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*SYNTHETIC POLYNUCLEOTIDES AND THE AMINO ACID CODE, VI**

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Previous experiments with synthetic polynucleotides of random nucleotide distribution have provided information about the base composition of the triplet code letters for 19 amino acids.[1] These results were confirmed by other investigators.[2] However, the sequence of bases in the code triplets is known only in the case of phenylalanine (UUU).[3] Through the use of nonrandom copolymers it should be possible to establish the base sequence of some code letters, the direction in which the code is read, and the colinearity of the synthesized polypeptides with the template polynucleotide.

The simplest approach to the problem is to place one or more triplets of known sequence at one end of a poly U chain. This is made possible by the fact that short oligonucleotides prime polynucleotide phosphorylase by acting as nuclei for growth of the polynucleotide chains.[4] By priming with a mixture of ApU and ApApU[5] we have obtained polymers which promoted the incorporation of small amounts of tyrosine besides phenylalanine, but not that of isoleucine, asparagine, or lysine. In contrast, random poly UA stimulated the incorporation of tyrosine and isoleucine to the same extent. This suggested that tyrosine incorporation may be caused by a beginning AUU sequence in these polymers.

Preparation of oligonucleotide primers of polynucleotide phosphorylase:—Poly UA(5:1), 16 mg, from a previously prepared batch[6] (actually determined U:A ratio, 4.8:1) was dissolved in 1.6 ml of 0.02 *M* Tris-HCl buffer, pH 7.4, and digested for 15 hr at room temperature with 0.32 mg of crystalline pancreatic ribonuclease (Worthington) after adding a few drops of toluene. The solution was then adjusted to pH 5.6 with acetic acid and made 0.01 *M* with sodium acetate. Ribonuclease digestion of the above polymer yields Up, ApUp, ApApUp, and traces of higher oligonucleotides (ApApApUp, etc.). In order to remove the terminal phosphate, monoesterified at ribose carbon 3′, the solution was incubated with prostatic phosphomonoesterase[7] until no more orthophosphate was released. Following this treatment the solution was adjusted to pH 7.9 with NaOH and digested with 0.1 mg of crystalline trypsin (Armour) for 15 min to destroy the ribonuclease and phosphomonoesterase. The phosphomonoesterase and trypsin digestions were conducted at 37°. The solution was shaken with phenol for 1 hr at room temperature. The aqueous layer was washed with ether and acidified to pH 3.0 with HCl. The oligonucleotides (mostly ApU and ApApU) together with the uridine were then adsorbed on 100 mg of charcoal,

eluted with a mixture of ethanol, ammonia, and water (50:2:48 by volume) and lyophilized. Poly UG (5:1)[8] was similarly treated to obtain a mixture of uridine, GpU, and GpGpU. For the work described in this paper the AU and GU oligonucleotide mixtures were used without prior separation of their components to prime the synthesis of the polymers referred to for convenience as AUU...U and GUU...U.

Other preparations: Poly AAU...U was prepared by incubation at 30° of a sample containing (in μmoles/ml), Tris-HCl buffer, pH 8.0, 150; $MgCl_2$, 29; EDTA, 1; UDP, 70; AU oligonucleotide primer, approximately 2.4 (ApU and ApApU); and *Azotobacter* polynucleotide phosphorylase (specific activity, 60), 0.17 mg (10 units). The reaction reached equilibrium within 1 hr. The polymer was isolated and lyophilized as previously described for other synthetic polynucleotides.[6] Poly GUU...U was similarly prepared with use of the GU oligonucleotide primer mixture. Poly U was prepared as in previous work.[6] The sedimentation coefficients of these polymers were: poly U, 10.77; poly AUU...U, 5.75; poly GUU...U, 8.2. The preparation of random poly UA has been described.[6] The sample used in this work was prepared from a mixture of UDP and ADP in 4:1 ratio. Actually determined U:A ratio, 3.8:1; $s_{20,w}$, 4.56 *S*.

