and Miss Gail Melson is deeply appreciated.

References

Biro, N. A., Szilagyi, L., and Balint, M. (1972), *Cold Spring Harbor Symp. Quant. Biol. 37,* 55.
Chou, P. Y., and Fasman, G. D. (1974), *Biochemistry 13,* 211.
Cohen, C., and Szent-Györgyi, A. G. (1957), *J. Amer. Chem. Soc. 79,* 248.
Cohen, C., Szent-Györgyi, A. G., and Kendrick-Jones, J. (1971), *J. Mol. Biol. 56,* 223.
Cowgill, R. W. (1967), *Biochim. Biophys. Acta 133,* 6.
Cowgill, R. W. (1968), *Biochim. Biophys. Acta 168,* 417.
Cowgill, R. W. (1972), *Biochemistry 11,* 4532.
Cowgill, R. W. (1974), *Biochemistry 13,* 2467.
Davis, B. J. (1965), *Ann. N. Y. Acad. Sci. 121,* 404.
Greenfield, N., and Fasman, G. D. (1969), *Biochemistry 8,* 4108.
Halsey, J. F., and Harrington, W. F., (1973), *Biochemistry 12,* 693.
Johnson, W. H., Kahn, J. S., and Szent-Györgyi, A. G. (1959), *Science 130,* 160.
Kahn, J. S., and Johnson, W. H. (1960), *Arch. Biochem. Biophys. 86,* 138.
Lowey, S., Kucera, J., and Holtzer, A. (1963), *J. Mol. Biol. 7,* 234.
Mihalyi, E., and Harrington, W. F. (1959), *Biochim. Biophys. Acta 36,* 447.
Mitchell, W. M. (1967), *Biochim. Biophys. Acta 147,* 171.
Olander, J. (1971), *Biochemistry 10,* 601.
Riddiford, L. M. (1966), *J. Biol. Chem. 241,* 2792.
Robson, B., and Pain, R. H. (1971), *J. Mol. Biol. 58,* 237.
Stafford, W. F., and Yphantis, D. A. (1972), *Biochem. Biophys. Res. Commun. 49,* 848.
Sluyterman, L. A. (1967), *Biochim. Biophys. Acta 139,* 418.
Szent-Györgyi, A. G., Cohen, C., and Kendrick-Jones, J. (1971), *J. Mol. Biol. 56,* 239.
Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244,* 4406.

Functional Differences in Protein Synthesis Between Rat Liver tRNA and tRNA from Novikoff Hepatoma†

Opendra K. Sharma, Laura Livingston Mays,‡ and Ernest Borek*

ABSTRACT: Synthesis of ovalbumin in fragmented oviduct magnum explants of immature, estrogen-stimulated chicks has been studied in the presence of exogenous tRNA. tRNA from Novikoff hepatoma specifically inhibited ovalbumin synthesis, determined by precipitation with antisera. In addition, the major protein(s) synthesized in the presence of hepatoma tRNA had higher electrophoretic mobility than ovalbumin, as shown by sodium dodecyl sulfate polyacrylamide gel electrophoresis. tRNAs from rat liver, rooster liver, and hen oviduct did not affect ovalbumin synthesis, although oviduct tRNA is stimulatory during the earlier stages of estrogen stimulation (Sharma *et al.* (1973), *J. Biol. Chem. 248,* 7622).

A comparison of elution profiles of aminoacyl-tRNAs from rat liver and Novikoff hepatoma, on methylated albumin Kieselguhr columns, has revealed the presence of new species of histidine tRNA, tyrosine tRNA, and asparagine tRNA among tRNAs extracted from hepatoma (Baliga *et al.,* 1969). Recently tyrosine tRNA and histidine tRNA have been shown to differ quantitatively in methylated ribonucleosides (Nau, 1974). The enzymes which introduce methyl groups at macromolecular level, tRNA methyltransferases are also aberrant in the hepatoma (Tsutsui *et al.,* 1966; Sharma, 1973). In a variety of other neoplasms tested, different isoacceptor species of tRNAs have been found (Borek and Kerr, 1972), and it has been suggested that chromatographically distinct atypical tumor tRNAs might play a regulatory role in protein synthesis. Morris hepatoma 5123D contains increased amounts of chargeable tRNA (Ouellette and Taylor, 1973). However, attempts to show functional differences in the behavior of tumor tRNAs compared to thier normal counterparts in protein synthesis have been unsuccessful either with synthetic messenger in an *Escherichia coli* cell-free system (Gonano *et al.,* 1971), or in hemoglobin synthesis on rabbit reticulocyte ribosomes (Mushinski *et al.,* 1970).

