charged with homologous enzymes. The NaCl gradients were linear from 0.40 to 0.65 M in 0.01 M sodium and magnesium acetate (pH 4.5) as indicated at the top of each column of graphs. The numbering system for these eight peaks of Ser-tRNA is indicated just below the top pattern in each row.

species of Leu-tRNA are not responsible for pattern alteration.

Discussion

In this report we show major differences in aminoacyl-tRNA patterns in very highly differentiated and specialized neoplastic cell lines which synthesize and secrete IgA-immunoglobulins. These differences take on particular significance in the light of the findings that the patterns of aminoacyl-tRNA from several different generations of the same tumor do not show differences nor do tRNAs from two sister line tumors.

The biological significance of such tRNA variations is not yet clear. We have suggested (Mushinski and Potter, 1969) that independent regulation of individual genes for each isoaccepting tRNA species could result in the overproduction of one tRNA species in one particular cell line or, conversely, its absence in another cell line.

Of course factors acting on the tRNAs after the transcription process is completed could also contribute to differences in elution patterns. Any dimers in the tRNA preparations

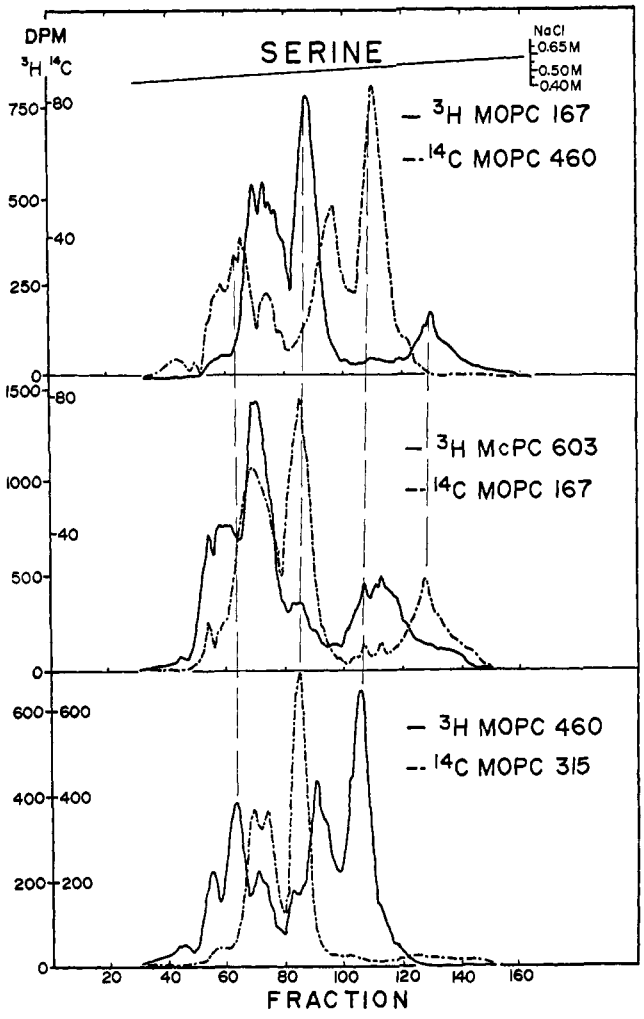

FIGURE 8: Ser-tRNA patterns chromatographed simultaneously on a Freon-Aliquat column as described in the text. The light vertical lines indicate the positions of prominent tRNA peaks for comparison with patterns where one or more of them is missing.

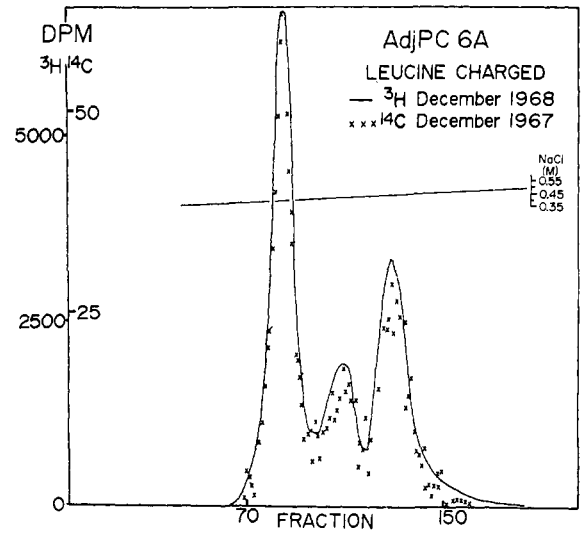

FIGURE 9: Double-label chromatography comparing one tRNA preparation from Adj. PC 6A, charged with leucine and stored at −20° for 1 year, with a freshly charged portion of the same tRNA preparation.

were removed before charging by Sephadex G-100 gel filtration, but variable aggregation or configuration alteration after charging could have been responsible for some pattern differences and must be studied further. We feel that having handled all preparations identically, we can make valid and meaningful comparisons of the chromatographic data.

