# Transfer Ribonucleic Acids from Mouse Plasmacytoma Tumors Producing κ and λ Immunoglobulin Chains†

Kenneth R. Bridges and George H. Jones*

ABSTRACT: The chromatographic patterns of tRNAs from two mouse plasmacytoma tumors, MOPC-41 and RPC-20, have been studied. These tumors were chosen because they produce κ and λ immunoglobulin chains of known amino acid sequence. RPC-5 chromatography of precharged tRNAs for the amino acids methionine, leucine, and glutamic acid revealed no differences in chromatographic profiles. Significant differences between the MOPC-41 and RPC-20 threonyl- and seryl-tRNAs were observed. To determine whether the observed chromatographic differences had functional significance, serine-containing proteins were synthesized in an RPC-20 microsomal system in response to RPC-20 and MOPC-41 tRNAs. Analysis of tryptic peptides from these incubations revealed no qualitative differences in the products formed in response to homologous and heterologous tRNA. However, some peptides seemed to be synthesized more rapidly in response to MOPC-41 than to RPC-20 tRNA. These results are discussed in terms of a regulatory role of seryl-tRNA in plasmacytoma protein synthesis.

The chromatographic properties of tRNAs from neoplastic cells have recently been examined in a number of laboratories. In particular, the reverse-phase chromatographic profiles of tRNAs from normal rat liver and from minimal deviation hepatomas have been compared for a number of amino acids (Volkers and Taylor, 1971; Gonano *et al.*, 1971). Chromatographic differences were observed for certain amino acids, but it was not possible to correlate these variations in terms of the function of the hepatoma translational machinery.

Similarly, several groups have examined the chromatographic properties of tRNAs from mouse plasmacytoma tumors (Yang and Novelli, 1968; Mushinski and Potter, 1969). The plasmacytoma systems have the advantage that they produce relatively large amounts of specific immunoglobulin molecules (Potter, 1967). Mushinski and Potter (1969) and Mushinski (1971) have studied the properties of plasmacytoma leucy-tRNAs in some detail. They have found variations in the chromatographic profiles of leucyl-tRNAs from several plasmacytomas producing κ immunoglobulin chains. Differences in the profiles of plasmacytoma and liver leucyl-tRNAs were also observed. Despite these variations, leucyl-tRNAs from the plasmacytoma and from mouse liver functioned identically in hemoglobin synthesis on rabbit reticulocyte ribosomes (Mushinski *et al.*, 1971).

The present study was undertaken to examine in more detail the nature and function of tRNAs from two mouse plasmacytomas, MOPC-41 and RPC-20. These systems possess two distinct advantages over those previously studied: (1) MOPC-41 and RPC-20 produce κ and λ immunoglobulin light chains, respectively, of known amino acid sequence (Edelman and Gall, 1969; Apella, 1971). It should thus be possible to study the biosynthesis of these specific immunoglobulin chains, making use of the protein chemical techniques which have been developed for the determination of their amino acid sequences. (2) tRNA-dependent cell-free protein synthesizing systems have been obtained from these tumors which are highly active in light-chain biosynthesis (Mach *et al.*, 1967). In the present study, tRNAs for five amino acids (methionine, leucine, glutamic acid, threonine, and serine) have been compared by reverse-phase (RPC-5) chromatography. Significant reproducible differences were observed in the chromatographic profiles of the seryl- and threonyl-tRNAs of MOPC-41 and RPC-20. The seryl-tRNAs from the two tumors were compared in terms of their function in an RPC-20 cell-free protein synthesizing system using endogenous mRNA. No qualitative differences were observed in the patterns of tryptic peptides obtained in response to the two tRNA samples. However, significant quantitative differences were found, suggesting that the nature of the tRNA supplied does have some effect on the rate of synthesis of specific proteins.

## Materials and Methods

*Plasmacytoma Tumors.* Mouse plasma cell tumors MOPC-41 and RPC-20 were obtained from Dr. Michael Potter, National Institutes of Health, Bethesda, Md., and were maintained by continuous transplantation into BALB/c female mice.