To study functional differences in tRNAs from rat liver and Novikoff hepatoma, we have used explanted oviduct magnum fragments from estrogen-stimulated immature chicks (Palmiter *et al.,* 1971). The oviduct magnum fragments in culture synthesize proteins identical with those made in the intact oviduct of chicks. In such a system, therefore, the control mechanisms within the cell remain intact and the probability of artifactual effects is minimized.

We have shown earlier that exogenous oviduct tRNA potentiated ovalbumin synthesis during the lag phase (Sharma *et al.,* 1973), in fragmented oviduct magnum explants of primary and secondary estrogen stimulated immature chicks. As an extension of these observations, tRNA from Novikoff hepatoma was added to estrogen-stimulated ovi-

† From the Department of Microbiology, University of Colorado Medical Center, Denver, Colorado 80220. *Received July 12, 1974.* This investigation was supported by National Institutes of Health Grant CA 14047 02 and Atomic Energy Commission Contract AT (11-1)-2066.

‡ Present address: Occidental College, Department of Biology, Los Angeles, California 90041.

Table I: Inhibition of Ovalbumin Synthesis by Novikoff Hepatoma tRNA.[a]

tRNA Source	^{14}C-Labeled Amino Acids Incorporated, per mg of Soluble Protein (cpm × 10^{-5})	^{14}C-Labeled Amino Acids Incorporated into Ovalbumin, per mg of Soluble Protein (cpm × 10^{-5})	Ovalbumin Synthesis, % of Total Protein Synthesis	Inhibition of Ovalbumin Synthesis (%)
None	2.54	1.35	53.1	
Rat liver				
Preparation I	2.78	1.50	54.0	
Preparation II	3.08	1.66	53.9	
Novikoff hepatoma				
Preparation I	2.15	0.72	33.5	36.9
Preparation II	2.14	0.79	36.9	30.5
None	2.84	0.88	31.0	
Hepatoma tRNA	2.47	0.42	17.0	45.2
Oviduct tRNA				
Preparation I	2.11	0.88	31.3	
Preparation II	3.06	0.89	29.1	

[a] Chicks were withdrawn for 28–35 days, following 10 days of primary estrogen stimulation. Secondary stimulation was by a single injection of 2 mg of estradiol benzoate, 48 hr prior to the experimental period. The incubation of magnum fragments was for 5 hr at 37°, tRNA was used at a concentration of 250 μg/ml. The inhibition of ovalbumin synthesis by exogenous Novikoff hepatoma tRNA was repeated with six different groups of animals. The data in Table I are the average of duplicate determinations of one of the experiments.

duct magnum explants past the lag phase when tRNA from oviducts no longer has a stimulatory influence. In the presence of hepatoma tRNAs, the ovalbumin synthesis was specifically inhibited and there was enhanced synthesis of product(s) smaller than ovalbumin. tRNAs from rat liver, rooster liver, and hen oviduct did not affect ovalbumin synthesis, although tRNA from oviduct specifically stimulated ovalbumin synthesis during the earlier stages of hormonal stimulation (Sharma *et al.*, 1973).

Materials and Methods

Animals. Four-day old Calhorn chicks, procured from Mayer Brother's Hatchery, Greeley, Colo., were injected intramuscularly below the knee daily with 1 mg of 17β-estradiol benzoate in sesame oil, and the injections were stopped after 10 days. Following a specified withdrawal period, the secondary stimulation consisted of a single injection of 2 mg of estradiol benzoate. Novikoff hepatoma was grown in the omentum of female Holtzman rats and the hepatoma was collected 5–6 days after the injection of the tumor.

17β-Estradiol benzoate was a product of Calbiochem, ^{14}C- and ^{3}H-labeled amino acid mixtures (algal profile) were purchased from Schwarz/Mann. $NaHCO_3$-free medium 199 (10×) and $NaHCO_3$-free Hank's balanced salt so- Company. Antiovalbumin serum was obtained from rabbits which had been injected with ovalbumin (5 mg) in complete Freund's adjuvant (Difco) every 2 weeks for at least 2 months. The rabbits were bled from the ear at least 2 weeks after the previous injection of ovalbumin and the separated serum was stored frozen at −18°.

