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*CELL-FREE PEPTIDE SYNTHESIS DEPENDENT UPON SYNTHETIC OLIGODEOXYNUCLEOTIDES**

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Since the use of randomly ordered template RNA preparations to direct cell-free protein synthesis has provided a relatively simple experimental approach for investigating the nature of the genetic code,[1, 2] it seemed that chemically defined DNA preparations also might facilitate such investigations. There have been several reports of cell-free amino acid incorporation dependent upon natural DNA;[3–8] however, the limited number of defined DNA preparations, such as poly d(AT), poly dC, and poly dG, which can be synthesized with DNA polymerase,[9, 10] have hindered investigations in this area. The chemical synthesis of oligodeoxynucleotides containing up to approximately 15 bases per chain by the dicyclohexylcarbodiimide method of Khorana and his associates,[11, 12] and the demonstration of an oligodeoxynucleotide-dependent synthesis of polyribonucleotides, catalyzed by RNA polymerase,[13–18] afforded an opportunity to study the ability of such oligodeoxynucleotides to stimulate cell-free amino acid incorporation. Since poly A serves as a template for polylysine synthesis,[19] we have used synthetic oligodeoxythymidylate to direct poly A, and then polylysine synthesis, as shown below:

$$\text{ATP} \xrightarrow[\text{RNA polymerase}]{\text{Oligo dT}} \text{Poly A} + \text{PP} \qquad (1)$$

$$\text{Lysine} \xrightarrow[\textit{E. coli} \text{ extracts, etc.}]{\text{Poly A}} \text{Polylysine} \qquad (2)$$

The characteristics of the poly A and polylysine synthesis directed by chemically synthesized oligodeoxynucleotides, natural DNA, and poly U are described below.

Materials and Methods.—Synthesis of oligodeoxynucleotides: Nucleoside mono- and triphos-

phates were obtained from Pabst Laboratories, Milwaukee, Wis., and Calif. Corp. Biochem. Research, Los Angeles, Calif., and the purity of each was checked before use by paper chromatography employing solvents A, B, and C (see below). Oligonucleotides of deoxythymidine-5′ phosphate and deoxyadenosine-5′ phosphate were synthesized by the dicyclohexylcarbodiimide method, as described by Khorana and his co-workers, and were purified by column chromatography.[11, 12, 20] The chain length of oligo dT_{13-14} was determined by measuring the ratio of total phosphate[21] to terminal phosphate released after hydrolysis with *E. coli* alkaline phosphatase (Worthington Biochem. Corp., Freehold, N. J.).[22, 23] Other fractions were identified from their column and paper chromatographic mobilities.[12] Incubation with rattlesnake venom phosphodiesterase (Worthington Biochem. Corp.) converted oligo dT to deoxythymidine-5′ phosphate.[24] An ϵ at 267mμ, pH 7, of 9.6×10^{-3} M^{-1} cm^{-1} was assumed for (pdT) residues in oligo dT when calculating (pdT) concentrations. Descending paper chromatography was performed with Whatman 40 and 3 MM paper for solvents A–D, and with Whatman DE-20 paper for solvent E. Solvent components were as follows: solvent A, n-propyl alcohol/concentrated NH_4OH/H_2O = 55/10/35 V/V; solvent B, isobutyric acid/1.0 M NH_4OH/0.1 M disodium ethylenediaminetetraacetate = 1,000/600/16 V/V; solvent C, isopropyl alcohol/concentrated NH_4OH/0.1 M boric acid = 7/1/2 V/V; solvent D, pyridine/n-butyl alcohol/acetic acid/water = 6/9/3/7 V/V; solvent E, 0.3 M ammonium formate.