*tRNA Preparation.* tRNA was prepared from freshly excised tumors by phenol extraction. Tumor (20–50 g) was homogenized in an equal volume of Medium A (0.05 M Tris-HCl–0.025 M KCl–0.005 M $MgCl_2$, pH 7.6) containing 0.2 M sucrose and poly(vinyl sulfate) (4 μg/ml). The tumors were disrupted with six strokes of a motor-driven Potter homogenizer and four strokes in a Dounce homogenizer. The homogenate was centrifuged for 20 min at 20,000*g* and the resulting supernatant was again centrifuged for 90 min at 100,000*g*. An aliquot of the 100,000*g* supernatant was saved for the preparation of aminoacyl-tRNA synthetases. The remaining 100,000*g* supernatant was extracted with an equal volume of water-saturated phenol for 15 min at room temperature. The aqueous and phenol phases were separated by centrifugation and the phenol phase extracted with 0.5 volume of medium A. Finally, the combined aqueous phases were extracted with an equal volume of phenol. tRNA was precipitated from the

† From the Department of Zoology, University of Michigan, Ann Arbor, Michigan 48104. *Received October 25, 1972.* Supported by Research Grant 5 R01 CA12752-02 from the National Cancer Institute, U. S. Public Health Service, and Grant IN-40L from the University of Michigan Cancer Research Institute.

aqueous phase by the addition of 0.1 volume of 1 N sodium acetate (pH 5.5) and 2.5 volumes of ethanol. After standing overnight at −10°, the tRNA precipitate was collected by centrifugation, dissolved in 2–5 ml of 0.5 M Tris-HCl (pH 8.8), and incubated for 2 hr at 37°. The solution was then diluted to 2.5 times its volume with distilled water and applied to a 1 × 15 cm column of DEAE-cellulose equilibrated with 0.2 M NaCl. The column was washed with this solution until the effluent was free of material absorbing at 260 nm, and the tRNA was eluted with 1 M NaCl. tRNA-containing fractions were pooled and desalted by passage through a 3 × 30 cm column of Sephadex G-25 in distilled water. Finally, the tRNA was lyophilized and redissolved in 0.001 M $MgCl_2$.

*Aminoacyl-tRNA Synthetases.* An aliquot of the 100,000*g* supernatant was passed through a 1 × 15 cm column of DEAE-cellulose equilibrated with medium A containing 0.2 M sucrose, 0.005 M 2-mercaptoethanol, and 0.3 M KCl. Enzyme-containing fractions were pooled and desalted by passage through a 1.8 × 30 cm column of Sephadex G-25, equilibrated with medium A, 0.2 M sucrose, and 0.005 M 2-mercaptoethanol. The synthetase containing fractions were pooled and stored in small aliquots at −70°.

*Aminoacylation of tRNA.* Reaction mixtures for charging tRNA contained per ml: Tris-HCl, pH 7.2, 100 μmol; ATP, 5 μmol; $MgCl_2$, 10 μmol; 2-mercaptoethanol, 6 μmol, 19 nonradioactive amino acids, 0.1 μmol; radioactive amino acid, 50 μCi ($^3H$ or $^{35}S$) or 10 μCi ($^{14}C$); tRNA, 0.50 mg, and aminoacyl-tRNA synthetase, 1 mg. Samples were incubated for 10 min at 37°. After incubation for preparation of [$^3H$]-aminoacyl-tRNA (RPC-20) and [$^{35}S$]- or [$^{14}C$]aminoacyl-tRNA (MOPC-41), 0.1 volume of 1 N sodium acetate was added to the reaction mixtures which were combined and immediately extracted with an equal volume of water-saturated phenol. Reextraction of the phenol and combined aqueous phases followed, and the final aqueous layer was freed of phenol and amino acids by passage through a 3 × 30 cm column of Sephadex G-25 in 0.01 M sodium acetate–0.01 M $MgCl_2$–0.001 M 2-mercaptoethanol (pH 5.5). tRNA-containing fractions were pooled, 2.5 mg of *Escherichia coli* tRNA was added, and the samples were either stored at −70° or applied directly to the reverse-phase column.