Incubation of Oviduct Explants. Chicks were decapitated following 24 hr of secondary estrogen stimulation and their oviducts were removed aseptically; the magnum portions of the oviduct were freed of adjoining tissue and were cut into small pieces. The magnum explants were incubated *in vitro* according to Palmiter *et al.* (1971) in antibiotic-free medium; 100–200 mg of the tissue was incubated in 2 ml of medium in 25-ml, rubber-stoppered erlenmeyer flasks. Each flask contained tissue from at least three oviduct magna. The incubations were carried out in duplicate at 37° for 3–5 hr with constant shaking and the flasks were gassed with 95% O_2–5% CO_2 at hourly intervals.

Preparation of Cell-Free Extract. At the end of the incubation the pieces of tissue were blotted on filter paper, were homogenized in 2 ml of sodium phosphate buffer (15 mM NaCl–10 mM sodium phosphate (pH 7.5)), and were centrifuged at 100,000*g* for 1 hr. The high-speed supernatant was used for subsequent analyses. The radioactivity in the Cl_3CCOOH insoluble protein was determined by the filter disc method of Bollum (1968). Protein content of the supernatant was estimated by the procedure of Lowry *et al.* (1951).

Quantitation of Ovalbumin by Precipitation with Antibody. The method used for the determination of labeled ovalbumin was essentially that of Rhoads *et al.* (1971).

Isolation of tRNAs. Transfer RNA was isolated by the procedure of Rogg *et al.* (1969), with a few modifications. After 2-propanol precipitation the RNA was incubated with RNase-free pancreatic DNase (Worthington) followed by Pronase and extracted with phenol. The tRNA was deacylated by incubation in 1.8 M Tris-HCl (pH 8.0) at 37° for 90 min and was further purified by gel filtration on a Sephadex G-100 (2.5 × 90 cm) column equilibrated with 0.01 M potassium acetate (pH 7.0). The purity of tRNAs was checked by polyacrylamide gel electrophoresis (Peacock and Dingman, 1967).

Fractionation of Novikoff Hepatoma tRNAs on DEAE-Sephadex. Novikoff hepatoma tRNA (100 mg) was fractionated on a 1.5 × 90 cm DEAE-Sephadex A-50 column according to Nishimura (1971). The tRNA was eluted with a linear gradient at room temperature consisting of 500 ml of 0.02 M Tris-HCl (pH 7.5), 0.008 M $MgCl_2$, and 0.375 M NaCl and 500 ml of 0.02 M Tris-HCl (pH 7.5), 0.017 M

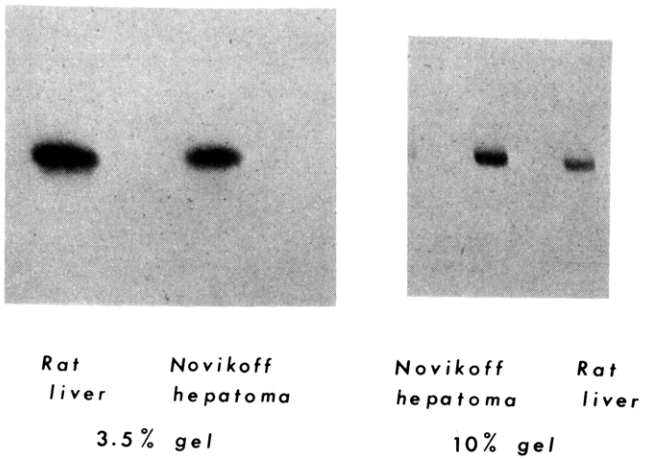

FIGURE 1: Polyacrylamide gel electrophoretograms of tRNA. tRNA (60–80 μg) was applied to the polyacrylamide gels and electrophoresed according to Peacock and Dingman (1967). The gels were stained with Methylene Blue.