Except for L-isoleucine, L-lysine, and L-tyrosine, which were uniformly labeled with C^{14} and of high specific radioactivity (1 mc/mg), obtained from the Schwarz Laboratories, Orangeburg, New York, the remaining C^{14}-labeled amino acids and other preparations were as previously noted.[6, 9]

Methods: The incubations were conducted in general as previously described[6] with use of the improved conditions noted in more recent publications of this series.[1, 9] Departures from these conditions not indicated in Table 1 were as follows: The samples with isoleucine-C^{14} had 5 μmoles of cold, highly purified L-leucine[10] and 0.14 μmole of cold L-tyrosine in addition to the usual mixture of 19 cold amino acids. The samples with tyrosine-C^{14} had 5 μmoles of each cold L-leucine and L-isoleucine[10] besides the amino acid mixture. Dialyzed *E. coli* supernatant was used. The incubation was for 1 hr at 37°. After stopping the reaction by addition of 5 ml of 10% trichloroacetic acid, followed by centrifugation, the precipitate was resuspended in 5% trichloroacetic acid, heated for 15 min at 95°, cooled, and the mixture centrifuged. The sediment was dissolved in 0.5 ml of 1.0 *N* NaOH and reprecipitated with trichloroacetic acid. The mixture was then heated and centrifuged as before, and the sediment was washed successively with water and 50% ethanol. It was finally suspended in 95% ethanol and the suspension plated for measurement of radioactivity.

TABLE 1

EFFECT OF POLY U, POLY AUU ...U, AND POLY UA ON THE INCORPORATION OF VARIOUS AMINO ACIDS IN *E. coli* SYSTEM*

Polynucleotide†	Amino acid‡				
	Phenylalanine	Isoleucine	Tyrosine	Asparagine	Lysine
None	600	22	25	58	35
U	22,000	29	35	62	26
AUU...U	20,000	29	63	63	27
UA(4:1)	9,400	1,400	1,540	500	200

* Values are expressed in μμmoles/mg ribosomal protein. They are averages of at least three (phenylalanine) or two (isoleucine, tyrosine) duplicate experiments.
† 20 μg of poly U and 40 μg of each poly AUU ...U and poly UA per sample.
‡ Per sample, isoleucine, tyrosine, and lysine, 12.5 millimicromoles; phenylalanine and asparagine, 50 millimicromoles.

Orthophosphate was determined by the method of Lohmann and Jendrassik.[11] Base analyses were performed by the method of Crossby *et al.*[12] following hydrolysis according to Marshak and Vogel.[13] Other methods were as in earlier work.[6]

Results.—The results of several experiments in which the effect of poly U, poly AUU ... U, and random poly UA on the incorporation of phenylalanine, isoleucine, tyrosine, asparagine, and lysine was compared, are summarized in Table 1. It may be seen that poly U did not significantly stimulate the incorporation of isoleucine, tyrosine, asparagine, or lysine. All these amino acids have a code letter containing U and A;[1] 2U1A for isoleucine and tyrosine, 1U2A for asparagine and

lysine. Poly AUU . . . U had no effect on the incorporation of isoleucine, asparagine, or lysine, beyond that of poly U, but it produced a small stimulation of the incorporation of tyrosine. This effect was quite consistent; it had been observed in several experiments preliminary to those of Table 1. Random poly UA promoted as expected the incorporation of isoleucine and tyrosine to the same extent. It promoted the incorporation of asparagine and lysine to a similar extent but, as already noted,[1] it stimulated the incorporation of the former to a greater extent than that of the latter.

In preliminary experiments with poly GUU . . . U, this polymer promoted the incorporation of small amounts of cysteine but had no effect on that of valine, glycine or tryptophan. These experiments will be reported in detail at a later date.

