*STUDIES ON POLYNUCLEOTIDES, LIX. FURTHER CODON ASSIGNMENTS FROM AMINO ACID INCORPORATIONS DIRECTED BY RIBOPOLYNUCLEOTIDES CONTAINING REPEATING TRINUCLEOTIDE SEQUENCES**

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The ribosomal binding technique using ribotrinucleotides developed by Nirenberg and Leder[1] has permitted a large number of codon assignments for different amino acids.[2, 3] An alternative approach to the study of the genetic code, which has been developed in this laboratory, involves the preparation of long ribopolynucleotides containing completely defined nucleotide sequences by a combination of chemical and enzymatic methods. These are subsequently used as messengers in the cell-free protein-synthesizing system so as to obtain defined polypeptides.[4] Thus a ribopolynucleotide containing a repeating trinucleotide sequence has been shown to direct the synthesis of three homopolypeptides,[5] and ribopolynucleotides containing two nucleotides in alternating sequences directed the synthesis of copolypeptides containing, invariably, two amino acids in alternating sequences.[6, 7] These initial studies provided an unambiguous demonstration of the basic properties of the genetic code and, furthermore, opened up a general method of wide scope for further studies of the genetic code. In particular, the approach offered an apparently completely reliable[8] method of deducing or verifying codon assignments obtained by the binding technique. Previous extensive experience had in fact shown the binding results with the trinucleotides not to be completely reliable and furthermore, for about 25 per cent of the total trinucleotides, no measurable effects were observed in the binding technique.[2, 3] It was therefore desirable to try to derive as much of the code as possible with polynucleotides containing repeating nucleotide sequences.[10–12] In the present paper, the results of amino acid incorporation experiments using seven[9] ribopolynucleotides containing repeating trinucleotide sequences are reported and the present status of the total codon assignments is reviewed.

Materials and Methods.—The double-stranded DNA-like polymers used were those listed in Table 1. Details of their synthesis from short-chain chemically synthesized deoxyribo-oligonucleotides and their full characterization will be described separately. As shown in Table 1, transcription of the DNA-like polymers by RNA polymerase was restricted to either of the two strands by providing in the reaction mixtures ribonucleoside 5′-triphosphates necessary only for the copying of that strand. Details of the individual preparations of the ribopolynucleotide messengers are given below and in legends to tables. Kinetics of synthesis and characterization of these polymers will be reported subsequently. Uniformly labeled L-C^{14}-amino acids used had specific activities similar to those described previously.[7]

Methods for the preparation of *E. coli* sRNA, *E. coli* ribosomes, and dialyzed 100,000 × *g* supernatant fraction have been described previously.[5, 6] DNA-dependent RNA polymerase was prepared from *E. coli* B according to Chamberlin and Berg.[13, 14] The specific activity corresponded to an incorporation of 2,000 and 2,700 mμmoles (two preparations) of C^{14}-CTP/hr/mg.[13]

General method for polypeptide synthesis: As in previous work,[6, 7] a two-stage procedure was used. Stage I contained, per ml: 40 μmoles of Tris-HCl (pH 8), 4 μmoles of $MgCl_2$, 1 μmole $MnCl_2$, 12 μmoles β-mercaptoethanol, the appropriate ribonucleoside 5′-triphosphates, and RNA polymerase (470 or 660 units/ml). The concentrations of the DNA-like polymers and of ribonucleoside triphosphates are given in the legends to tables. In a parallel small-scale experiment

TABLE 1

DOUBLE-STRANDED DNA-LIKE POLYMERS AND SINGLE-STRANDED RIBOPOLYNUCLEOTIDE PRODUCTS FORMED BY TRANSCRIPTION WITH RNA POLYMERASE

DNA-like polymer*, †	Ribonucleoside triphosphates used in RNA polymerase reaction	Ribopolynucleotide†
Poly d-TAC:GTA	(1) ATP + UTP + CTP	Poly r-UAC
	(2) ATP + UTP + GTP	Poly r-GUA
Poly d-ATC:GAT	(1) ATP + UTP + CTP	Poly r-AUC
	(2) ATP + UTP + GTP	Poly r-GAU
Poly d-TTG:CAA	(1) UTP + GTP	Poly r-UUG
	(2) ATP + CTP	Poly r-CAA
Poly d-TTC:GAA	(1) UTP + CTP	Poly r-UUC
	(2) ATP + GTP	Poly r-GAA

* The DNA-like polymers are written so that the colon separates the two complementary strands. The complementary sequences in the individual strands are written so that antiparallel base-pairing is evident.

