STUDIES ON POLYNUCLEOTIDE-STIMULATED AMINO ACYL TRANSFER FROM SOLUBLE-RNA TO RAT LIVER RIBOSOMES*

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Recent evidence has provided experimental support for the concept of a "messenger RNA" concerned with the transmission of information from DNA to protein.[1-5] The demonstrations that biosynthetically prepared polyribonucleotides of varying base compositions stimulate the incorporation of individual amino acids in an amino acid-incorporating system from *E. coli*[6-15] suggest that such polymers act as synthetic "messengers." These results have provided an experimental approach to the problem of "coding" in protein synthesis whereby a certain sequence of nucleotides in RNA can determine the sequence of amino acids in a polypeptide chain. The relative agreement between some of the assigned nucleotide "code" units derived from studies with *E. coli* and amino acid replacement data from a number of species[12, 13, 16-19] and other studies [20, 21] suggest that the "genetic code" might apply to more than one species. This study was initiated since it appeared of considerable importance to determine whether comparable evidence could be obtained in mammalian cells. The use of a highly purified rat liver system, available in this laboratory,[22, 23] that catalyzes the transfer of amino acids from amino acyl *s*-RNA to ribosomal protein was considered since it is relatively free of interfering reactions which are also involved in protein synthesis. The amino acyl transfer reaction represents an isolated reaction, dissociated from amino acid activation and the synthesis of *s*-RNA amino acid, which leads to the incorporation of amino acids into proteins. This communication describes the characterization of such a system which responds markedly to addition of various polynucleotides. While this work was in progress, several reports appeared which demonstrated that the incorporation of free amino acids in crude sub-cellular mammalian systems was stimulated by polynucleotide preparations.[24-27]

Materials and Methods.—The preparation of amino acyl *s*-RNA labeled with C^{14}-amino acids, deoxycholate-extracted ribosomes, pH 5 supernatant, and two purified soluble protein factors, designated amino acyl transferases (or polymerases) I and II, from rat liver has been described previously.[22, 23] The resolution of transferases I and II from the pH 5 supernatant fraction and the purification of transferase II, which has also been obtained from microsomal extracts,[23, 28] will be described in detail in a subsequent communication. Polyuridylic acid (poly U), polycytidylic acid (poly C), polyuridylic-guanylic acid (poly UG, 5:1), polyuridylic-cytidylic acid (poly UC, 5:1), and polyuridylic-adenylic acid (poly UA, 5:1) were obtained from Dr. Severo Ochoa and his colleagues; their generous gifts are gratefully acknowledged.

Incubation procedures are described in detail below. At the end of the incubation, the perchloric acid-insoluble fraction was prepared and the residual protein was obtained from it after several washes including two extractions with 5 per cent trichloroacetic acid at 90°. The residual proteins were plated directly and were

counted long enough to achieve at least a 10 per cent precision; appropriate corrections for self-absorption and background (1.6 to 2.0 cpm) were applied.

Results and Discussion.—Previous studies demonstrated the requirements for GTP and a soluble fraction of the cell for amino acyl transfer to cytoplasmic ribonucleoprotein particles.[22, 23] Recent reports from this laboratory[23, 28] have presented evidence indicating the presence of two activities essential for amino acyl transfer. One of these, transferase I, catalyzes amino acyl transfer to intact microsomes, but is inactive in experiments with purified ribosomes. The other activity, transferase II, has been obtained from the soluble portion of the cell and from deoxycholate-soluble extracts of microsomes and is required, in addition to transferase I, for amino acyl transfer to ribosomes. Thus, amino acyl transfer is obtained with crude soluble preparations after removal of the amino acid-activating enzymes (pH 5 supernatant) or with the two purified factors (amino acyl transferases I and II) in the presence of GTP and glutathione.

The effect of polyuridylic acid on amino acyl transfer was examined with two concentrations of ribonucleoprotein (Table 1). Addition of poly U to incubations of pH 5 supernatant with high concentrations of ribosomes (experiment A) resulted in slightly higher incorporation of phenylalanine as compared to controls without poly U. Incubations in the presence of transferases I and II did not reveal any stimulation on addition of poly U. When similar incubations were carried out with relatively low concentrations of ribosomes (experiment B), addition of poly U in the presence of pH 5 supernatant led to a marked (2–4 fold) stimulation of phenylalanyl transfer. In contrast to the marked stimulation observed in incubations with pH 5 supernatant, slight but significant increases were observed on addition of poly U to incubations with combined transferases I and II.