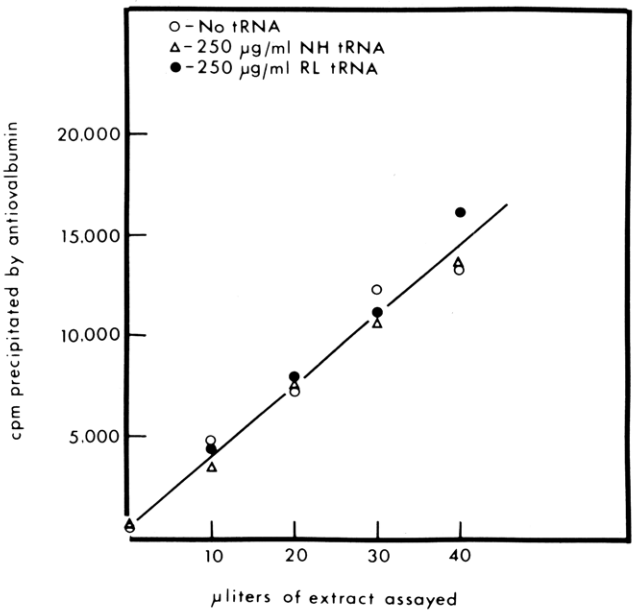

FIGURE 2: Effect of exogenous tRNA on antiovalbumin precipitation reactions. The high-speed supernatants from the oviduct magnum explants, cultured *in vitro* (without exogenous tRNA) were used for precipitation reactions. To the samples of high-speed supernatant, hepatoma tRNA or rat liver tRNA was added to a concentration of 250 μg/ml, and aliquots were taken to determine the ovalbumin precipitable by antiserum as described under Materials and Methods.

$MgCl_2$, and 0.60 M NaCl. The flow rate was adjusted to 12 ml/hr and 6.5-ml fractions were collected. From the pooled fractions tRNA was recovered by precipitation with 2.5 volumes of ethanol containing 2% potassium acetate. The precipitated tRNA was washed twice with ethanol and once with ether. It was dissolved in sterile water and stored frozen.

Results and Discussion

Addition of exogenous Novikoff hepatoma tRNA to the oviduct magnum explants of immature chicks, following 48 hr of secondary estrogen stimulation, resulted in the inhibition of ovalbumin synthesis (Table I). Ovalbumin synthesis is expressed as the per cent of total soluble protein synthesis in order to compensate for possible variations due to the amount of tissue, pool size of indigenous amino acids, and the penetration of tRNA. The inhibition of ovalbumin synthesis was consistent and was repeated in six different experiments. The tRNAs from rat liver, rooster liver (data not shown), and oviduct did not affect the ovalbumin synthesis, confirming earlier observations, where oviduct tRNA did not stimulate ovalbumin synthesis following 48 hr of secondary estrogen stimulation, although oviduct tRNA specifically enhanced ovalbumin synthesis during earlier time periods (Sharma *et al.*, 1973). The tRNAs used were electrophoretically pure, no visible traces of 5S RNA or high molecular weight RNA were evident on polyacrylamide gels (Figure 1). The ovserved inhibition of ovalbumin synthesis by hepatoma tRNA was not due to the interference with ovalbumin precipitation by antiserum, since large quantities of Novikoff hepatoma tRNA or rat liver tRNA could be added to the precipitation reaction without any effect (Figure 2).

The inhibition of ovalbumin synthesis by Novikoff hepatoma tRNA was abolished by prior ribonuclease treatment of tRNA (Table II). In control experiments ovalbumin syn-

Table II: Effect of Ribonuclease-Treated Hepatoma tRNA on Ovalbumin Synthesis.[a]

Additions	^{14}C-Labeled Amino Acids Incorporated, per mg of Soluble Protein (cpm × 10^{-5})	^{14}C-Labeled Amino Acids Incorporated into Ovalbumin, per mg of Soluble Protein (cpm × 10^{-5})	Ovalbumin Synthesis, % of Total Soluble Protein Synthesis	Inhibition of Ovalbumin Synthesis (%)
No RNA	3.24	2.08	64.2	
Hepatoma tRNA (250 μg/ml)	3.45	1.59	46.1	28.3
RNase (exposed to heat)	2.90	1.65	56.9	
Hepatoma tRNA treated with RNase and exposed to heat	2.95	1.66	56.3	

[a] Novikoff hepatoma tRNA (2 mg) was incubated with 20 μg of pancreatic ribonuclease (Worthington) in 0.05 M Tris-HCl (pH 7.5) for 1 hr at 37° (volume 200 μl) and was subsequently heated in a boiling water bath for 15 min. In a separate tube, 20 μg of pancreatic ribonuclease in 0.05 M Tris-HCl (pH 7.5) (volume 200 μl) was treated as described above; 50 μl aliquots were added to the incubation medium. The oviduct magnum explants from chicks 48 hr after secondary estrogen stimulation (withdrawn from estrogen for 30 days, following 10 days of primary stimulation) were incubated for 3 hr at 37°. The data are the average of duplicate determinations.