Synthesis of polyadenylic acid (stage I): Tetralithium 8-C^{14}-ATP was obtained from Schwarz BioResearch, Inc., Orangeburg, N. Y. (0.15–1.5 mcuries/mmole). Calf thymus DNA was purified according to the method of Kay *et al.*[25] Poly A, poly U, and poly C, prepared with polynucleotide phosphorylase, were obtained from Miles Chem. Co., Clifton, N. J. *E. coli* RNA polymerase was purified 100- to 150-fold by the procedure of Chamberlain and Berg.[16] The final specific activity after the DEAE column step was 800–1600 units[16]/mg protein when assayed with calf thymus DNA. Each RNA polymerase stage I reaction mixture contained the following in a total volume of 0.125 ml; 0.04 M Tris pH 7.8; 4×10^{-3} M $MgCl_2$; 1×10^{-3} M $MnCl_2$; 1.2×10^{-2} M mercaptoethanol; 1.6×10^{-3} M ATP; oligo- or polynucleotide where specified, and 20 μg RNA polymerase protein ($\sim$20 units[16]). Reaction mixtures were incubated for 15 min at 37° unless otherwise noted. Poly A synthesis was determined concomitantly in separate reaction mixtures containing C^{14}-ATP in place of C^{12}-ATP. After incubation at 37° for 15 min, reaction mixtures were chilled, and 2.0 ml of cold H_2O and 1.0 ml of 15% TCA were added to each tube. After standing for 10 min at 3°, the contents of each tube were filtered with suction through a Millipore filter (0.45-μ pore size, 25 mm diameter, Millipore Co., Bedford, Mass.) and washed with 25 ml of 5% TCA at 3°. Filters were glued to planchettes, dried, and radioactivity was determined in a thin-window, gas-flow counter (Nuclear-Chicago Corp., Des Plaines, Ill.) with a counting efficiency of 25%.

The average chain length of C^{14}-poly A synthesized with RNA polymerase in a stage I incubation was determined in the following manner. A reaction mixture was heated at 90–95° for 5 min and then was chilled in ice. Precipitated protein was removed by centrifugation, and the supernatant fluid was lyophilized. The residue was dissolved in water and chromatographed on Whatman DE-20 paper with solvent E for 2.5 hr to remove C^{14}-ATP from the C^{14}-poly A which remained at the origin.[18] The C^{14}-poly A was hydrolyzed in 0.3 N KOH for 20 hr at 37°, and the solution was adjusted to pH 10–11 with Dowex 50 (H^+). Unlabeled carrier adenosine, adenosine-3′(2′)-phosphate, and adenosine-3′(2′)-5′-diphosphate (the gift of Dr. Leon Heppel) were added, and the components of the solution were separated by chromatography on Whatman DE-20 paper in solvent E for 6 hr, and also on Whatman 40 paper in solvent A for 24 hr. The chromatograms were cut into 0.5-in. strips, placed in vials containing toluene-PPO-POPOP phosphor solution,[26] and the radioactivity of each sample was determined in a liquid scintillation counter (Packard Instrument Co., Inc., La Grange, Ill.).

Synthesis of C^{14}-polylysine (stage II): The sources and specific radioactivities of C^{14}-amino acids, and the preparation of DNase-treated, preincubated, cell-free, *E. coli* extracts used in stage II (protein synthesizing) reaction mixtures have been described previously.[1]

Stage II components were added to stage I reaction mixtures immediately after stage I tubes had been incubated at 37° for 15 min. Thus, both stage I and II reactions were performed consecutively in the same tube. Each combined stage I and II reaction mixture contained the following components in a total volume of 0.25 ml: 6×10^{-2} M Tris, pH 7.8; 2×10^{-3} M $MgCl_2$;

1.2×10^{-2} *M* magnesium acetate; 5×10^{-4} *M* $MnCl_2$; 1.2×10^{-2} *M* mercaptoethanol; 2.8×10^{-3} *M* ATP; 5×10^{-2} *M* KCl; 5×10^{-3} *M* PEP; 5 μg crystalline PEP kinase (Calif. Corp. Biochem. Research); 2×10^{-4} *M* each of 19 L-amino acids; 2×10^{-4} *M* C^{14}-L-lysine (Nuclear-Chicago Corp.) of specific radioactivity 4–8 mcuries/mmole; 20 μg RNA polymerase protein and 1.1 mg *E. coli* extract protein. Reaction mixtures were incubated at 37° for the times indicated, and C^{14}-lysine incorporated into protein was then precipitated and counted by the method of Gardner *et al.*[19] unless otherwise specified. All assays were performed in duplicate.