† The order in which the base initials appear in the abbreviations for the repeating polymers shows the repeating trinucleotide sequences of which the polymers are composed. Thus, poly r-UAC is the abbreviation for a ribopolynucleotide containing the repeating trinucleotide sequence, uridylyl-adenylyl-cytidylyl. As the terminal nucleotides of the ribopolymers are not known, poly r-UAC could equally well be abbreviated as poly r-ACU or poly r-CUA.

one of the ribonucleoside 5′-triphosphates was used in the C^{14}-labeled form so as to determine the amount of ribopolynucleotidic product formed in the main experiment.

After incubation at 37°, the components of Stage I were cooled in ice and added directly to the components for Stage II, such that the reaction mixture now contained, per ml: 60 μmoles Tris-HCl, pH 8, 12 μmoles $MgCl_2$, 0.5 μmole $MnCl_2$, 12 μmoles β-mercaptoethanol, 50 μmoles NH_4Cl, 0.25 μmole GTP, 2 μmoles ATP, 5 μmoles sodium phosphoenolpyruvate, 20 μg phosphoenolpyruvate kinase, 16 OD_{260} units ribosomes, 13 OD_{260} units of crude sRNA, and 0.76 mg of 100,000 × *g* supernatant protein. In addition, RNA polymerase, the DNA template, and excess triphosphates introduced from Stage I were present.

Most C^{14}-labeled amino acids were added at concentrations of 2 μc/ml, with the following exceptions: asn, gln, and ser at 1 μc/ml; met at 0.2 μc/ml; and H^3-try at 20 μc/ml. Cold amino acids, where added, were 50 mμmoles per ml of each. The reaction mixture was incubated at 37°.

Assay of polypeptide synthesis: (1) *Assay by acid insolubility:* Aliquots (0.02 ml) were taken from the incubation mixtures at the times indicated in the tables. These were pipetted directly onto 1/4 of a Whatman no. 3, 2.3-mm disk which had just been saturated with about 0.03 ml of 1 *N* NaOH. The disk segments were allowed to dry at room temperature and then washed using trichloroacetic acid-tungstic acid procedure, dried, and counted.[5, 7]

(2) *Assay by chromatography:* Aliquots (0.02 ml) of the reaction mixture from Stage II were treated with 0.01 ml of 1 *N* NaOH. After 30 min at 37°, 0.025 ml of the solution was applied to a paper chromatogram or to a strip of DEAE-cellulose paper. For the latter purpose, the aliquot was neutralized with pyridinium Dowex-50 ion exchange resin. The solvent for paper chromatography was *n*-butanol–pyridine–acetic acid–water (15:10:3:12) (Solvent I). For chromatography on DEAE-cellulose paper, the strips were irrigated with 0.2 *M* ammonium formate.

The chromatographic strips were scanned for radioactivity using the Packard 7200 radiochromatogram scanner. The new radioactive peak near the origin produced in the presence of the ribopolynucleotide template was cut out and counted for radioactivity in the Packard 3002 scintillation counter. The control reaction mixtures, in which the deoxypolynucleotide template was absent, were treated analogously and the portion of the chromatogram corresponding to the radioactive product formed in presence of the template was also measured for radioactivity.

Characterization of polyaspartic acid: Complete acidic hydrolysis of polyaspartic acid was performed in 10 *N* HCl for 18 hr at 120°. Identification of resulting aspartic acid was carried out by paper electrophoresis at pH 1.9 (buffer used: acetic acid–formic acid–water, 3:1:16) and by paper chromatography in Solvent I and in *n*-butanol–acetic acid–water (4:1:2).