Table III: Inhibition of Ovalbumin Synthesis by Hepatoma tRNA in the Presence of Actinomycin D.[a]

	^{14}C-Labeled Amino Acids Incorporated, per mg of Soluble Protein (cpm × 10^{-5})	^{14}C-Labeled Amino Acids Incorporated into Ovalbumin, per mg of Soluble Protein (cpm × 10^{-5})	Ovalbumin Synthesis, % of Total Soluble Protein Synthesis	Inhibition of Ovalbumin Synthesis (%)
No tRNA	1.80	1.12	62.2	
Hepatoma tRNA	1.81	0.79	43.6	30

[a] Chicks were withdrawn for 35 days, following 10 days of primary estrogen stimulation. Secondary stimulation was for 48 hr by single injection of 2 mg of estradiol benzoate. The oviduct magnum explants were incubated in culture medium in the absence of radioactive amino acids. The cultures contained actinomycin D (10 μg/ml). Following 5 hr of preincubation, two flasks received 500 μg of hepatoma tRNA. To each flask 10 μCi of ^{14}C-labeled amino acid mixture and 10 μCi of [^{3}H]uridine were added; incubation was carried out for another hour. Incorporation of [^{3}H]uridine into RNA was inhibited more than 90%. The data are the average of duplicate determinations.

Table IV: Inhibition of Ovalbumin Synthesis by Different Concentrations of Novikoff Hepatoma tRNA.[a]

tRNA Source	tRNA Concentration (μg/ml)	^{14}C-Labeled Amino Acids Incorporated, per mg of Soluble Protein (cpm × 10^{-5})	^{14}C-Labeled Amino Acids Incorporated into Ovalbumin, per mg of Soluble Protein (cpm × 10^{-4})	Ovalbumin Synthesis, % of Total Soluble Protein Synthesis	Inhibition of Ovalbumin Synthesis (%)
None		4.10	10.33	25.2	
Novikoff	25	4.26	10.19	23.9	5.5
hepatoma	100	4.06	2.60	6.4	74.6
	250	4.69	2.95	6.3	75.0
	500	4.34	2.31	5.3	78.9
Rat liver	250	4.15	10.62	25.6	0
	500	4.32	10.90	25.2	0
Novikoff hepatoma + rat liver	250 + 250	4.32	6.05	14.0	44.4

[a] Incubation was for 5 hr at 37°, other details are same as in Table I.

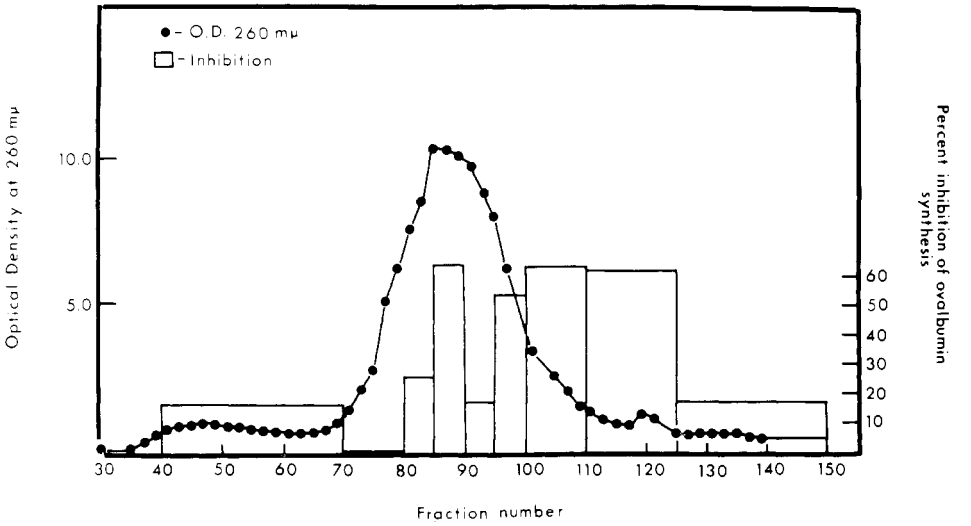

FIGURE 3: Inhibition of ovalbumin synthesis by hepatoma tRNA, fractionated on DEAE-Sephadex. Each fraction of tRNA was tested at a concentration of 250 μg/ml in the incubation medium.

thesis was inhibited 11% by the addition of heat-treated ribonuclease alone. However, no further decrease in ovalbumin synthesis was observed in the presence of ribonuclease-treated hepatoma tRNA. Ovalbumin synthesis was also

studied during conditions of simultaneous inhibition of RNA synthesis by actinomycin D. Actinomycin D (10 μg/ml) had no effect on the inhibition of ovalbumin synthesis by hepatoma tRNA (Table III).