Characterization of the product synthesized from C^{14}-lysine: After 30 min of incubation at 37°, each stage II reaction mixture was deproteinized by the addition of 2 ml of cold 10% TCA solution. The supernatant solution was extracted three times with an equal volume of ether, concentrated *in vacuo*, and then the residue was cochromatographed with a partial tryptic digest of chemically synthesized C^{12}-polylysine (YEDA Research and Development Co., Ltd., Rehovoth, Israel) on Whatman 3 MM paper in solvent D for 56 hr (similar to the solvent described by Waley and Watson[27]). C^{14}-Lysine moves rapidly in this solvent and can be separated easily from polylysine of chain length greater than 11 which remains at the origin. The C^{14}-product together with unlabeled carrier polylysine was eluted from the origin with 0.1 *N* HCl, then dried and hydrolyzed for 12 hr in 6 *N* HCl at 110°. The residue from the dried reaction mixture was rechromatographed in the same manner. The radioactivity of each chromatogram was determined as described earlier. C^{14}-material eluted from the origin was characterized further by paper chromatography and was incubated at 37° for 9 hr with 20 μg of trypsin in a 0.25 ml reaction mixture containing 0.04 *M* Tris pH 7.8, 4×10^{-3} *M* $MgCl_2$, and 10^{-3} *M* $MnCl_2$. The C^{14}-products of the tryptic digestion were identified by paper chromatography as described above.

The identity of the C-terminal end group of the synthesized C^{14}-polypeptide was established by a hydrazinolysis procedure.[28] The polypeptide was treated *in vacuo* at 106° for 20 hr. The amino acid hydrazides and free amino acids in aqueous solution at pH 11.0 were applied to a Dowex-1 column (OH^-), and the free C^{14}-lysine then was eluted with 1.0 *M* acetic acid.

Pancreatic DNase was obtained from the Worthington Biochem. Corp.; pancreatic RNase from Sigma Chem. Co., St. Louis, Mo.; chloramphenicol succinate from Parke, Davis and Co., Detroit, Mich.; and actinomycin D from Merck, Sharp and Dohme Co., Rahway, N. J. Puromycin HCl was the generous gift of Dr. F. H. Bergmann. Protein was determined by a micromodification of the method of Lowry *et al.*[29]

Results.—Characteristics of poly A synthesis: The data of Figure 1 show that C^{14}-AMP incorporation was dependent upon the addition of oligo dT_{13-14} to stage I reaction mixtures, and that C^{14}-AMP incorporation was proportional to the amount of oligo dT added within the range of 2.4 or less mμmoles of nucleotide residues in oligo dT_{13-14}. Incorporation stopped after 30 min of incubation.

The average chain length of the C^{14}-product synthesized in the presence of oligo dT_{13-14} was determined as described in the *Materials and Methods* section. After hydrolyzing the C^{14}-products of the reaction in KOH and separating the mononucleotides by paper chromatography, 339, 308, and 22,900 counts per min of

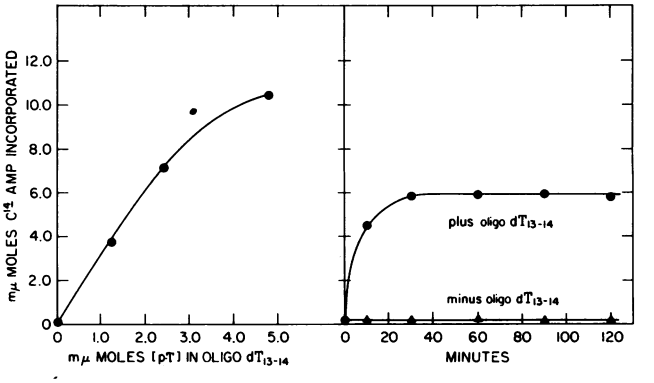

FIG. 1.—Characteristics of C^{14}-poly A synthesis in RNA polymerase (stage I) reaction mixtures. Components of stage I reaction mixtures are described under *Materials and Methods*. In the figure on the left, reaction mixtures were incubated at 37° for 15 min. In the figure on the right, the symbols represent the following: ▲, minus polymer; ●, plus 1.2 mμmoles of (pdT) in oligo dT_{13-14}.

