Tokyo) for the supply of globoside I and Forssman antigen which made this investigation possible.

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2′-*O*-Methyl Polynucleotides as Templates for Cell-Free Amino Acid Incorporation*

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ABSTRACT: The 2′-*O*-methyl-containing heteropolymers, poly(Cm,U) and poly(Am,C), and the three homopolymers, poly(Am), poly(Cm), and poly(Um), were tested for template activity in a cell-free amino acid incorporation system from *Escherichia coli* B. The heteropolymer, poly(Cm,U), directed the incorporation of significant levels of phenylalanine, serine, leucine, and proline, and small amounts of isoleucine and tyrosine. The total incorporation of amino acids was slightly greater with poly(Cm,U) than with poly(C,U). The heteropolymer poly(Am,C) directed the incorporation of proline, threonine, and histidine, but its template activity was lower than that of poly(A,C). Poly(Cm,U) was active as a template for a longer period of time than poly(C,U) in directing the incorporation of phenylalanine. Both poly(Am,C) and -(Cm,U) were degraded more slowly than their unmethylated analogs when incubated in reaction mixtures used for cell-free protein synthesis. Poly(Am), poly(Cm), and poly(Um) had no template activity when tested under conditions that were optimum for the template activity of the corresponding nonmethylated polymers. However, neomycin induced the template activity of the homopolymer, poly(Um), and stimulated the amino acid incorporation directed by the heteropolymers poly(Cm,U) and poly(Am,C). Thus RNA polymers only partially methylated in the 2′ position can still direct the incorporation of amino acids into protein while complete 2′-*O*-methylation renders an RNA molecule inactive as a template.

Since Nirenberg and Matthaei's discovery in 1961 of the template activity of poly(U) in a cell-free extract, RNA polymers of known nucleotide composition have been extensively used as artificial messengers to study the properties of the RNA code and the mechanism of protein synthesis. Many of these studies have examined the changes in template properties following modifications of the RNA polymer. Most of the modifications have been in the ring moiety, *e.g.*, poly(m^2A), poly(m^6A), and poly(m^3U) (McCarthy *et al.*, 1966). However, several investigations have employed polymers containing modifications in the ribose–phosphate backbone. Single-stranded DNA has been reported by McCarthy *et al.* (1966), and Morgan *et al.* (1967), to lack template activity, except in the presence of certain amino glycoside antibiotics, such as streptomycin or neomycin. In another study, Knorre *et al.* (1967), examined the effect of acetylation of the 2′-hydroxyl group in RNA. They found that neither poly(U) that was 88% acetylated in the 2′-hydroxyl position, nor poly(A) that was 98% acetylated, directed the incorporation of amino acids in a cell-free system from *Escherichia coli* B.

In contrast to 2′-*O*-acetyl ribonucleotides and deoxyribonucleotides, 2′-*O*-methyl ribonucleotides are found in RNA isolated from natural sources (Smith and Dunn, 1959; Hall, 1964; Wagner *et al.*, 1967). Nucleotides containing 2′-*O*-methylribose have been used to synthesize both 2′-*O*-methyl homopolymers (Rottman and Heinlein, 1968; Janion *et al.*, 1970), and heteropolymers containing both normal and methylated nucleotides (Rottman and Johnson, 1969). Since natural RNA species contain methyl groups on both the base and sugar moieties, the synthesis of these polymers has made it possible to ex-

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amine the effects of sugar methylation on the physical and biological properties of RNA, unobscured by the effects of base methylation. Thus 2′-*O*-methyladenosine oligonucleotides were shown to stimulate the binding of lysyl-tRNA to ribosomes (Price and Rottman, 1970), indicating that 2′-*O*-methylation does not destroy template activity. However the binding assay only monitors recognition of aminoacyl-tRNA by codons in the presence of ribosomes and not the ability of adjacent codons to direct the subsequent polymerization of amino acids. Therefore the present investigation was undertaken to determine the effect of 2′-*O*-methyl nucleotides on the ability of RNA polymers to direct the incorporation of amino acids into peptides.

Materials and Methods

Preparation of S-30 Extracts. Cultures of *E. coli* B were grown in a 12-l. Microfermenter (New Brunswick Scientific Co.) with forced aeration at 37° in a medium containing 1.0% tryptone, 0.5% yeast extract, 0.35% K_2HPO_4, and 0.15% KH_2PO_4 (pH 7.0). At mid-log phase (A_{660} *ca.* 0.5) the antibiotic rifampicin (Calbiochem) was added to a final concentration of 30 μg/ml. Rifampicin, which specifically inhibits initiation of RNA synthesis (Di Mauro *et al.*, 1969), was added to reduce endogenous mRNA levels. Fifteen minutes after adding rifampicin, the culture was chilled rapidly to 4° and the cells harvested by centrifugation. The yield was approximately 1 g of cells (wet weight) per l.