Since the possibility existed that endogenous "messenger RNA" on rat liver ribosomes might interfere with the interaction of exogenous polynucleotide with ribosomal particles, preincubation procedures were assayed in attempts to facilitate such interactions. As shown in Table 2, preincubation of both high (experiment A) and low (experiment B) concentrations of ribosomes in the presence of pH 5 supernatant led to a drastic decrease in phenylalanine incorporation as compared to the results obtained without a preincubation period (Table 1). Ribosomes preincubated with transferases I and II did not exhibit such a loss of activity. The addition of poly U to ribosomes preincubated with pH 5 supernatant resulted in a marked stimulation of amino acyl transfer, particularly in experiments with less

TABLE 1

THE EFFECT OF POLYURIDYLIC ACID ON AMINO ACYL TRANSFER

Enzyme preparation	Poly U additions	Cpm Incorporated into Protein Experiment A	Experiment B
None	−	100	44
None	+	114	−
pH 5 supernatant	−	734	461
" " "	+	858	1,144
Transferases I and II	−	612	327
" " " "	+	565	433

Incubations contained 0.08 mg (2,500 cpm) of C^{14}-phenylalanyl s-RNA, ribosomes, 1.2 μmoles of GTP·$MgCl_2$, 10 μmoles of glutathione, and 10 mg of pH 5 supernatant protein or 0.003 mg of transferase I plus 4 mg of soluble transferase II protein where indicated, in a total volume of 2.5 ml. Poly U additions, indicated by + signs, consisted of 0.2 mg of polyuridylic acid. Experiment A was carried out with 7 mg of ribosomes and experiment B with 0.7 mg of ribosomes per flask. Incubations were carried out for 20 min at 37°.

TABLE 2

EFFECT OF PREINCUBATION AND POLYURIDYLIC ACID ON AMINO ACYL TRANSFER

Preincubation components	Incubation additions	Cpm Incorporated into Proteins Experiment A	Experiment B
Ribosomes	s-RNA-Phe-C^{14}	119	9
"	" + poly U	125	0
" + pH 5 supernatant	"	255	75
" "	" + poly U	817	1,165
" + Transferases I & II	"	883	274
" " "	" + poly U	850	372

Ribosomes, 1 μmole of GTP·$MgCl_2$ and 10 μmoles of glutathione were incubated at 37° as such, in the presence of 10 mg of pH 5 supernatant protein, or with 0.003 mg of transferase I plus 4 mg of soluble transferase II protein. After an 8-min preincubation, 0.08 mg (2,500 cpm) of C^{14}-phenylalanyl s-RNA and, where noted, 0.2 mg of polyuridylic acid were added and the incubation was continued for an additional 20 min. Experiment A was carried out with 7 mg of ribosomes and Experiment B with 0.7 mg of ribosomes, in a final volume of 2 ml.

than 1 mg of ribosomes. Preincubation in the absence of transferring preparations led to irreversible inactivation. The loss of activity on preincubation and the marked recovery on addition of poly U are consistent with the degradation of an endogenous ribosomal component and its replacement by the synthetic polynucleotide. It is interesting to note that the purified transferases I and II do not catalyze the decrease in ribosomal activity and subsequently fail to exhibit a significant poly U effect, suggesting the presence of an additional factor in the pH 5 supernatant responsible for this effect. Studies on the nature of this factor are presently in progress and will be reported subsequently.

As discussed above, the poly U-dependent stimulation of amino acyl transfer is more apparent with lower concentrations of ribosomes. Similar results are presented in Figure 1. Although the stimulatory effect of poly U on amino acyl transfer was more marked at lower ribosomal concentrations, optimal incorporation in the presence of poly U was observed in incubations containing 0.7 mg of preincubated ribosomes. With increasing ribosomal concentrations, the poly U-dependent incorporation was decreased while endogenous incorporation increased.