*Reverse-Phase Chromatography.* RPC-5 column packing was prepared by method C of Pearson *et al.* (1971), using Plaskon CTFE powder (Allied Chemical Corp.) and Adogen 464 (Ashland Chemical Co.). Chromatography was performed in a 1.2 × 100 cm water-jacketed column maintained at 25°. tRNAs were eluted with a linear gradient composed of 400 ml of 0.5 M NaCl in 0.01 M sodium acetate–0.01 M $MgCl_2$–0.001 M 2-mercaptoethanol (pH 5.5) and 400 ml of 0.8 M NaCl in the same buffer. Fractions of 3 ml were collected at a flow rate of 15 ml/hr. Alternate fractions were assayed by the addition of 0.5 mg of total yeast RNA and trichloroacetic acid to 10%. Precipitates were collected on glass-fiber filters and counted in a toluene–liquifluor scintillation cocktail.

*Cell-Free Protein Synthesis.* Amino acid incorporation into protein was measured in a microsomal system prepared from tumor RPC-20 as described by Mach *et al.* (1967). Reaction mixtures contained per ml: Tris-HCl, pH 7.6, 50 μmol; $MgCl_2$, 10 μmol; KCl, 60 μmol; 2-mercaptoethanol, 5 μmol; ATP, 5 μmol; GTP, 0.5 μmol; creatine phosphate, 10 μmol, creatine kinase, 50 μg; 19 nonradioactive amino acids, 0.1 μmol; [$^3H$]serine, 25 μCi, or [$^{14}C$]serine, 5 μCi; RPC-20 tRNA, 0.06 mg, or MOPC-41 tRNA, 0.05 mg; RPC-20 synthetase preparation, 0.36 mg; RPC-20 microsomes, 2.64 mg of protein. Mixtures were incubated for 10 min at 37° without microsomes to allow charging of tRNA. Microsomes were then added and the incubation was continued at 37° for 20 min. The reaction was stopped by adding trichloroacetic to a final concentration of 10% and heating at 90° for 15 min. Precipitated samples were washed three times with 10% trichloroacetic acid, three times with ethanol–ether, once with ether, and dried.

*Trypsin Digestion and Dowex 1 Chromatography.* Labeled proteins prepared as described above were dissolved in performic acid. $^{14}C$- and $^3H$-labeled samples were mixed and allowed to stand 1 hr at room temperature. The performic acid was diluted with water and the samples were lyophilized. Dried samples were suspended in 0.5% ammonium bicarbonate and digested with 100 μg of trypsin for 6 hr at 37°. The digestion was stopped by lyophilization and the dried peptides were dissolved in 2% pyridine–1% collidine (pH 9.0). The resulting solution was applied to a 1 × 30 cm column of Dowex 1-X2 (100–200 mesh) and the peptides were eluted with a three chamber gradient composed of 2% pyridine–1% collidine (pH 8.5) (2 × 130 ml) in the mixing chambers and pyridine–acetate (pH 5.0) (130 ml) in the reservoir. The column was maintained at 37° and eluted at a flow rate of 36 ml/hr, and 1.6-ml fractions were collected. The column was stripped with 4 N acetic acid. Aliquots of alternate fractions were counted in a Triton X-100 containing scintillation cocktail.

*Counting of Radioactive Samples.* Liquid scintillation counting was performed in a Beckman LS-250 liquid scintillation counter equipped with automatic quench correction. Corrections were made for crossover of $^{14}C$ counts into the tritium channel. Tritium crossover was negligible.