The harvested cells were washed once in a buffer containing 20% sucrose, 0.05 M Tricine, [*N*-tris(hydroxymethyl)methylglycine, adjusted to pH 7.8 with concentrated NH_4OH], and 0.01 M magnesium acetate, centrifuged, and resuspended in 10 ml of the above buffer per g (wet weight) of packed cells. Lysozyme was added to a final concentration of 0.1 mg/ml followed by EDTA to a final concentration of 1.0 mM. After 3-min incubation at 0°, the cells were centrifuged at 3000*g* and resuspended (2 ml/g wet weight of cells) in a buffer consisting of 0.05 M Tricine (pH 7.8), 0.04 M KCl, 0.04 M NH_4Cl, 0.01 M magnesium acetate, 2.0 mM dithiothreitol, and approximately 10 μg/ml of DNase (type I, Worthington Biochemical Co.). The cell suspension was passed through a French pressure cell (Aminco), at 8000–10,000 psi after which it was centrifuged at 30,000*g* for 30 min and the supernatant (S-30) withdrawn. The S-30 extract was incubated for 6 hr at 0° prior to freezing. This procedure increased the incorporating activity threefold when poly(A,C) or poly(Am,C) were used as templates. After this incubation the S-30 was frozen in 0.3-ml fractions in a Dry Ice–acetone bath, and stored in liquid nitrogen. The protein content of the S-30 extract was determined by the method of Lowry *et al.* (1951).

Assay for Template Activity of Polymers. The conditions for amino acid incorporation were similar to those of Nirenberg and Matthaei (1961), and the assay included a modification of Bollum's paper filter disk method (1966). Each reaction mixture contained the following components in a total volume of 70 μl: 0.05 M Tricine (adjusted to pH 7.8 with NH_4OH), 0.04 M KCl, 0.04 M ammonium acetate, 5.0 mM phosphoenolpyruvate, 1.0 mM ATP, 0.2 mM GTP, 2.0 mM each of nineteen [^{12}C]amino acids, 2.0 mM of the ^{14}C-labeled amino acid being studied (5–40 mCi/mmole), 2.0 mM dithiothreitol, 1.0 A_{260} unit of *E. coli* B tRNA, 5 μg of phosphoenolpyruvate kinase (Sigma Chemical Co.), 10 mM magnesium acetate with poly(C,U), poly(Cm,U), poly(U), or poly(Um) as templates, 11.5 mM magnesium acetate with poly(A,C), and 13.5 mM with poly(Am,C). The polymer concentration varied, and is given for each experiment. S-30 (10–15 μl) was used, which represents about 0.3 mg of protein. After addition of the S-30, the reaction mixtures were incubated at 37° for 20 min, chilled rapidly in an ice bath, and a 60-μl sample from each reaction mixture was spotted on Whatman No. 3MM disks (2.3 cm diameter). The disks were dried and placed in a beaker of 5% trichloroacetic acid (approximately 10 ml/disk), and heated at 90–95° for 20 min. The disks were placed on a wet filter paper in a Büchner funnel and washed with 5% trichloroacetic acid, ethanol, and finally diethyl ether. When poly(A) or poly(Am) was the template, 5% trichloroacetic acid–0.25% sodium tungstate (pH 2.0) was used in place of 5% trichloroacetic acid. The dried disks were placed in 10 ml of toluene containing 0.4% 2,5-bis[2(5-*tert*-butylbenzoxazolyl)]thiophene and counted in a Beckman LS-100 scintillation counter. Background incorporation of amino acids in the absence of added polynucleotide has been substracted from the values reported.

Preparation of Polymers. To determine if the introduction of relatively small amounts of 2′-*O*-methyl nucleotides had an effect on the template activity of an RNA polymer, similar 2′-*O*-methyl and nonmethylated copolymers were synthesized and their template activities compared. Poly(Am,C), poly(A,C), poly(Cm,U), and poly(C,U) were synthesized as described previously (Rottman and Heinlein, 1968; Rottman and Johnson, 1969). Poly(Um) was prepared by the polymerization of UmDP with polynucleotide phosphorylase using conditions similar to those used for poly(Am). UmDP was made by deamination of CmDP (Basilio *et al.*, 1962). Poly(Cm) was prepared as described by Janion *et al.* (1970) in which Mn^{2+} was used in place of Mg^{2+}. Heteropolymers formed from a mixture of 2′-*O*-methyl and nonmethylated nucleotides reflect a tendency for paired incorporation of the 2′-*O*-methyl species (Rottman and Johnson, 1969). This was suppressed in the poly(Am,C) by using dimethyl sulfoxide in the polymerization reaction, but no way has been found to eliminate clustering of the (Cm) bases in the poly(Cm,U), and hence there was a higher per cent of Cm–Cm sequences in the poly(Cm,U) polymer than C–C sequences in the poly(C,U) polymer.