Discussion.—Examination of the polypeptides synthesized in the presence of poly AUU . . . U, and either tyrosine-C^{14} or phenylalanine-C^{14} with the remaining cold amino acids, for radioactive end groups was difficult because of their poor solubility. However, in preliminary experiments with use of the dinitrofluorobenzene technique phenylalanine, but not tyrosine, was found in N-terminal groups. With random poly UA, on the other hand, both phenylalanine and tyrosine were found at N-terminal positions. Hydrazinolysis detected the presence of appreciable tyrosine at C-terminal positions. These experiments are still underway. The results so far suggest that AUU is the sequence that corresponds to tyrosine. Since synthesis of the polypeptide chain in cell-free reticulocyte and *E. coli* systems is known to start with the N-terminal amino acid,[14-16] it would appear that the RNA code is read starting from the end with an unesterified 3′ hydroxyl group, i.e., from right to left in a sequence like ApUpUp . . . . UpU.

Summary.—Polymers prepared with polynucleotide phosphorylase by priming the synthesis of poly U with a mixture of oligoribonucleotides containing ApU and ApApU, promoted the incorporation of phenylalanine and small amounts of tyrosine by the cell-free *E. coli* system. The polymers had no effect on the incorporation of isoleucine, asparagine, and lysine. In contrast, random poly UA stimulated the incorporation of isoleucine and tyrosine to the same extent. Since the base composition of the triplet code letters for tyrosine or isoleucine is 2U1A, the results suggest that tyrosine incorporation may be caused by a beginning AUU sequence in the primed polymers.

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*POLYURIDYLIC ACID STIMULATION OF PHENYLALANINE INCORPORATION IN ANIMAL CELL EXTRACTS**

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Recent studies of the genetic code by the use of synthetic polyribonucleotides in a subcellular *E. coli* amino acid incorporating system[1-4] have raised the question of whether or not the results obtained can be applied to other species. In preliminary studies, other investigators were unable to obtain polyuridylic acid (poly U) stimulation of phenylalanine incorporating in a subcellular rat liver system,[2, 5] though an effect was obtained in a mixed system, employing *E. coli* supernatant and rat liver ribosomes.[2] In collaboration with Dr. Elliott Osserman, we have recently developed a subcellular amino acid incorporating system obtained from a plasma cell tumor of C_3H mice.[6] During the course of these studies, it was found that poly U caused up to a 40-fold stimulation of phenylalanine incorporation by extracts of this tumor. The purpose of this paper is to describe the characteristics of this phenomenon. Studies demonstrating a poly U stimulation of phenylalanine incorporation in extracts of a human plasma cell tumor, a rat tumor, normal rat and mouse liver, and rabbit reticulocytes are also described.

Materials and Methods.—Mice bearing the plasma cell tumor X5563[7] were sacrificed by cervical fracture and the excised tumor (wet weight 1–2 gm) was rinsed with a few ml of cold (4°C) homogenizing medium. The latter is the "standard buffer" of Nirenberg *et al.*[1] (tris-HCl 0.01 *M*, pH 7.8, magnesium acetate 0.01 *M*, potassium chloride 0.06 *M*, and mercaptoethanol 0.006 *M*) plus sucrose 0.25 *M*. All subsequent procedures were performed at 4°C. The tissue was minced and transferred to a Potter-Elvehjem homogenizer with the aid of one volume of homogenizing medium. After homogenizing for 45 sec at approximately 1,500 rpm, employing a loosely fitting Teflon pestle, 2 more volumes of this medium were added. The homogenate was centrifuged at 10,000 × *g* for 15 min and the supernatant fluid recentrifuged at 30,000 × *g* for 30 min. The resulting supernatant fraction (S-30) was centrifuged at 105,000 × *g* for 2 hr to obtain the microsomal supernatant (S-100) and microsomal (P-100) fractions. The latter was washed by suspension in the same medium and recentrifuged at 105,000 × *g* for 2 hr. Cell fractions could be stored for at least one week at −20°C without appreciable loss in activity. The S-30 fractions of the other tissues listed in Table 4 were prepared in essentially the same manner as that described above.