Varying amounts of hepatoma tRNA were tested for inhibition of ovalbumin synthesis (Table IV). Maximum inhibition was observed at a concentration of 100 μg/ml of hepatoma tRNA in the incubation medium; a fivefold higher concentration of rat liver tRNA had no effect on ovalbumin synthesis. However, mixtures of rat liver and Novikoff hepatoma tRNA (250 μg of each) caused a 30% lowering of the inhibition expected with hepatoma tRNA alone.

The effect of Novikoff hepatoma tRNA on ovalbumin synthesis was observed in the presence of indigenous oviduct tRNA, evidently hepatoma tRNA competed successfully with the homologous oviduct tRNA.

The hepatoma tRNA was fractionated on a DEAE-Sephadex column and the fractions were tested for their ability to inhibit ovalbumin synthesis (Figure 3). The various fractions tested were not equally inhibitory in ovalbumin synthesis.

The soluble proteins in the high-speed supernatants synthesized by oviduct magnum explants in culture were examined by SDS[1] gel electrophoresis. The proteins synthesized in the presence of externally added tRNA were labeled with ^{14}C amino acids and those without exogenous tRNA with ^{3}H amino acids (Figure 4). The proteins synthesized in the presence of rat liver tRNA were similar to those synthesized in the absence of exogenous tRNA. In controls and with rat liver tRNA the major protein(s) synthesized (30–34% of the radioactive material) had the electrophoretic mobility of ovalbumin. However, the distribution of radioactivity was different in incubations with hepatoma tRNA. The major protein(s) synthesized (X) had a higher mobility than ovalbumin and this was confirmed in five different experiments. Ovalbumin and the new product(s) were not separated well on SDS gel electrophoresis under the conditions used, but the radioactivity in the ovalbumin region of the gel was reduced to 17% compared to control incubations which had 30–34% of the total radioactivity. Whether the faster moving component was a new protein or was also present in control samples is not clear. If it is synthesized normally by the oviduct magnum, then it could only be a minor component in the control experiments. From the electrophoretic mobility of this product on SDS gel electrophoresis using conalbumin, ovalbumin, and cytochrome *c* as markers, a molecular weight of 36,000 could be deduced whereas ovalbumin has a molecular weight of 45,000. It is conceivable that it is a "precursor" of ovalbumin or a counterfeit protein accumulated in the presence of hepatoma tRNA due to incorrect amino acid insertions.

Bridges and Jones (1973) have investigated proteins synthesized *in vitro* by mouse plasmacytomas MOPC-41 and RPC-20, which produce different immunoglobulin chains. Serine containing peptides were synthesized by using aminoacylated serine tRNA from each plasmacytoma in the presence of a RPC-20 protein synthesizing system. No qualitative differences in the newly synthesized peptides were observed. However, quantitative differences in the serine containing peptides synthesized in the presence of tRNA from each plasmacytoma were apparent. On the other hand, Mushinski *et al.* (1970) found that isoacceptor