Analysis of Polymers. The heteropolymers poly(A,C), poly(Am,C), poly(C,U), and poly(Cm,U) were analyzed for their nucleotide composition after completely digesting each polymer to its component nucleosides using a combination of snake venom phosphodiesterase and *E. coli* alkaline phosphatase. The nucleosides were separated by paper chromatography on acid-washed Whatman No. 1 paper in the following systems: 1-butanol–water–ammonia (86:14:5, v/v) for the separation of A, Am, and C; ethyl acetate–1-propanol–H_2O (4:1:2, v/v, upper phase) for the separation of C and U; isopropyl alcohol–ammonia–0.1 M boric acid (7:1:2, v/v) for the separation of Cm and U. Ultraviolet-absorbing material on the chromatogram, corresponding to known standards, was eluted and quantitated as described previously (Rottman and Johnson, 1969). Prior to the digestion all polymers were chromatographed on Whatman No. 1 paper in 1-propanol–concentrated NH_4OH–H_2O (55:10:35, v/v) to determine that they were free of monomer.

The sedimentation coefficients (*s* value) of the polymers were determined as follows. Approximately 3 A_{260} units of polymer was layered over a 4.8-ml linear sucrose gradient of 5–20% sucrose in 0.1 M potassium acetate and 0.02 M Tris-acetate (pH 9.0) and centrifuged at 38,000 rpm for 18 hr in a Spinco SW39 rotor. *E. coli* tRNA was included as a standard and the *s* values of the polymers determined from their posi-

TABLE I: Incorporation of Amino Acids Directed by Poly(Cm,U) and Poly(C,U).[a]

[^{14}C]Amino Acid	Coding Triplet	Poly(C,U)			Poly(Cm,U)		
		pmoles of Amino Acid Incorpd[b]	% of Total Incorpn		pmoles of Amino Acid Incorpd[b]	% of Total Incorpn	
			Exptl	Theor[c]		Exptl	Theor
Phenylalanine	UU$^{C}_{U}$	2090	69.0	70.5	2530	75.6	73.0
Serine	UC$^{U}_{C}$	400	13.2	13.4	244	7.3	12.5
Leucine	CU$^{U}_{C}$	430	14.3	13.4	414	12.4	12.5
Proline	CC$^{U}_{C}$	110	3.5	2.7	160	4.7	2.1
Total		3030			3348		

[a] Conditions for the S-30 assay are described in Materials and Methods. Approximately 0.2 A_{260} unit of polymer was used per reaction. [b] Incorporation is expressed as picomoles of amino acid incorporated per milligram of S-30 protein. [c] Theoretical per cent incorporation is calculated from the polymer base ratios, which were 1:5.3 and 1:5.8 (C or Cm to U) for poly(C,U) and poly(Cm,U), respectively. Background incorporation of amino acids in the absence of added polynucleotides has been subtracted from the values reported. Polynucleotide stimulated incorporation ranged from twice background for proline to fifteen-times background for phenylalanine.

tions in the gradient relative to that of tRNA. Since Jones *et al.* (1964) have shown that the template activity of a polymer is influenced by its chain length, pairs of heteropolymers were used which were of similar size as indicated by similar *s* values. The *s* values of the polymers used in this study were as follows: poly(Cm,U), 4.0; poly(C,U), 4.0; poly(Am,C), 5.2; poly(A,C), 5.3; poly(Am), 5.0; poly(Cm), 4.0; and poly(Um), 9.2. The sedimentation profiles indicated that the polymers were heterodisperse.

Results

Poly(Cm,U)- and Poly(C,U)-Directed Incorporation of Amino Acids. The template activity of both the poly(Cm,U) and poly(C,U) polymers was determined by measuring the incorporation of phenylalanine, leucine, serine, and proline. These four are the only amino acids that can be coded for by a polymer containing C and U. The results, in Table I, are compared to the values calculated from the base ratio of the polymers. As shown in Table I, the total amino acid incorporation directed by the (Cm,U) polymer was slightly greater than the incorporation directed by the (C,U) polymer. Therefore there seems to be no overall inhibition in the template activity of poly(Cm,U) by the Cm nucleoside. The poly(Cm,U) directed incorporation of serine, leucine, and proline into peptides shows that the nucleoside Cm can replace C in a triplet, since these amino acids are coded for by triplets which contain C.