Results and Discussion.—General comments on amino acid incorporations: Tables 2–5 show the amino acid incorporations directed by the ribopolynucleotides listed in Table 1. For each ribopolynucleotide every one of the 20 amino acids was tested in labeled form, both alone and in the presence of 19 cold amino acids. Aliquots were taken at two time intervals as shown in the tables, principally to confirm the

TABLE 2

AMINO ACID INCORPORATIONS DIRECTED BY POLY r-UUC

	Labeled Amino Acid Incorporated ($\mu\mu$moles/ml)							
	40 min				80 Min			
Labeled amino acid	Minus template	Plus template	Minus template*	Plus template*	Minus template	Plus template	Minus template*	Plus template*
Leu	50	480	66	330	55	430	65	340
Phe	120	1700	500	1300	170	1470	110	1350
Ser	52	190	60	260	55	180	57	240

The concentration of poly d-TTC:GAA at Stage I was 48 mμmoles/ml. UTP and CTP, the two triphosphates added, were present at concentrations of 320 and 160 mμmoles/ml, respectively. At the end of Stage I incubation, poly r-UUC was formed in the amount of 56 mμmoles/ml. During Stage II, the concentration of poly r-UUC was 28 mμmoles/ml.

* In the presence of 19 cold amino acids.

incorporation results. All incorporations are shown that were more than 30 per cent above the background values in the absence of the DNA templates. It is seen that, in general, the presence of 19 cold amino acids did not significantly influence incorporation of the different labeled amino acids (cf. previous results with poly r-AAG[5]). This would be expected, since the individual amino acids are being incorporated into homopolypeptide chains.

As seen in Table 4, poly r-UAC directed the incorporation of five amino acids, and again poly r-AUC (Table 5) directed the incorporation of methionine in addition to the three expected amino acids. These additional incorporations are without doubt due to the transcription of the second strand of the DNA template during Stage II when GTP is present in the reaction mixture. This was confirmed by the fact that when labeled GTP was added to the Stage II reaction mixture in the experiment in which poly r-UAC and the DNA template, poly d-TAC:GTA, were introduced from Stage I, GTP was incorporated into acid-insoluble product.

Theoretically, for ribopolynucleotide messengers containing repeating trinucleotide sequences, the incorporation of a maximum of three amino acids to form homopolypeptides would be expected, if control on initiation of polypeptide chain synthesis is absent. As is seen in Tables 2–5, as a rule, three amino acids were in fact incorporated with each ribopolynucleotide. It is seen, however, that there was great variation in the extent of amino acid incorporations directed by the same ribopolynucleotide. There could be several reasons for these variations. As shown recently by Salas *et al.*[15] with block polynucleotides, the starting point of the reading of the messenger is not quite random: the first triplet from the 5′-hydroxyl end is largely not read, and reading starts preferentially with the second triplet. Therefore, in the repeating trinucleotide polymers, each individual chain could be expected to direct the incorporation largely of one amino acid, the nature of which would be determined by the trinucleotide codon at the 5′-hydroxyl end. The variations in the incorporations of the three amino acids would then be partly explained by the different proportions of polynucleotide chains containing the three different codons at the 5′-hydroxyl terminus.

Another reason for the variations in amino acid incorporations probably is that the concentrations of tRNA's recognizing the three codons present in each ribopolynucleotide are different. An abundant tRNA species may simply determine the "phasing" of the reading of the messenger, thus giving preferential incorporation of the corresponding amino acid. Low incorporations would therefore correspond to low tRNA levels for those codons. It is notworthy that there appears to be, on the whole, a correlation between the response in the binding assay given by a trinu-

TABLE 3

AMINO ACID INCORPORATIONS DIRECTED BY RIBOPOLYNUCLEOTIDES PREPARED FROM POLY d-TTG:CAA

		Labeled Amino Acid Incorporated ($\mu\mu$moles/ml)							
		30 min				60 min			
Ribopolynucleotide template	Amino acid	Minus template	Plus template	Minus template*	Plus template*	Minus template	Plus template	Minus template*	Plus template*
Poly r-UUG	Cys	42	109	50	132	35	142	42	116
	Leu	43	355	76	314	55	366	97	385
	Val	25	797	48	553	35	1,140	60	803
Poly r-CAA	Thr	60	990	120	830	60	1,270	110	1,140
	Gln	200	230	90	240	120	260	80	290
	Asn	520	940	630	860	420	1,180	420	792

For the preparation of poly r-UUG the concentration of poly d-TTG:CAA in Stage I was 60 mμmoles/ml, and the triphosphates UTP and GTP were present at 640 and 320 mμmoles/ml, respectively. After 90 min, 130 mμmoles/ml of poly r-UUG had been synthesized, and therefore the concentration in Stage II was 65 mμmoles/ml. For the synthesis of poly r-CAA the concentration of poly d-TTG:CAA in Stage I was 110 mμmoles/ml, ATP 4 μmoles/ml, and CTP 640 mμmoles/ml. After 90 min 138 mμmoles of poly r-CAA had been synthesized and the concentration in Stage II was therefore 69 mμmoles/ml.