The effect of varying concentrations of pH 5 supernatant on ribosomal preincubations is shown in Figure 2. The values obtained with ribosomes preincubated with 1 to 2.5 mg of pH 5 supernatant protein, in the presence and absence of poly U, are quantitatively similar to those obtained in nonpreincubated experiments (Table 1); higher concentrations led to lower incorporation values in the absence of poly U, and the response to poly U also appeared to be proportional to the concentration of pH 5 supernatant.

Figure 3 shows the dependence of phenylalanyl transfer to preincubated ribosomes on the concentration of poly U. Significant stimulation was observed with 0.02 mg of polynucleotide per ml of incubation fluid and maximal incorporation was observed with approximately 0.1 mg per ml. The addition of poly C over a similar concentration range had no effect on this process. Possibilities existed that the extent of polynucleotide-stimulated amino acyl transfer observed above might reflect less than maximal incorporation due to the degradation of C^{14}-amino acyl s-RNA or added polynucleotide or to the inactivation of transferring enzymes or ribosomes during the incubation. Alternatively, the amino-acid acceptor sites on the ribosomal particle might have become saturated, thus causing incorporation to cease. The experiments described in Figure 4 represent an attempt to examine these possibilities. Amino acyl transfer was completed within 5 min of incubation

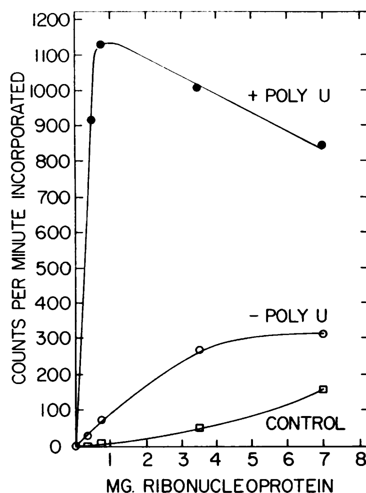

FIG. 1.—Effect of polyuridylic on amino acyl transfer to varying concentrations of preincubated ribosomes. Ribosomes (0.3 to 7 mg) were incubated at 37° in the presence of 1 μmole of GTP·$MgCl_2$, 10 μmoles of glutathione, and 10 mg of pH 5 supernatant protein in a total volume of 1.6 ml. After 8 min of preincubation, 0.08 mg of C^{14}-phenylalanyl *s*-RNA with (closed circles, + Poly U) or without (open circles, − Poly U) 0.2 mg of polyuridylic acid was then added and incubated for an additional 20 min in a total volume of 2 ml. The control (squares) represents incubations carried out in a similar manner, in the absence of pH 5 supernatant.

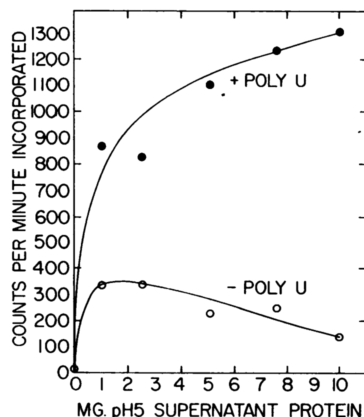

FIG. 2.—Effect of polyuridylic acid on amino acyl transfer to ribosomes preincubated with varying concentrations of pH 5 supernatant protein. Approximately 0.7 mg of ribosomes were incubated at 37° for 8 min in the presence of GTP, glutathione, and the concentrations of pH 5 supernatant protein shown, as described in Figure 1. C^{14}-phenylalanyl *s*-RNA with (closed circles, + Poly U) or without (open circles, − Poly U) polyuridylic acid was then added and incubated for an additional 20 min.

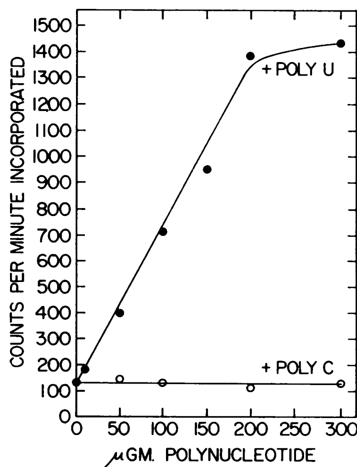

FIG. 3.—Effect of polyuridylic acid concentration of amino acyl transfer to preincubated ribosomes. Approximately 0.7 mg of ribosomes were incubated at 37° for 8 min in the presence of GTP, glutathione, and pH 5 supernatant as described in Figure 1. C^{14}-Phenylalanyl *s*-RNA and varying concentrations of polyuridylic acid (closed circles, + Poly U) or polycytidylic acid (open circles, +Poly C) were then added and incubated for an additional 20 min.