Poly(Cm,U) and poly(C,U) were used as templates to determine whether polymers containing Cm were misread more than polymers containing C. The incorporation of several amino acids was measured whose code words corresponded to these listed in Table I with one of the 5′ or internal C residues replaced by A or G. These amino acids were valine (GUN,N = A, U, C or G), tyrosine $\left(\text{UA}^{\text{U}}_{\text{C}}\right)$, arginine (CGN, N = A, U, C or G), and isoleucine $\left(\text{AU}^{\text{U}}_{\text{C}}\right)$. No incorporation of valine or arginine was detected when either poly(C,U) or poly(Cm,U) was used as a template. Tyrosine and isoleucine were incorporated to a slight extent when either poly(C,U) or poly(Cm,U) was used as a template. Therefore, it is evident that the nucleoside Cm is not extensively misread in the 5′ or internal position as A or G, nor does it cause misreading of adjacent U bases in the polymer.

Since a fixed time assay was used to obtain the results in Table I, experiments were done to determine if the difference in template activity was time dependent. The time course of incorporation of phenylalanine was measured using both poly(Cm,U) and poly(C,U) as templates. The results shown in Figure 1 indicate that although the initial rate of incorporation of phenylalanine is similar for both polymers, incorporation continues for a longer period of time when poly(Cm,U) is present.

The longer duration of the template activity of poly(Cm,U) as compared to poly(C,U) may be related to increased protection of the (Cm,U) polymer from degradation by nucleases in the S-30 extract. To test this, [^{14}C]uracil-labeled copolymers of (C,U) and (Cm,U) were synthesized and their degradation to trichloroacetic acid soluble products measured, using the same conditions employed in the amino acid incorporation assay. The results in Figure 2A indicate that the (Cm,U) polymer is degraded at a much slower rate than the (C,U) polymer, suggesting that the 2′-*O*-methyl group increased the nuclease resistance of the (Cm,U) polymer. Thus it seems likely that poly(Cm,U) can direct the incorporation of phenylalanine for a longer period of time than poly(C,U) because of its slower rate of degradation.

Incorporation of Amino Acids Directed by Poly(Am,C) and Poly(A,C). In contrast to the template activity of poly(Cm,U) relative to poly(C,U), the (Am,C) polymer was found to be much less active as a template than the corresponding (A,C) polymer. Table II contains a summary of the results of experiments which measured the template activity of the (A,C) and (Am,C) polymers. The poly(Am,C)-directed incorporation of histidine and threonine into peptides in the absence of neomy-

TABLE II: Incorporation of Amino Acids Directed by Poly(Am,C) and Poly(A,C) in the Presence and Absence of Neomycin.[a]

[^{14}C]Amino Acid	Coding Triplet	Poly(A,C)[b]		Poly(Am,C)	
		−Neomycin	+Neomycin	−Neomycin	+Neomycin
Proline	CCN	600	314	156	314
Threonine	ACN	53	82	18	82
Histidine	CA^{C}_{U}	43	143	8	121
Serine	UCN, AG^{U}_{C}	ND[c]	206	ND	164
Arginine	CGN, AG^{A}_{G}	ND	54	ND	48
	(N = A, C, U, or G)				

[a] Conditions for the S-30 assay are described in Materials and Methods. Poly(Am,C) (0.12 A_{265} unit) and poly(A,C) (0.20 A_{265} unit) were used per reaction. Neomycin concentration was 5 μg/ml. Incorporation is expressed as picomoles of amino acid incorporated per milligram of S-30 protein. [b] Both poly(A,C) and poly(Am,C) had base ratios of 1:13 (A or Am to C). [c] ND = none detected. Background incorporation of amino acids in the absence of added polynucleotide has been subtracted from the values reported. Polynucleotide-stimulated incorporation ranged from one-times background for histidine to ten-times background for proline.

cin indicates Am can be read as A in a triplet, since both amino acids require A in their code words. The lower stimulation of amino acid incorporation by poly(Am,C) compared to poly(A,C) indicates that the 2′-*O*-methyl groups have an inhibitory effect on the template activity of the (Am,C) polymer.

To determine if the nucleoside Am was misread in the 5′ or internal position, or caused misreading of the cytosine residues in the polymer, the incorporation of the amino acids serine, arginine, alanine, and leucine, was measured. The codons for these amino acids are, respectively, UCN, CGN, GCN, CUN, where N = A, U, C, or G. Neither poly(Am,C) nor poly(A,C) stimulated significant incorporation of any of these four amino acids. Thus the nucleoside Am does not appear to be read as G or U, and apparently does not increase the misreading of the polymer to any detectable extent.