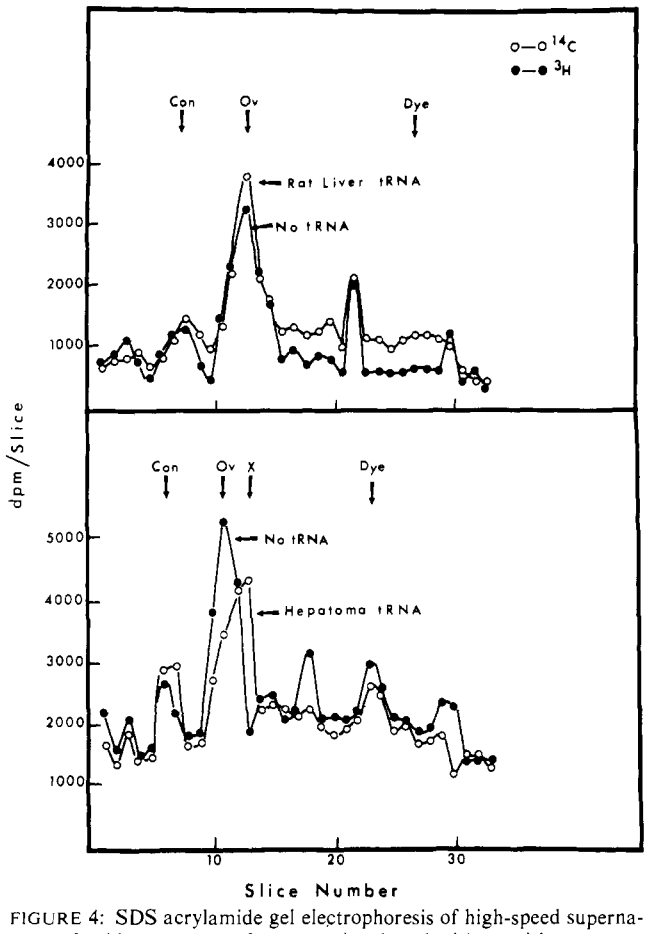

FIGURE 4: SDS acrylamide gel electrophoresis of high-speed supernatants of oviduct magnum fragments incubated with or without exogenous tRNA. The gels were loaded with mixed samples, the control sample being labeled with ^{3}H amino acids and the sample with exogenous tRNA labled with ^{14}C amino acids. The gel on top was run with ^{3}H, 30,6000 cpm and ^{14}C, 35,000 cpm. The gel on the bottom was loaded with ^{3}H, 43,000 cpm, and ^{14}C, 41,000 cpm. The gels were run as described by Palmiter *et al.* (1971), sliced into 2.9 mm slices, digested overnight at 37° with NCS, and counted in a toluene-2,5-diphenyloxazole-1,4-bis[2-(5-phenyloxazolyl)]benzene scintillation fluid. The counter was set so that no counts from ^{3}H appeared in the ^{14}C channel; the ^{3}H being counted at 10% efficiency and the ^{14}C at 51% efficiency. The ^{3}H counts were corrected for a 17% spillover from the ^{14}C, and both were then converted to dpm. The recovery of the loaded radioactivity was 50% for both labels from the gel with the control and rat liver tRNA samples. The recovery of both labels from the gel with the control and hepatoma tRNA samples was 80%. In the experiment from which these samples were taken, ovalbumin synthesis was inhibited 69% by 250 μg/ml of hepatoma tRNA, while rat liver tRNA had no effect on ovalbumin synthesis. The electrophoretic mobility of conalbumin (Con), ovalbumin (Ov), and X, on the gel is indicated by arrows.

leucyl-tRNA from different plasmacytomas and mouse liver functioned identically in hemoglobin biosynthesis in rabbit reticulocyte protein synthesizing system. The plasmacytomas MOPC 46B and MPOC 149 were used because they secrete K type light immunoglobulin chains which differ in at least one position in leucine content. It should be noted that these experiments were carried out in a heterologous rabbit reticulocyte system and, therefore, their results may stem from the absence of some subtle recognition system.

We have no direct evidence for the entry of tRNA in the cells during the incubation of explanted oviduct fragments. However, mammalian cells in culture are known to take up *E. coli* tRNA but whether they remain functional is unknown (Herrera *et al.*, 1970). In a permeable mutant of *E.*

[1] Abbreviation used is: SDS, sodium dodecyl sulfate.

coli exogenous suppressor tRNA has been shown to be functional (Yamamoto *et al.*, 1971).

The mechanism(s) of inhibition of ovalbumin synthesis and the accumulation of other protein(s) under the influence of hepatoma tRNA is obscure: studies with an isolated protein synthesizing system might provide an insight. It would also be of interest to use purified novel isoacceptor tRNAs from Novikoff hepatoma (Baliga *et al.*, 1969) in ovalbumin synthesis to ascertain whether they simulate the effects of the total population of tRNAs from that source.

References

Baliga, B. S., Borek, E., Weinstein, I. B., and Srinivasan, P. R. (1969). *Proc. Nat. Acad. Sci. U. S. 62,* 899.

Bollum, F. J. (1968), *Methods Enzymol. 12B,* 169.

Borek, E., and Kerr, S. J. (1972), *Advan. Cancer Res. 15,* 163.

Bridges, K. R., and Jones, G. H. (1973), *Biochemistry 12,* 1208.

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

Herrera, F., Adamson, R. H., and Gallo, R. C. (1970), *Proc. Nat. Acad. Sci. U. S. 67,* 1943.

Lowry, O. H. Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). *J. Biol. Chem. 193,* 265.

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

Nau, F. (1974), *Biochemistry 13,* 1105.

Nishimura, S. (1971), *Proced. Nucleic Acid Res. 2,* 542.

Ouellette, A. J., and Taylor, M. W. (1973), *Biochemistry 12,* 3542.