adenosine, adenosine-3′(2′)-5′ diphosphate, and adenosine-3′(2′)-monophosphate, respectively, were found. Thus, oligo dT of chain length 13–14 base residues stimulated the synthesis of poly A of average chain length 60–70 (pA) residues. These results confirm similar observations made by Furth, Hurwitz, and Goldmann[13] and also by Falaschi, Adler, and Khorana.[18]

Characteristics of polylysine synthesis: After incubating stage I reaction mixtures at 37° for 15 min, stage II components were added, and the effect of the newly synthesized poly A upon C^{14}-lysine incorporation into protein was observed. As shown in Figure 2, no increase in C^{14}-lysine incorporation was found in the absence

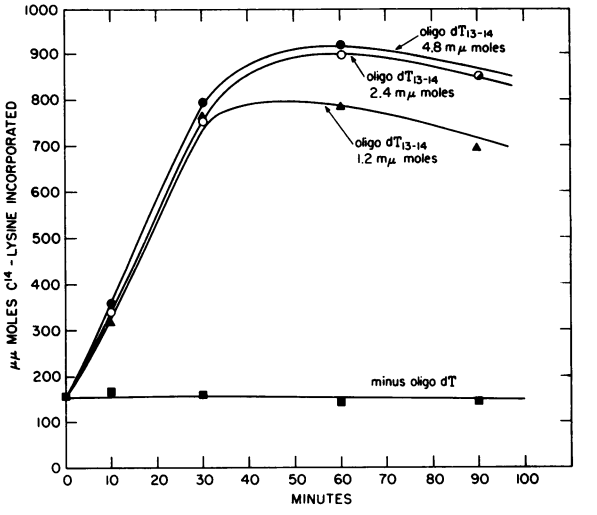

FIG. 2.—Characteristics of oligo dT_{13-14} directed synthesis of C^{14}-polylysine. ■, minus oligo dT; ▲, plus 1.2 mμmoles (pdT) residues in oligo dT_{13-14}; O, plus 2.4 mμ moles (pdT) residues in oligo dT_{13-14}; ●, plus 4.8 mμmoles (pdT) residues in oligo dT_{13-14}. Components of stage I and stage II reaction mixtures are described under *Materials and Methods*. Stage I reaction mixtures were incubated at 37° for 15 min before addition of stage II components. The length of stage II incubation, in minutes, is shown on the abscissa.

of oligo dT, whereas the addition of 1.2 mμmoles of (pdT) residues in oligo dT_{13-14} stimulated C^{14}-lysine incorporation at a linear and almost optimal rate for 30 min. In separate reaction mixtures, C^{14}-AMP incorporation into poly A was determined at the end of the stage I incubation. No incorporation was observed in the absence of oligo dT, whereas the addition of 1.2 mμmoles of base in oligo dT_{13-14} stimulated the incorporation of 3.8 mμmoles of AMP into poly A, which in turn directed the incorporation of 0.64 mμmoles of C^{14}-lysine into protein. Additional experiments demonstrated that oligo dT directed little or no C^{14}-AMP incorporation into poly A in stage II reaction mixtures. No C^{14}-lysine incorporation into protein was found in stage I reaction mixtures; thus, both incubations were required.

The specificity of amino acid incorporation also was investigated. Poly A was synthesized in stage I reaction mixtures containing oligo dT_{13-14}, and its effect upon the incorporation of 19 C^{14}-amino acids (cysteine not tested) was determined. The incorporation of each C^{14}-amino acid was assayed separately, and all reaction mixtures contained the regular complement of 19 unlabeled amino acids minus the one C^{14}-amino acid present. Only C^{14}-lysine was directed into protein; thus, marked amino acid specificity was observed.