To determine if the inhibition noted in Table II was time dependent, the incorporation of proline was measured as a function of time when directed by either poly(Am,C) or poly(A,C). The results, shown in Figure 3, indicate that the rate of incorporation of proline is significantly slower when directed by poly(Am,C) than when directed by poly(A,C). Since poly(Cm,U) was more resistant than poly(C,U) to degradation by the S-30 extract, it was of interest to see if the same held true for poly(Am,C) when compared to poly(A,C). The results of degradation studies performed with [^{14}C]cytosine-labeled

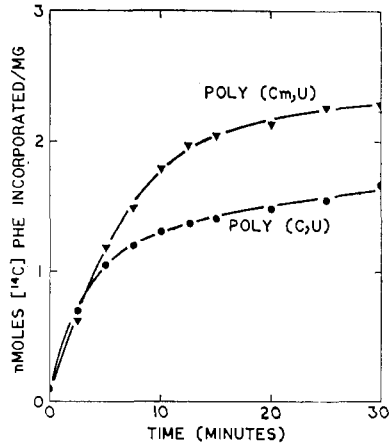

FIGURE 1: Time dependence of incorporation of [^{14}C]phenylalanine directed by poly(C,U) or poly(Cm,U). Conditions for the S-30 assay are given in Materials and Methods. The reaction volume was 700 μl, which contained 2 A_{260} units of poly(C,U) or poly(Cm,U), and 2.9 mg of S-30 protein. After addition of the S-30, 60-μl samples were withdrawn at the times indicated and spotted on Whatman No. 3MM disks, which were then treated as described in Materials and Methods. Incorporation is expressed as nanomoles of amino acid incorporated per milligram of S-30 protein.

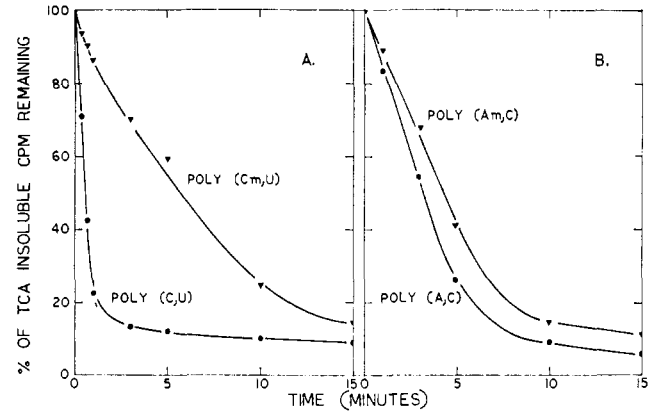

FIGURE 2: Degradation of polymers by S-30 extracts. Labeled polymers, in 700 μl of S-30 incorporation buffer (substituting [^{12}C]- for [^{14}C]amino acid), were incubated at 37°. At the times shown, 60-μl samples were withdrawn, added to 1.0 ml of cold 5% trichloroacetic acid (TCA), and filtered through a Millipore filter (Type HAWP, 0.45 μ pore size). The filter was washed three times with cold 5% trichloroacetic acid, dried, and counted. In part A 2.7 mg of S-30 and 5.0 A_{260} units of ^{14}C-labeled (C,U) or (Cm,U) were used. The specific radioactivity of both the [^{14}C]poly(C,U) and poly(Cm,U) was 1.0×10^4 cpm/A_{260} unit. In part B 5.0 mg of S-30 and 5.0 A_{265} units of ^{14}C-labeled poly(A,C) or poly(Am,C) were used. The specific radioactivity of poly(A,C) was 3×10^4 cpm/A_{265} unit and of poly(Am,C), 1.7×10^4 cpm/A_{265} unit.

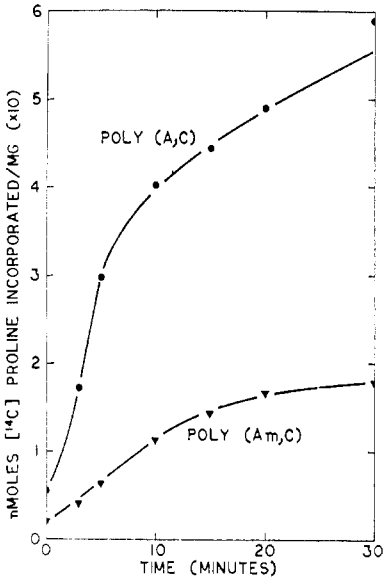

FIGURE 3: Time dependence of incorporation of [^{14}C]proline directed by poly(A,C) or poly(Am,C). Conditions of the S-30 assay are given in Materials and Methods. The reaction volume was 700 μl, which contained 2.6 A_{265} units of poly(A,C) or 1.3 A_{265} units of poly(Am,C) and 5.8 mg of S-30 protein. After addition of the S-30, 60-μl samples were withdrawn at the times indicated and spotted on Whatman No. 3MM disks, which were then treated as described in Materials and Methods. Incorporation is expressed as nanomoles of amino acid incorporated per milligram of S-30 protein.