Radioisotopes used in this study were as follows: [$^{35}S$]- and [$^3H$]methionine (Amersham/Searle, 30.5 and 5 Ci per mmol, respectively); [$^3H$]- and [$^{14}C$]leucine (New England Nuclear, 35.5 Ci/mmol and 327 Ci/mmol, respectively); [$^3H$]- and [$^{14}C$]threonine (New England Nuclear, 2.38 Ci/mmol and 182 Ci/mmol, respectively); [$^3H$]- and [$^{14}C$]glutamic acid (Schwarz/Mann, 15 Ci/mmol and 260 Ci/mol, respectively); [$^3H$]- and [$^{14}C$]serine (New England Nuclear, 1.23 Ci/mmol and 128 Ci/mol, respectively).

## Results

*RPC-5 Chromatography of MOPC-41 and RPC-20 tRNAs.* The chromatographic profiles of isoaccepting tRNAs for the amino acids methionine, leucine, glutamic acid, threonine, and serine were examined in this study. These amino acids were not selected randomly. Methionine was selected because of its established role in the initiation of protein biosynthesis (Jackson and Hunter, 1970). It was thus felt that any observed differences in the Met-tRNAs might be related to some regulatory function at the level of initiation. Leucine was chosen because a relatively large number of codons have been assigned to it (Nirenberg *et al.*, 1966) and because variations in plasmacytoma leucyl-tRNAs have been observed by other workers (Mushinski and Potter, 1969). Glutamic acid was chosen because it has been shown to be the precursor of L-pyroglutamic acid "*in vivo*" (Twardzik and Peterkofsky, 1972). L-Pyroglutamic acid is the amino-terminal amino acid of the RPC-20 light chain (Apella, 1971), and it has been postulated that its formation might involve the participation of a specific glutamyl-tRNA (Twardzik and Peterkofsky, 1972). Finally, serine and threonine were chosen because of their high frequencies of appearance in the light chains in question and because of reported chromatographic differences observed

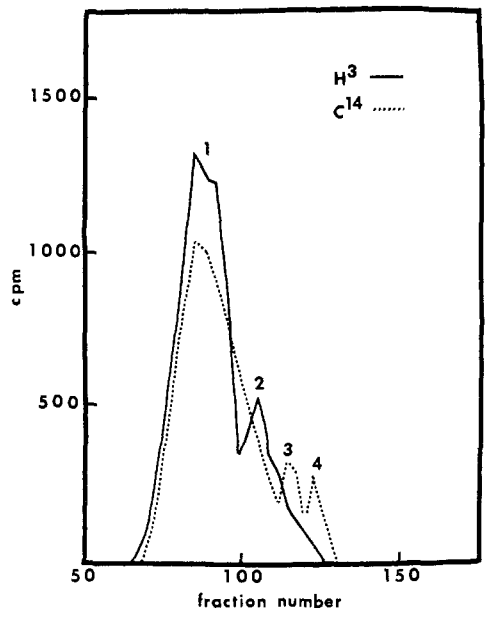


FIGURE 1: RPC-5 column chromatography of RPC-20 and MOPC-41 threonyl-tRNAs. Aminoacyl-tRNA was prepared as described in Materials and Methods. *E. coli* tRNA (2.5 mg) was added to the labeled tumor preparations prior to chromatography. Aminoacyl-tRNAs were eluted with a linear gradient of 0.5–0.8 M NaCl in 0.01 M sodium acetate–0.01 M $MgCl_2$–0.001 M 2-mercaptoethanol (pH 5.5). RPC-5 chromatography was performed at 25°, and 90,000 cpm of RPC-20 [$^3$H]threonyl-tRNA and 46,000 cpm of MOPC-41 [$^{14}$C]threonyl-tRNA were applied.