Palmiter, R. D., Oka, T., and Schimke, R. T. (1971), *J. Biol. Chem. 246,* 724.

Peacock, A. C., and Dingman, C. W. (1967), *Biochemistry 6,* 1818.

Rhoads, R. E., McKnight, G. S., and Schimke, R. T. (1971), *J. Biol. Chem. 246,* 7407.

Rogg, H., Wehrli, W., and Staehelin, M. (1969), *Biochim. Biophys. Acta 195,* 13.

Sharma, O. K. (1973), *Biochim. Biophys. Acta 299,* 415.

Sharma, O. K., Mays, L. L., and Borek, E. (1973), *J. Biol. Chem. 248,* 7622.

Tsutsui, E., Srinivasan, P. R., and Borek, E. (1966), *Proc. Nat. Acad. Sci. U. S. 56,* 1003.

Yamamoto, M., Ishizawa, M., and Endo, H. (1971), *J. Mol. Biol. 58,* 103.

Recognition of the 3′ Terminus of 2′-*O*-Aminoacyl Transfer Ribonucleic Acid by the Acceptor Site of Ribosomal Peptidyltransferase†

David Ringer, Kevin Quiggle, and Stanislav Chládek*

ABSTRACT: The interaction of the 3′ terminus of 2′- and 3′-*O*-aminoacyl-tRNA with the peptidyltransferase A site of *Escherichia coli* ribosomes has been studied using the following aminoacyl oligonucleotides as models of the 3′ terminus of AA-tRNA: C-A-Phe, C-A(2′Phe)H, C-A(2′H)Phe, C-A(2′Phe)Me, C-A(2′Me)Phe, C-A(2′Gly)H, and C-A(2′H)Gly. The transfer of Ac-[^{14}C]Phe from the Ac-[^{14}C]Phe-tRNA · poly(U) · 70S ribosome complex to puromycin (10^{-4} and 10^{-5} M) was inhibited by C-A-Phe, C-A(2′Phe)H, C-A(2′H)Phe, C-A(2′Gly)H, and C-A(2′H)Gly. Kinetic analysis of the inhibition of Ac-[^{14}C]Phe-puromycin formation by C-A(2′Phe)H failed to show simple competitive inhibition. Binding of C-A-C-C-A-[^{14}C]Phe to 70S ribosomes in the presence of an excess of deacylated tRNA was also inhibited by C-A-Phe, C-A(2′Phe)H, C-A(2′H)Phe, C-A(2′Phe)Me, and C-A(2′Me)Phe. It appears that the acceptor site of peptidyltransferase can recognize the 3′ terminus of either 2′- or 3′-*O*-AA-tRNA, with preference for the 2′ isomer. It therefore follows that 2′-*O*-AA-tRNA may be bound to ribosomes prior to peptide bond formation and that 3′-*O*-AA-tRNA, which is used exclusively by peptidyltransferase as an acceptor, is supplied by 2′ → 3′ transacylation occurring at the peptidyltransferase A site.

It was predicted by Zamecnik (1962) that 2′-*O*-aminoacyl-tRNA may be formed by enzymic aminoacylation of tRNA and that 3′-*O*-aminoacyl-tRNA might be used in later stages of protein biosynthesis. Studies of Wolfenden *et al.* (1964), McLaughlin and Ingram (1965), and Griffin *et al.* (1966) have demonstrated the extremely rapid 2′ ⇄ 3′ isomerization of the aminoacyl group on the terminal adenosine unit of tRNA. The finding of Nathans and Neidle (1963) that puromycin, a nonisomerizable analog of 3′-*O*-aminoacyl-tRNA, inhibits polypeptide synthesis, whereas its 2′ isomer is inactive, has often been referred to by various authors as proof that only 3′-*O*-aminoacyl-tRNA can participate in protein synthesis at the ribosomal level. Indeed, Phe-tRNA-C-C-3′-dA (the 2′ ester)[1] prepared by

† From the Michigan Cancer Foundation, Detroit, Michigan 48201. *Received July 30, 1974.* This investigation was supported in part by U.S. Public Health Service Research Grant No. GM-19111-02 from the National Institutes of General Medical Sciences, General Research Support Grant No. RR-05529-12 from the National Institutes of Health, and in part by an institutional grant to the Michigan Cancer Foundation from the United Foundation of Greater Detroit. This paper is No. XX in the series, Aminoacyl Derivatives of Nucleosides, Nucleotides, and Polynucleotides. For a preceding report of this series (paper XIX), see Chládek *et al.* (1974).