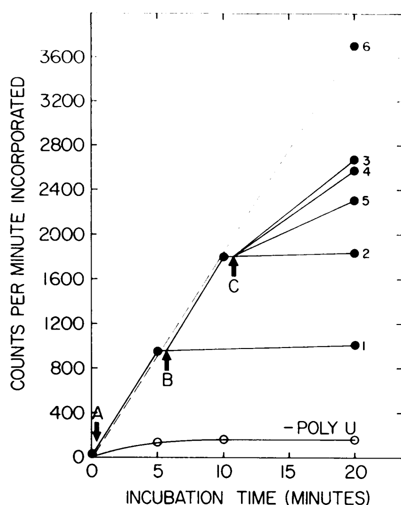

FIG. 4.—The effect of multiple additions on amino acyl transfer to preincubated ribosomes. Approximately 0.7 mg of ribosomes were preincubated for 8 min as described in Figure 1. Series 1 received 0.08 mg of C^{14}-phenylalanyl *s*-RNA and 0.2 mg of polyuridylic acid at the end of the preincubation period, A, and were assayed after 5 and 20 min of incubation. Series 2 received polyuridylic acid at A and C^{14}-phenylalanyl *s*-RNA at A and B. Series 3 received polyuridylic acid at A, and C^{14}-phenylalanyl *s*-RNA at A, B, and C. Series 4 received polyuridylic acid at A and C, and C^{14}-phenylalanyl *s*-RNA at A, B, and C. Series 5 received polyuridylic acid at A and C, and C^{14}-phenylalanyl *s*-RNA at A and B. Experiment number 6 represents an incubation with 0.24 mg of C^{14}-phenylalanyl *s*-RNA added at A. A control series in the absence of polyuridylic acid (open circles, – Poly U) received C^{14}-phenylalanyl *s*-RNA at 0, 5, and 10 min of incubation.

(Figure 4, series 1). Addition of a second dose of C^{14}-phenylalanyl *s*-RNA to the labeled suspensions, after 5 min of incubation (point B), resulted in the further incorporation of amino acid into protein as shown in series 2. The extent of incorporation during the second 5-min labeling period was quantitatively similar to that observed during the first 5 min. A third addition of C^{14}-phenylalanyl *s*-RNA was made after 10 min of incubation (point C); again, the extent of incorporation during this third labeling period was quantitatively similar to those observed during the first and second 5-min incubations. The addition of poly U and C^{14}-phenylalanyl *s*-RNA at point C (series 4) did not lead to a greater increase in ribosomal labeling than that observed with C^{14}-phenylalanyl *s*-RNA alone and the addition of Poly U only (series 5) was not as effective. The results presented here indicate that the limiting component in the incubation system is the labeled substrate, C^{14}-phenylalanyl *s*-RNA, probably due to the degradation of *s*-RNA-amino acid under these conditions. It would also appear that the poly U responsible for the enhanced incorporation, perhaps ribosomal-bound polynucleotide, is not excessively broken down during these incubations. Further, ribosomal amino acid-acceptor sites do not appear to be saturated, as evidenced also by incubation series 6 which received three times the usual dose of C^{14}-phenylalanyl *s*-RNA at the beginning of the incubation.