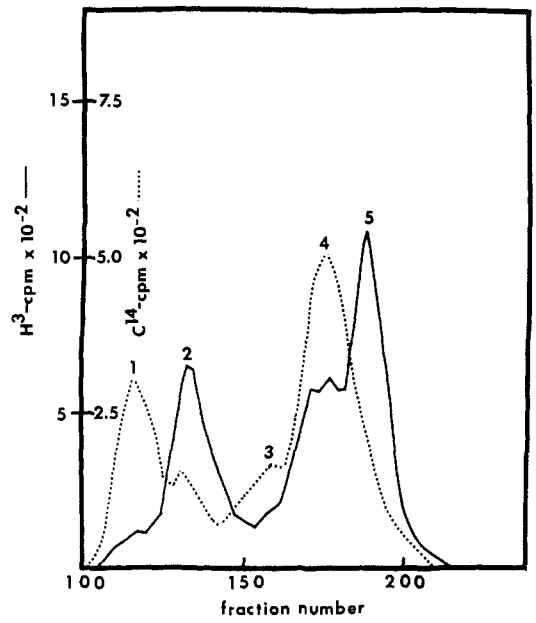


FIGURE 2: RPC-5 column chromatography of RPC-20 and MOPC-41 seryl-tRNAs. Conditions were as in the legend to Figure 1 and 55,000 cpm of RPC-20 [$^3$H]seryl-tRNA and 31,000 cpm of MOPC-41 [$^{14}$C]seryl-tRNA were applied.

in seryl-tRNA profiles from other tumors (Yang and Novelli, 1968).

The chromatographic patterns shown in Figures 1 and 2 were reproducible with different batches of tRNA and synthetase and with different RPC-5 column preparations. (It was observed, however, that using the column for more than four or five separations resulted in a marked decrease in resolution.) The nature of the profile obtained did not depend on whether a homologous or heterologous (different tumor or mouse liver) aminoacyl-tRNA synthetase preparation was used, and essentially identical patterns were obtained when the isotopes used to label the tRNAs were reversed. Pearson *et al.* (1972) suggest performing the RPC-5 separations at 37°. No increase in resolution was observed in the present study when the temperature was increased from 25 to 37°. However, the tRNAs did elute at higher salt concentrations at the higher temperature.

Examination of the chromatographic patterns for the amino acids methionine, leucine, and glutamic acid showed no differences (data not shown). It is significant that the chromatographic profiles for the MOPC-41 and RPC-20 glutamyl-tRNAs are identical, considering the postulated role of tRNA in the formation of amino-terminal L-pyroglutamic acid (Twardzik and Peterkofsky, 1972). This finding may mean that there is no tRNA species specifically involved in the formation of L-pyroglutamic acid, or that MOPC-41 synthesizes some L-pyroglutamic acid containing proteins, even though its light chain does not possess this rare amino acid.

Significant chromatographic differences were observed for the tumor threonyl- and seryl-tRNAs. Specifically, inspection of Figure 1 shows that whereas both MOPC-41 and RPC-20 contain the major species of threonyl-tRNA, only RPC-20 contains peak 2 and only MOPC-41 contains peaks 3 and 4. Similarly, Figure 2 shows that although MOPC-41 possesses a relatively large amount of serine peak 1 and a smaller amount of peak 2, these proportions are reversed in RPC-20. Further, serine peak 4 is significantly diminished in RPC-20 relative to MOPC-41, and peak 5 is completely absent from the MOPC-41 tRNA preparation.

*Cell-Free Incorporation of Serine into RPC-20 Proteins.* Studies on the amino acid sequence of the RPC-20 and MOPC-41 L chains have revealed that serine and threonine make up about 25% of their total amino acid content, and that the relative amounts of the two amino acids are reversed in the two L chains. Specifically, the MOPC-41 L chain contains 17.8% serine and 8.5% threonine, while the RPC-20 L chain contains 11.2% serine and 14% threonine. By way of contrast, both L chains contain about 7% leucine (Edelman and Gall, 1969; Apella, 1971). It thus seemed possible that the observed chromatographic differences in the seryl- and threonyl-tRNAs might reflect the different contents of these amino acids in the two L chains. In other words, the differences in the chromatographic profiles for the seryl- and threonyl-tRNAs might be a direct reflection of a difference in the nature of the code words for these amino acids in the L-chain mRNA (and other mRNAs) in the tumors. If this is so, one might expect to see qualitative or quantitative differences in the nature of the proteins synthesized using endogenous mRNA from one tumor and tRNA from the other.