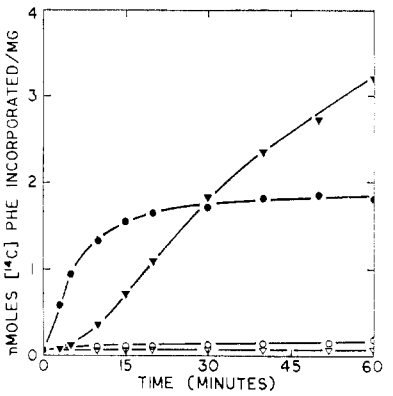

FIGURE 4: Time dependence of incorporation of [^{14}C]phenylalanine directed by poly(U) or poly(Um) with and without neomycin. Conditions of the S-30 assay are given in Materials and Methods. The reaction volume was 350 μl, which contained 2.0 A_{260} units of poly(U) or poly(Um) and 2.0 mg of S-30 protein. Neomycin concentration was 5 μg/ml. After addition of the S-30, 30-μl samples were withdrawn at the times indicated and spotted on Whatman No. 3MM disks, which were then treated as described in Materials and Methods. ● Poly(U), (▽) poly(Um), (○) poly(U) + neomycin, and (▼) poly(Um) + neomycin. Incorporation is expressed as nanomoles of amino acid incorporated per milligram of protein.

poly(Am,C) and poly(A,C) are shown in Figure 2B. Although poly(Am,C) was degraded at a slower rate than poly(A,C), the differences in the rates of degradation are not as pronounced as the differences between poly(Cm,U) and poly(C,U).

In determining the optimum conditions for the amino acid incorporation assays, differences were found between poly(A,C) and poly(Am,C) for both the polymer saturation level and the Mg^{2+} concentration. Poly(A,C) saturated the reaction at 0.25 A_{265} unit/70-μl reaction, and had an optimum Mg^{2+} concentration of 11.5 mM, while poly(Am,C) saturated the reaction at 0.12 A_{265} unit/70 μl, and had an optimum Mg^{2+} concentration of 13.5 mM. A lower saturation level was also found for poly(Cm,U) (0.12 A_{260} unit/70 μl) compared to poly(C,U) (0.25 A_{260} unit/70 μl). However, the optimum Mg^{2+} concentration of 10 mM was the same for both poly(Cm,U) and poly(C,U).

Use of 2′-O-Methyl Homopolymers as Templates. The three 2′-*O*-methylribose homopolymers, poly(Am), poly(Cm), and poly(Um), were tested for template activity under conditions which were optimum for the corresponding nonmethylated polymer. None of the three methylated homopolymers exhibited any template activity under these conditions, which would have permitted detection of 1% of the poly(U)-directed incorporation, and 5% of the poly(C)- or poly(A)-directed incorporation. Since template activity is highly dependent on the Mg^{2+} concentration in the assay, the 2′-*O*-methyl homopolymers were also tested for template activity using a range of Mg^{2+} concentrations from 4 to 20 mM. No activity was found at any Mg^{2+} concentration.

Effects of Neomycin on Template Activity of Homopolymers. McCarthy *et al.* (1966) and Morgan *et al.* (1967) have shown that neomycin enables DNA to act as a template for *in vitro* protein synthesis. Hence it was of interest to see if neomycin would influence the template activity of 2′-*O*-methyl-containing polymers. At neomycin concentrations of 5 μg/ml, neither poly(Cm) nor poly(Am) exhibited any template activity, but poly(Um) now served as an efficient template for the incorporation of phenylalanine. A time study of the template activity of poly(Um) plus neomycin as compared to poly(U) is shown in Figure 4. The results indicate that the rate of phenylalanine incorporation was slower when directed by poly(Um) plus neomycin, but incorporation continued for a longer period of time, perhaps due to the stability of the template. Thus the incorporation of phenylalanine directed by poly(U) is essentially over after 20 min, while the incorporation of phenylalanine directed by poly(Um) plus neomycin is still continuing after 60 min. To determine the relative ambiguity of the neomycin-facilitated translation of poly(Um), the incorporation of the amino acids, leucine, isoleucine, serine, and tyrosine, was measured using both poly(Um) and poly(U) as templates. The results, in Table III, show that the total incorporation of the amino acids listed is about the same when the template was either poly(Um) with neomycin or poly(U) without neomycin. This implies that the total ambiguity in translation of poly(Um) in the presence of neomycin is about the same as that of poly(U) in the absence of neomycin. The pattern of miscoding has changed, however, and the predominant miscoded amino acid with poly(Um) is serine as compared to leucine with poly(U).