* In the presence of 19 cold amino acids.

cleotide and the incorporation for that amino acid directed by a polynucleotide containing the same trinucleotide codon in repeating sequence.

Amino acid incorporations directed by poly r-UUC: As seen in Table 2, poly r-UUC directed the incorporation of phenylalanine, serine, and leucine. Of the three codons UUC, UCU, and CUU, which this polymer contains, the previous assignments[2, 3] of UUC and UCU to phenylalanine and to serine, respectively, are confirmed and the assignment of the CUU codon to leucine is now clearly deduced. CUU failed to give any binding of leucyl-tRNA to ribosomes, and this assignment has hitherto been doubtful.[2, 3]

Amino acid incorporations directed by ribopolynucleotides derived from poly d-TTG:CAA: Table 3 shows the incorporations directed by poly r-UUG and poly r-CAA. Poly r-UUG directed the incorporations of leucine, cysteine, and valine, and the results provide confirmation of the previous assignment of UUG to leucine,[2, 3] UGU to cysteine,[2, 3, 7] and GUU[2, 3] to valine.

Poly r-CAA stimulated the incorporation of threonine, glutamine, and asparagine. Experiments with different preparations of ribosomes and S100 supernatant fractions showed variations in the extent of incorporation of glutamine and asparagine, while threonine incorporation was always high. The presence of an asparaginase in *E. coli* extracts has been reported,[16] and it seems highly probable that different batches of *E. coli* cells vary in their content of amidases for these two amino acids. From the incorporation data, the previously deduced assignments of ACA to threonine,[2, 3, 7] CAA to glutamine,[2, 3] and AAC to asparagine[2, 3] are confirmed. The assignment of AAC to asparagine has been clearly demonstrated by previous amino acid incorporation results of Salas *et al.*[15]

Amino acid incorporations directed by ribopolynucleotides derived from poly d-TAC:GTA: Poly r-UAC stimulated the incorporation of threonine, tyrosine, and leucine (Table 4) (as discussed above, the additional small incorporation of serine and valine is due to poly r-GUA formation during Stage II). Of the three codons present in poly r-UAC, the assignments of UAC and ACU to tyrosine and threonine, respectively, have been made previously.[2, 3] However, no assignment could be made to CUA, which failed to give any effect in the binding assay.[2, 3] The present results permit clear assignment of CUA to leucine.

Poly r-GUA stimulated the incorporation of the two amino acids serine and

TABLE 4

AMINO ACID INCORPORATIONS DIRECTED BY RIBOPOLYNUCLEOTIDES PREPARED FROM POLY d-TAC:GTA

Ribopoly-nucleotide template	Labeled amino acid	Labeled Amino Acid Incorporated (μμmoles/ml) 40 min: Minus template	40 min: Plus template	40 min: Plus template*	80 min: Minus template	80 min: Plus template	80 min: Plus template*
Poly r-UAC	Leu	51	238	256	59	255	265
	Thr	41	5,640	5,000	45	5,590	5,610
	Tyr	62	524	871	73	512	926
	Ser†	44	63	145	51	85	171
	Val†	35	140	138	39	456	367
Poly r-GUA	Ser	41	180	530	46	290	610
	Val	29	11,100	10,900	33	10,600	9,650

The concentration of poly d-TAC:GTA at Stage I was 36.4 mμmoles/ml, while that of each of the ribonucleoside triphosphates was 320 mμmoles/ml. For the formation of poly r-UAC, the triphosphates present were ATP, UTP, and CTP, while for poly r-GUA synthesis, ATP, UTP, and GTP were added. After incubation for 1 hr the amount of poly r-UAC formed was 60 mμmoles/ml and the concentration of poly r-GUA was 66 mμmoles/ml. With the addition of the components for Stage II, the concentrations of the ribopolynucleotide templates were halved.