It has been possible to test this hypothesis using a microsomal protein synthesizing system from tumor RPC-20. Previous reports indicate that up to 50% of the protein synthesized in this system is light chain (B. Mach and P. Vassalli, in preparation). Serine was chosen as the amino acid for this study. The data of Figure 3 show that under the conditions of the amino acid incorporation assay, both MOPC-41 and RPC-20 tRNAs were capable of stimulating serine incorporation into protein. On a weight basis, MOPC-41 was slightly more stimulatory than RPC-20 tRNA. It was also established

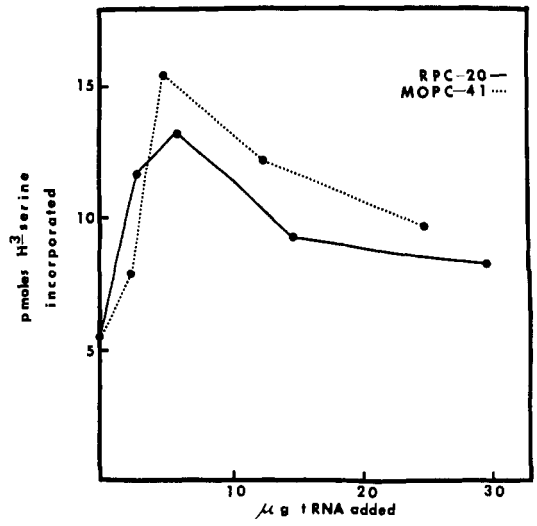


FIGURE 3: Stimulation by RPC-20 and MOPC-41 tRNAs of serine incorporation by RPC-20 microsomes. Reaction mixtures of 0.1 ml were prepared as described in Materials and Methods, except that varying amounts of RPC-20 and MOPC-41 tRNA were added to selected tubes. After a 10-min preincubation at 37°, microsomes were added to all tubes, and the incubations were continued for 20 additional min. Protein was precipitated with 10% trichloroacetic acid at 90° for 15 min, and the precipitates were collected on glass-fiber filters. Dried filters were counted in a toluene–liquifluor scintillant.