Effect of Neomycin on the Template Activity of Heteropolymers. Table IV lists the results of experiments to determine the effect of neomycin on the incorporation of amino acids directed by poly(Cm,U) and poly(C,U). With poly(Cm,U) the effect of neomycin depended on its concentration and the amino acid being tested. Low (0.5–5.0 μg/ml) concentrations of neomycin stimulated the incorporation of all amino acids, whereas higher concentrations of neomycin were inhibitory. The neomycin concentration for maximum stimulation of incorporation depended on the amino acid, and is given in Table IV. With poly(C,U) as a template however, the incorporation of the amino acids listed in Table IV was inhibited at all concentrations of neomycin. The results of similar experiments using poly(Am,C) and poly(A,C) are listed in

TABLE III: Effect of Neomycin on the Misreading of Poly(Um) and Poly(U).[a]

[14C]Amino Acid Incorpd	Poly(U)		Poly(Um)	
	−Neomycin	+Neomycin	−Neomycin	+Neomycin
Leucine	510	330	ND[b]	260
Isoleucine	100	82	ND	51
Serine	61	150	ND	570
Tyrosine	50	ND	ND	ND
Total	721	562		881

[a] Conditions for the S-30 assay are described in Materials and Methods. Approximately 0.50 A_{260} unit of polymer was used per reaction. Neomycin concentration was 5 μg/ml. Results are expressed as picomoles of amino acid incorporated per milligram of S-30 protein. [b] ND = none detected. Background incorporation of amino acids in the absence of added polynucleotide has been subtracted from the values reported. Polynucleotide-stimulated incorporation ranged from two-times background for isoleucine to five-times background for leucine.

TABLE IV: Effect of Neomycin on the Incorporation of Amino Acids Directed by Poly(Cm,U) and Poly(C,U).[a]

[14C]Amino Acid	Poly(C,U)		Poly(Cm,U)	
	−Neomycin	+Neomycin	−Neomycin	+Neomycin
Phenylalanine	1684	897	1868	2182
Serine	355	129	310	414
Leucine	548	330	494	1330
Proline	132	77	126	209

[a] Conditions for the S-30 assay are described in Materials and Methods. Approximately 0.50 A_{280} unit of polymer was used per reaction. Neomycin concentration was 0.5 μg/ml for phenylalanine, 2.5 μg/ml for leucine and proline, and 5.0 μg/ml for serine. Incorporation is expressed as picomoles of amino acid incorporated per milligram of S-30 protein. Background incorporation of amino acids in the absence of added polynucleotide has been subtracted from the values reported. Polynucleotide stimuated incorporation ranged from two-times background for proline to ten-times background for phenylalanine.

Table II. At a concentration of 5 μg/ml, neomycin stimulated the poly(Am,C)-directed incorporation of all the amino acids tested. This concentration of neomycin stimulated the poly(A,C)-directed incorporation of four of the five amino acids tested, but inhibited the incorporation of the fifth, proline.

Discussion

Both poly(Cm,U) and poly(Am,C) served as templates for amino acid incorporation, as shown in Tables I and II. Compared to their nonmethylated analogs, poly(Am,C) was less active as a template than poly(Cm,U). Other experiments employing poly(Um,U), which was prepared by deamination of poly(Cm,U), indicate that poly(Um,U) also served as a template (data not shown). The effect of 2′-*O*-methyl groups on the template activity of a polymer may depend on which of the four nucleotides is 2′-*O*-methylated, the nature of adjacent nucleotides, and the base composition of the polymer. The important conclusion to be drawn from these studies is that low levels of 2′-*O*-methyl nucleotides in an RNA polymer do not eliminate its template activity and under certain conditions can be stimulatory.

If methylation of the 2′-sugar position caused a significant change in the conformation of the bases in the polymer, it might alter the hydrogen-bonding capabilities of the component nucleotides and thus increase misreading. The results of Tables I and II, however, indicate that both Am and Cm can replace their respective nonmethylated analogs without causing an increase in misreading of the polymer.