Variability of degree of methylation could contribute to variation in elution patterns of tRNAs (Nau *et al.*, 1969), but no careful comparison among myelomas for methylase differences has been reported. Methylating enzymes are present in the DEAE enzyme fractions used for tRNA charging in the work reported here; ATP and methionine are present in the reaction mixture; and no change in chromatographic pattern is noticed if heterologous enzymes are used to charge the tRNA. This suggests that any variation that may exist in methylases from different tumors is unlikely to play a significant role in determining the chromatographic patterns unique to each tumor. However it is known that full methylation *in vitro* may require the presence of considerable *S*-adenosylmethionine, and methylase differences may not be evident at low enzyme concentrations (F. Nau, personal communication). For these reasons we will pursue a more careful investigation into the role of methylases in influencing tRNA profiles.

It should be noted that the nature of tRNA pattern variation among the plasma cell tumors resembles that seen among different normal organs. The Tyr-tRNA patterns of all the normal organs are very similar, and, in like manner, the patterns of all the tumors' Tyr-tRNA resemble each other strongly. Leu-tRNA patterns are somewhat more variable among normal organs, and a similar degree of moderate variability is seen among the IgA-producing myeloma Leu-tRNAs illustrated here and among Leu-tRNAs from κ-chain-producing plasmacytomas reported earlier (Mushinski and Potter, 1969). Considerable variability is seen here among Ser-tRNA patterns of normal tissues and, this is even more exaggerated among plasma cell tumors.

Plasma cell tumors represent end stages of development and specialization much like normal, well-differentiated organs. Although the tumors studied here have certain similarities in as much as they all secrete IgA-immunoglobulins, each tumor's protein product has an unique amino acid sequence. MOPC 315 and MOPC 460 make antibody-like anti-DNP immunoglobulins (Eisen *et al.*, 1968), and MOPC 167 and McPC 603 secrete proteins which precipitate pneumococcal C polysaccharide (Potter and Leon, 1968). Yet each of the proteins with similar antibody-like specificity can be distinguished from the other by its strength of antigen binding and by the haptens by which the specific antigen binding can be inhibited. Further, amino acid sequence studies on the proteins from MOPC 167 and McPC 603 have revealed differences in the amino-terminal end of their light chains (Hood *et al.*, 1970).

These unique immunoglobulins represent the concentration of a considerable proportion of the energy and synthetic activity of each plasma cell tumor. Possibly, then, these cells have a much more limited diversity of physiological functions than the normal organs studied here. Since the predominant function of each plasmacytoma is devoted to the production of a unique myeloma protein for secretion, the tRNAs among different protein producers might reflect this by appearing more unique in chromatographic pattern than the tRNAs from various organs whose functions are diverse and complicated. Not having the production of one single protein predominate, the normal organs' tRNAs might reflect a com-

bination of many functions and end up as a general sort of pattern more or less similar in all organ systems. It will be interesting to test this hypothesis by comparing the tRNA patterns of clones of lymphoid cells making a single antibody or endocrine organs which export a limited number of protein hormones.

However, even if this hypothesis were established it would be difficult to decide whether the tRNA variations were due to the variable requirements of the different cells (different messengers to be translated) or whether the synthesis of each of the tRNAs was independently regulated at the transcription level and the different tRNA species then regulated which messengers could be translated or controlled other cellular functions. In a previous study (Mushinski *et al.*, 1970) we could not show any particular effect on the *in vitro* translation of rabbit hemoglobin messenger using tRNA from one or another mouse plasmacytoma. Clearly further biological and biochemical studies are needed to better understand the meaning of these different distributions of isoaccepting tRNAs.

Acknowledgments

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In Vivo Synthesis, Molecular Weights, and Proportions of Mitochondrial Proteins in *Neurospora crassa**

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ABSTRACT: Mitochondria of wild-type *Neurospora crassa* synthesized three major proteins, comprising 10% of their total protein, with molecular weights of 33,500, 27,700, and 17,500 in the relative proportions 64:20:16. Minor proteins, about 1% of the total, revealed weights of 11,000, 21,000, and 25,000. A mitochondrial mutant *mi*-1 also synthesized three proteins. For these, the overall specific activities were identical with those of the three proteins from wild type, but their proportion and electrophoretic mobility differed. Mitochondrial proteins in wild type of molecular weights between 2500 and 10,000 were synthesized by cytoribosomes but did not appear in the cytosol itself. They comprised 12% by weight of the total mitochondrial protein, and they apparently were not products of either turnover or incomplete synthesis.

Although it is now well established that mitochondria synthesize some of their own proteins, that these proteins are localized in the inner membrane and are insoluble in water, and that they comprise between 5 and 15% by weight of the total mitochondrial protein (Ashwell and Work, 1970), the physicochemical, genetical, and functional properties of these proteins are not presently well defined. Definition of these properties should contribute to an understanding of mitochondrial biogenesis (Roodyn and Wilkie, 1968) and differentiation (Flavell, 1971).

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