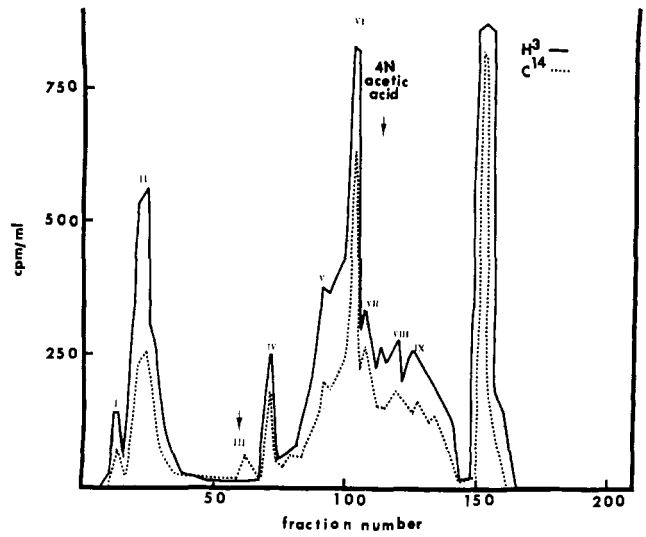


FIGURE 4: Dowex 1 column chromatography of serine containing peptides synthesized by RPC-20 microsomes. Reaction mixtures for serine incorporation were prepared as described in Materials and Methods. A 0.4-ml reaction mixture contained RPC-20 tRNA and [³H]serine and a 0.6-ml reaction mixture contained MOPC-41 tRNA and [¹⁴C]serine. Labeled proteins from the two incubations were combined, oxidized with performic acid, and digested with trypsin. Tryptic peptides were separated on a 1 × 30 cm column of Dowex 1-X2 using a three chamber gradient. Each of two mixing chambers contained 130 ml of 2% pyridine–1% collidine (pH 8.5). The reservoir contained 130 ml of pyridine–acetate (pH 5.0). The column was stripped with 4 N acetic acid. Elution was carried out at 37°, 36 ml/hr, and 1.6-ml fractions were collected. Selected fractions were counted in a Triton X-100 containing scintillant. The column input was 43,000 cpm of [³H]serine and 21,500 cpm of [¹⁴C]serine, giving an ³H:¹⁴C ratio of 2.00. The arrow denotes a small peak of ¹⁴C radioactivity which is absent from the ³H profile.

that the 10-min preincubation was sufficient to allow maximal charging of both the RPC-20 and MOPC-41 tRNA by RPC-20 synthetase (data not shown).

Large-scale incubations were then carried out containing [³H]serine and RPC-20 tRNA and [¹⁴C]serine and MOPC-41 tRNA. The labeled proteins from these incubations were combined and digested with trypsin. Tryptic digests were fractionated on Dowex 1 as described in Materials and Methods, yielding the pattern shown in Figure 4. No qualitative differences were observed in the patterns obtained from the incubations with RPC-20 and MOPC-41 tRNA, with the exception of the small ¹⁴C peak III, which was absent from the RPC-20 profile. This peak was observed reproducibly, but was always present in very small amounts. It may be an artifact of the column procedure. To determine whether the different tRNAs might exert differential effects on the rate of protein synthesis, fractions from the Dowex column corresponding to the peaks in Figure 4 were pooled, concentrated to dryness, dissolved in 1 ml of water, and counted in a Triton X-100 scintillation cocktail. The results of this analysis are shown in Table I. Peaks I, II, and X give ³H:¹⁴C ratios which are close to the input ratio of 2.00. The remaining seven peaks give lower values ranging from 0.5 for peak III (but see above) to 1.67 in peak VII. These findings suggest that some proteins (or peptides) of the RPC-20 system are synthesized at a higher rate in response to MOPC-41 than RPC-20 tRNA. Again this difference in rate may be a reflection of the availability of specific serine codons in the endogenous RPC-20 mRNA.

## Discussion

Of the MOPC-41 and RPC-20 aminoacyl-tRNAs examined in this study, only the seryl- and threonyl-tRNAs were chromatographically different. Quantitative differences in the serine containing RPC-20 peptides synthesized "*in vitro*" in response to the two tRNA preparations were also observed. These data argue for the role of seryl-tRNA in determining the rates of protein biosynthesis in the RPC-20 (and presumably the MOPC-41) plasmacytoma.

TABLE I: $H^3$:$C^{14}$ Ratios of Serine-Containing Peptides Synthesized by RPC-20 Microsomes.[a]

| Peak | Cpm ³H | Cpm ¹⁴C | ³H:¹⁴C |
|---|---|---|---|
| I | 155 | 73 | 2.12 |
| II | 1155 | 548 | 2.04 |
| III | 39 | 78 | 0.50 |
| IV | 253 | 182 | 1.40 |
| V | 748 | 476 | 1.48 |
| VI | 1333 | 818 | 1.63 |
| VII | 582 | 348 | 1.67 |
| VIII | 792 | 636 | 1.24 |
| IX | 684 | 670 | 1.02 |
| X | 5177 | 2768 | 1.88 |

[a] Fractions corresponding to peaks I–X of Figure 4 were pooled, concentrated to dryness, dissolved in water, and counted in a Triton X-100–toluene scintillant. Counts were corrected for ¹⁴C crossover into the tritium channel. The table shows counts recovered and $H^3$:$C^{14}$ for each peak.