The effects of several polymers on the transfer of various *s*-RNA-bound C^{14}-amino acids are summarized in Table 3. Although the specific radioactivities of the C^{14}-amino acyl *s*-RNA preparations varied between 18,000 cpm and 40,000 cpm per mg of *s*-RNA, approximately 3,000 cpm of each was used per incubation. Amino acyl transfer in the absence of polynucleotides was less than 10 per cent of that observed in control flasks which were not preincubated. The results obtained here indicate that the transfer of individual *s*-RNA-bound amino acids is stimulated by specific polynucleotide preparations. For example, incorporation of phenylalanine under these conditions is stimulated by all of these polymers, glycine by poly UG, and serine by poly UC. These partial results appear to be in general

TABLE 3

THE EFFECT OF VARIOUS POLYNUCLEOTIDES ON THE TRANSFER OF INDIVIDUAL s-RNA-BOUND AMINO ACIDS

s-RNA-bound C^{14}-amino acid	Control	Polynucleotide Added: None	Poly U	Poly UG (5:1)	Poly UC (5:1)	Poly UA (5:1)
Glycine	390	0	0	*85*	0	0
Isoleucine	798	24	12	16	16	*45*
Leucine	2,625	24	33	*320*	*403*	*108*
Lysine	1,020	24	8	0	12	9
Phenylalanine	594	42	*3,449*	*1,056*	*1,872*	*182*
Serine	381	20	0	8	*125*	0
Threonine	522	0	0	0	0	0
Tyrosine	207	8	0	0	0	*20*
Valine	315	0	28	*535*	0	0

Approximately 0.7 mg of ribosomes were incubated at 37° for 8 min in the presence of GTP, glutathione, and pH 5 supernatant as described in Figure 1. After 8 min, C^{14}-amino acyl s-RNA and 0.3 mg of the polynucleotide noted were added and incubated for an additional 20 min. In this experiment, the Poly U used was purchased from Miles Laboratories. The results are expressed as increase in specific radioactivity (cpm per mg) of ribosomal protein; values obtained in the presence of polynucleotides have therefore been corrected by subtracting the values obtained in the absence of polynucleotide (None). The control values refer to amino acyl transfer to ribosomes, in the absence of polynucleotide and a preincubation period, as described in Table 1.

agreement with those obtained with *E. coli*.[6–15] The relative incorporation obtained here with poly UA, however, does not appear to be as marked as in the *E. coli* system or as with other active polymers in this system. It should be emphasized that Poly C had no effect on the incorporation of any of the amino acids tested. These data further suggest that the polynucleotide-enhanced incorporation is specific for individual amino acids and does not involve the simultaneous transfer of all of the s-RNA-bound amino acids; the C^{14}-amino acyl s-RNA preparations were similar with respect to endogenous, bound amino acids and differed only in the nature of the radioactive amino acid.

The low level of endogenous amino acid incorporation obtained with preincubated ribosomes and the marked stimulation observed in the presence of synthetic polymers reflect polynucleotide effects comparable to those obtained with free amino acids and markedly higher than those reported previously for amino acyl transfer[9] in *E. coli* systems. The observations described above suggest that this mammalian system is particularly well suited for studies designed to investigate the "universality" of the genetic code.

Summary.—A rat liver system that catalyzes amino acyl transfer from amino acyl s-RNA to ribosomal protein is stimulated by the addition of polyuridylic acid. The stimulation is markedly greater when ribosomes are preincubated with pH 5 supernatant, particularly in experiments with low concentrations of ribosomes. When amino acyl transfer is examined with several polynucleotide preparations, varying in base composition, the incorporation of individual s-RNA-bound amino acids appears to be enhanced in the presence of specific polynucleotides. Partial characterization of this polynucleotide-stimulated mammalian system has been carried out. The results suggest that on preincubation, a component of ribosomes, possibly messenger RNA, is removed by a soluble factor which is different from the amino acyl transferring enzymes. The stimulation observed on addition of polynucleotides to preincubated ribosomes suggests that the natural messenger RNA is replaced by these synthetic messenger molecules.

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*RIBONUCLEIC ACID POLYMERASE OF AZOTOBACTER VINELANDII, I. PRIMING BY POLYRIBONUCLEOTIDES**

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The initial investigations[1–5] on RNA polymerase demonstrated that DNA[6] primes and directs the synthesis of RNA. Subsequently it was shown that native DNA is a more effective primer than is the heat denatured, single stranded form.[7] Recently Nakamoto and Weiss[8] reported that poly C and turnip yellow mosaic virus RNA could direct the synthesis of polyribonucleotides with polymerase preparations from *Micrococcus lysodeikticus*. The effect of polyribonucleotides has been confirmed and extended with a 500-fold purified RNA polymerase from