* In the presence of 19 cold amino acids.

† The slight incorporation of these amino acids is due to the formation of poly r-GUA during Stage II of the incorporation experiment.

valine. The assignment of GUA to valine is clear from the previous binding data[2, 3] and UAG has been shown to be a chain-terminating codon in *Escherichia coli* B.[17, 18] The third codon, AGU, present in poly r-GUA is therefore assigned to serine. AGU failed to give any binding of seryl-tRNA[2, 3] and was reported to direct binding of cysteinyl-tRNA.[2] Streisinger and co-workers[19] have previously deduced the assignment of AGU for serine from their work with phase-shift mutations.

Amino acid incorporations directed by ribopolynucleotides derived from poly d-ATC:GAT: The incorporation of histidine, isoleucine, and serine was stimulated by poly r-AUC (Table 5), ignoring methionine, whose incorporation clearly resulted from the synthesis of poly r-GAU during Stage II. From these results, the previous assignments[2, 3] of CAU to histidine, AUC to isoleucine, and UCA to serine are confirmed.

By the acid-insolubility assay, poly r-GAU stimulated the incorporation of only methionine, while the incorporation of aspartic acid was demonstrated by paper-chromatographic assay.[20] Incorporation of aspartic acid into oligo- and poly-aspartic acid was further confirmed by anion exchange chromatography on DEAE-cellulose paper where radioactive peaks moving close to the origin and slower than aspartic acid were detected. Polyaspartic acid was further characterized by complete acidic hydrolysis. Aspartic acid was the only radioactive product formed as shown by its mobility on paper electrophoresis and in two chromatographic solvents. The incorporations of methionine and aspartic acid confirm the assignments of AUG to methionine and GAU to aspartic acid.

AUG has recently been concluded to be an initiator codon in protein synthesis,[21–23] and due to its phasing effect, poly r-GAU might have stimulated the incorporation of methionine only. However, the incorporation of aspartic acid shows that under the conditions of present experiments (0.01 *M* Mg^{++}), poly r-GAU can be read in other frames. Therefore the incorporation of an amino acid corresponding to UGA would be expected. From the general structure of the genetic code the most likely candidates seem to be cysteine and tryptophan. To date, attempts to demonstrate the incorporation of any amino acid other than aspartic acid and methionine in the presence of poly r-GAU have given negative results.

TABLE 5

AMINO ACID INCORPORATIONS DIRECTED BY RIBOPOLYNUCLEOTIDES PREPARED FROM POLY d-ATC:GAT

Ribopolynucleotide template	Labeled amino acid	Labeled Amino Acid Incorporated (μμmoles/ml) 40 min				80 min			
		Minus template	Plus template	Minus template*	Plus template*	Minus template	Plus template	Minus template*	Plus template*
Poly r-AUC	His	46	400	45	600	44	410	45	640
	Ileu	32	4,240	62	4,410	32	4,640	66	4,680
	Ser	44	210	54	280	45	190	57	290
	Met†	660	4,000	440	4,130	630	6,660	610	7,250
Poly r-GAU	Asp‡	71	1,770	—	—	—	—	—	—
	Met	550	13,100	450	5,070	690	12,000	600	6,600

The concentration of poly d-ATC:GAT at Stage I was 84 mμmoles/ml, while that of each of ribonucleoside triphosphates was 640 mμmoles/ml. For the formation of poly r-AUC, the triphosphates added were ATP, UTP, and CTP, while for the synthesis of poly r-GAU, ATP, UTP, and GTP were added. After Stage I, poly r-AUC was formed in the amount of 84 mμmoles/ml and the concentration of poly r-GAU was 140 mμmoles/ml. These concentrations were reduced to one half during Stage II when other components were added.

* In the presence of 19 cold amino acids.

† This incorporation is due to the formation of poly r-GAU during Stage II of the incorporation experiment.

‡ This incorporation was assayed by paper chromatography in solvent I.