Similar effects of tRNA on the rate of protein synthesis have been reported by Anderson (1969) and Anderson and Gilbert (1969). Anderson observed that the concentration of tRNA could regulate the rate of translation of synthetic polynucleotides in a crude *E. coli* protein synthesizing system. tRNA dependent stimulation of the rates of poly(phenylalanine) and polyarginine synthesis was observed in response to poly(U) and to poly(A,G). In the latter case, Anderson suggested that the codons A-G-A and A-G-G might be regulatory in *E. coli*. In a rabbit reticulocyte cell-free system, Anderson and Gilbert demonstrated that the $\alpha$ and $\beta$ chains of hemoglobin, normally produced in equal amounts, were produced in unequal amounts when a particular tRNA fraction was used in place of total rabbit liver tRNA. They postulated again that certain codons in the $\alpha$ and $\beta$ chain mRNAs regulate the rates of synthesis of the two chains when presented with certain species of tRNA.

Such an explanation seems plausible in the present situation. Serine is a common amino acid in the RPC-20 and MOPC-41 L-chains representing about 15% of the total amino acid content. It is conceivable that the observed difference in the rate of synthesis of RPC-20 protein in response to MOPC-41 tRNA is due to a difference in the nature of the "regulator" serine codon(s) in the RPC-20 and MOPC-41 messages.

The results presented above are in contrast to those of Mushinski *et al.* (1970) who found that the leucyl-tRNAs from different plasmacytomas functioned identically in hemoglobin biosynthesis on reticulocyte ribosomes. They observed neither qualitative nor quantitative differences in the nature of the hemoglobin peptides obtained. It should be noted however, that the results obtained in a heterologous system, using hemoglobin mRNA, need not apply to the plasmacytoma. Indeed, Mushinski *et al.* note that their data do not preclude the existence of functional differences between tRNAs from different plasmacytomas in immunoglobulin biosynthesis.

Recently, several laboratories have reported isolation of the mRNA for the MOPC-41 light chain (Stavnezer and Huang, 1971; Swan *et al.*, 1972). It should soon become possible to verify the hypothesis set forth above, and to resolve the question of the regulatory role of tRNA by examining the effects of individual seryl-tRNA species on the translation of the light-chain mRNA *in vitro*.

## References


Anderson, W. F. (1969), *Proc. Nat. Acad. Sci. U. S. 62*, 566.

Anderson, W. F., and Gilbert, J. (1969), *Biochem. Biophys. Res. Commun. 36*, 456.

Apella, E. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 590.

Edelman, G. M., and Gall, W. E. (1969), *Annu. Rev. Biochem. 38*, 415.

Gonano, F., Chiarugi, V. P., Pirro, G., and Marini, M. (1971), *Biochemistry 10*, 900.

Jackson, R., and Hunter, T. (1970), *Nature (London) 227*, 672.

Mach, B., Koblet, H., and Gros, D. (1967), *Cold Spring Harbor Symp. Quant. Biol. 32*, 269.

Mushinski, J. F. (1971), *Biochemistry 10*, 3917.

Mushinski, J. F., Galizzi, A. and von Ehrenstein, G. (1970), *Biochemistry 9*, 489.

Mushinski, J. F., and Potter, M. (1969), *Biochemistry 8*, 1684.

Nirenberg, M., Caskey, T., Marshall, R., Brimacombe, R., Kellog, D., Doctor, B., Hatfield, D., Levin, J., Rottman, F., Pestka, S., Wilcox, M., and Anderson, F. (1966), *Cold Spring Harbor Symp. Quant. Biol. 31*, 11.

Pearson, P. L., Weiss, J. F., and Kelmers, A. D. (1971), *Biochim. Biophys. Acta 228*, 770.

Potter, M. (1967), *Methods Cancer Res. 2*, 105.

Stavnezer, J., and Huang, R. C. C. (1971), *Nature (London) 230*, 172.

Swan, D., Aviv, H., and Leder, P. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 1967.

Twardzik, D., and Peterkofsky, A. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 274.

Volkers, S. A. S., and Taylor, M. R. (1971), *Biochemistry 10*, 488.

Yang, W., and Novelli, G. D. (1968), *Biochem. Biophys. Res. Commun. 31*, 534.