Nakada (1965) has postulated that in *E. coli*, methylation of nascent rRNA destroys its template activity. 2′-*O*-Methyl nucleotides comprise from 0.1 to 1.9% of the component nucleotides of rRNA (Starr and Sells, 1969). Our results, which show that even higher levels of 2′-*O*-methyl nucleotides (7–15%) do not completely inhibit template activity, suggest that the amount of 2′-*O*-methyl nucleotides found in rRNA would not be sufficient to prevent translation.

Neomycin had no effect on poly(Am) or poly(Cm) template activity, but promoted that of poly(Um) and stimulated the template activity of both methylated heteropolymers. The promotion of poly(Um) template activity was not accompanied by any appreciable increase in miscoding. In a similar finding, Morgan *et al.* (1967) reported that poly(dT) is inactive as a template, but in the presence of neomycin it efficiently directed the incorporation of phenylalanine. They also noted that neomycin did not cause misreading of this polymer. Davis (1966) has reported that neomycin can cause inhibition of template activity with only a slight increase in miscoding. Our results are in essential agreement with the conclusion that neomycin can affect template efficiency without significantly increasing miscoding.

Bobst *et al.* (1969a–c) have shown that poly(Am) has more secondary structure than poly(A), and Zmudzka *et al.* (1969) obtained similar results for poly(Cm). Thus the failure of the 2′-*O*-methyl homopolymers to serve as templates may be due to their increased secondary structure, since Szer and Ochoa (1964) have reported that increased secondary structure in RNA decreases its template ability.

The degradation studies using poly(Cm,U) and poly(Am,C) indicate that even in a crude cell-free protein-synthesizing system, known to contain a variety of nucleases (Barondes and Nirenberg, 1962) methylated nucleotides confer nuclease resistance to a polymer. We have also noted that the methylated homopolymers are very resistant to mixtures of alkaline phosphatase, snake venom phosphodiesterase, and micrococcal nuclease. Thus 2′-*O*-methylation may provide a mechanism for stabilizing a template without affecting its fidelity of translation. Further studies are in progress to investigate the effect of 2′-*O*-methyl nucleotides on the stability of RNA toward various nucleases.

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Absorption and Circular Dichroism Studies on Nucleohistone IV*

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ABSTRACT: Reconstituted calf thymus nucleohistone IV was studied by absorption and circular dichroism spectroscopy. The binding of histone IV to DNA does not change either the absorption or the circular dichroism spectrum of DNA at a detectable level. Based on this fact both the absorption and circular dichroism spectrum of histone IV, in its conformation as part of nucleohistone IV were obtained, and found to be independent of coverage. The absorption spectrum of complexed histone IV shows an anomalously large 70% hypochromism for the 190-nm $\pi \rightarrow \pi^*$ band of the amide linkage. In contrast, the $n \rightarrow \pi^*$ absorption near 220 nm of the carbonyl of the amide group is not significantly affected by complex formation. The circular dichroism spectrum of histone IV in the complex is also anomalous, and is not typical of α helix, β structure, or random coil, or a linear combination of these.

The implications of these spectral observations are discussed. In addition, studies are presented which show that histone IV binding to DNA does not protect the DNA from salt perturbation. Molar extinction coefficients for both free and complexed histone IV are reported here, for the first time, through the amide absorbance region, down to 180 nm. There is a high probability that the amides interact with the phosphates or sugars rather than the bases.

Histones complexed with DNA repress the latter in RNA transcription (Huang and Bonner, 1962; Barr and Butler, 1963; Allfrey *et al.*, 1963). Histone–DNA interaction is therefore biologically interesting, and this physical interaction has been extensively investigated (Zubay and Doty, 1959; Bonner and Ts'o, 1964; Huang *et al.*, 1964; Akinrimisi *et al.*, 1965; Ohlenbusch *et al.*, 1967; Olins, 1969; Shih and Bonner, 1970; Fasman *et al.*, 1970a,b; Li and Bonner, 1971; Adler *et al.*, 1971). In addition, polypeptides such as polylysine and polyarginine have been used as model molecules for the study of basic protein–DNA interaction (Tsuboi *et al.*, 1966; Leng and Felsenfeld, 1966; Olins *et al.*, 1967, 1968; Evett and Isenberg, 1969; Evett *et al.*, 1970).

Among the classes of histones, histone IV is particularly interesting because the primary sequence is known (DeLang *et al.*, 1969; Ogawa *et al.*, 1969). However very little physical information on histone IV is as yet known. A proton magnetic resonance study of histone IV (Boublik *et al.*, 1970) indicates

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