Comments on total codon assignments: Table 6 summarizes all the amino acid incorporations and their codon assignments which have been obtained so far from work with ribopolynucleotides containing repeating nucleotide sequences. Included are the results of unpublished work with polymers containing repeating tetranucleotide sequences. The total number of codon sequences deduced or confirmed so far by work with polymers of repeating nucleotide sequences is 28. Several of these constitute codon sequences for which no conclusions could be drawn from the binding data with the trinucleotides: prominent examples are CUC, CUU, CUA, UUA, and AGU and AGA. It should be added that when results of incorporations with repeating polymers are examined individually, unique assignments cannot be made. Thus, for example, from the incorporation of glutamic acid and arginine by poly r-AG, it is not possible to decide which of the two codons AGA and GAG stands for which amino acid. The decision between these alternatives can be made by taking into account results obtained by an independent method, such as the binding technique.

The total codon assignments for the 20 amino acids which have been deduced from the binding data[2, 3] and the amino acid incorporation data obtained with defined polymers are assembled in Table 7. Most of the codon assignments made can now be regarded as certain. There are, however, still a few assignments for which the evidence is weak or indirect, for example, AGC and AGG, where no effect was observed in the binding assay. AUA, for which binding failed to give clear evidence, has been shown to stand for isoleucine from work with block polymers.[24] This work has also confirmed the assignments of several additional codons.[25] One outstanding question mark in Table 7 is the function for UGA. As discussed above, work with a repeating trinucleotide polymer containing this trinucleotide sequence has failed so far to give any positive result. The question

TABLE 6

AMINO ACID INCORPORATIONS DIRECTED BY RIBOPOLYNUCLEOTIDES WITH REPEATING NUCLEOTIDE SEQUENCES AND CODON ASSIGNMENTS*

Ribopolynucleotide	Amino acids incorporated and codon assignment
Ribopolynucleotides with Repeating Trinucleotide Sequences†	
Poly r-UAC	tyr (UAC), thr (ACU), leu (CUA)
Poly r-GUA	val (GUA), ser (AGU)
Poly r-AUC	ileu (AUC), ser (UCA), his (CAU)
Poly r-GAU	asp (GAU), met (AUG)
Poly r-UUG	leu (UUG), cys (UGU), val (GUU)
Poly r-CAA	gln (CAA), thr (ACA), asn (AAC)
Poly r-UUC	phe (UUC), ser (UCU), leu (CUU)
Poly r-AAG	lys (AAG), glu (GAA), arg (AGA)
Ribopolynucleotides with Repeating Dinucleotide Sequences‡	
Poly r-UC	ser (UCU), leu (CUC)
Poly r-AG	arg (AGA), glu (GAG)
Poly r-UG	val (GUG), cys (UGU)
Poly r-AC	thr (ACA), his (CAC)
Ribopolynucleotides with Repeating Tetranucleotide Sequences§	
Poly r-UAUC	tyr (UAU), leu (CUA), ileu (AUC), ser (UCU)
Poly r-UUAC	leu (UUA), tyr (UAC), thr (ACU), leu (CUU)

* Codon assignments in parentheses.
† Present work except for poly r-AAG (ref. 5).
‡ Previous work; refs. 6 and 7.
§ Unpublished work from this laboratory.

whether this is a nonsense or a rare codon in *E. coli* B remains open.

Summary.—The messenger activity of seven ribopolynucleotides containing repeating trinucleotide sequences has been tested in the cell-free protein-synthesizing system. The incorporation of a total of 19 amino acids was thus observed. This and related work on polymers with repeating nucleotide sequences has permitted the assignment of 28 codons. While a majority of these serve to confirm the earlier assignments, several comprise codons for which evidence from binding studies or other methods has so far been doubtful. Except for UGA, all of the possible trinucleotide codons have now been given assignments (Table 7).

TABLE 7

TOTAL CODON ASSIGNMENTS

First letter	Second Letter: U	C	A	G	Third letter
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Ochre	?	A
	Leu	Ser	Amber	Try	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ileu	Thr	Asn	Ser	U
	Ileu	Thr	Asn	Ser	C
	Ileu	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

The assignments not underlined are on the basis of binding experiments only. Doubly underlined assignments are from binding data and have been confirmed by cell-free polypeptide synthesis using completely defined polymers. Singly underlined assignments are deduced from incorporation experiments with defined polymers but gave essentially no binding.

* Paper LVIII in this series is by Gupta, N., and H. G. Khorana, these PROCEEDINGS, **56**, 772 (1966).

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[9] Results with poly r-AAG, the eighth ribopolynucleotide in Table 1, have been reported previously.[5]

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