Characteristics of C^{14}-lysine-containing products: Both the C^{14}-products synthesized in stage II reaction mixtures and synthetic polylysine were soluble in cold 10% TCA and could be precipitated by the addition of a sodium tungstate-TCA

solution.[19] Paper chromatography of polylysine as described in the *Materials and Methods* section permits separation of lysine peptides of different chain lengths. Peptides containing approximately 11 or more lysine residues remain at the origin, whereas the mobilities of smaller peptides are as follows: lysine > di > tri > tetra > penta > hexa > hepta > octa > nona > deca-lysine. The scales at the top and bottom of Figure 3 show the chromatographic mobilities of lysine peptides in this system. As seen in Figure 3*A*, when stage I and II reaction mixtures were incubated without oligo dT, little C^{14}-lysine or C^{14}-product remained at the origin after chromatography; however, when incubations were performed in the presence of oligo dT_{13-14}, most of the C^{14}-product synthesized remained at the origin.

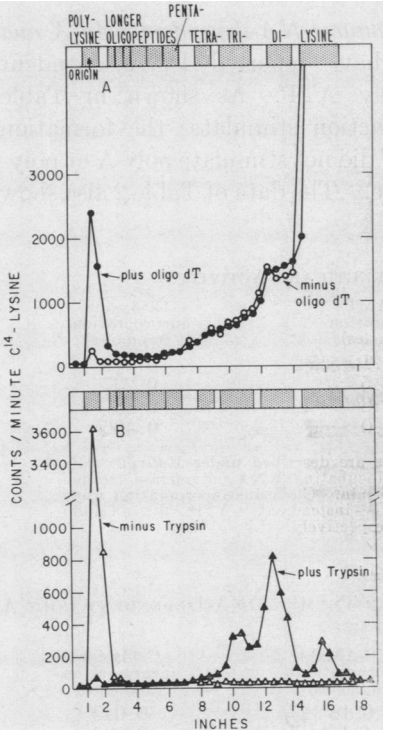

FIG. 3.—Chromatographic analysis of the C^{14}-polylysine in stage I plus stage II reaction mixtures under the direction of oligo dT_{13-14}. Fig. 3*A* depicts the chromatographic analysis of reaction mixtures plus and minus oligo dT_{13-14}. The symbols represent the following: ○, minus oligo dT_{13-14}; ●, plus oligo dT_{13-14}. Reactions were performed as described in the *Materials and Methods* section. After incubation of stage II reaction mixtures at 37° for 30 min, the reactions were terminated by the addition of 2 ml of cold 10% TCA. The supernatant solution was extracted three times with equal volumes of ether, concentrated *in vacuo*, and the residue was compared chromatographically with a partial tryptic digest of chemically synthesized C^{12}-polylysine as described in the *Materials and Methods* section.

In Fig. 3*B*, the C^{14}-products formed in stage I and stage II reaction mixtures were chromatographed as described in the previous paragraph. The C^{14}-product remaining at the origin was eluted and one aliquot was digested with trypsin. The symbols represent the following: △, rechromatography of the isolated product; ▲, tryptic digest of the isolated C^{14}-product. Methods of chromatographic analysis, C^{14}-product isolation, and tryptic digestion are described in the *Materials and Methods* section.

Incubation with trypsin converts polylysine to free, di-, and tri-lysine. The data of Figure 3*B* show that digestion with trypsin converted the isolated C^{14}-product almost quantitatively to peptides which migrated with free, di-, tri-, and tetra-lysine. Tetra-lysine would be expected if digestion were not complete.

In separate experiments, an aliquot of the C^{14}-product, which had been eluted from the origin after paper chromatography, was hydrolyzed completely in HCl, and another aliquot was used to characterize carboxyl-terminal residues. The hydrolysate obtained with HCl was subjected to paper chromatography, and only one C^{14}-spot, having the characteristic mobility of free lysine, was found. In addition, the C^{14}-product was shown to contain carboxyl-terminal radioactivity by a hydrazinolysis method.[28] These experiments strongly suggest that the C^{14}-product is C^{14}-polylysine.

Effect of molecular weight upon the activity of oligo dT: Falaschi, Adler, and Khorana demonstrated[18] that oligo dT chains containing less than 4 (pdT) residues did not stimulate poly A synthesis but that longer chains were stimulatory. The activity was proportional to chain length until maximum stimulation was reached with oligo dT fractions containing 14 or more (pdT) residues per chain. The data of Table 1 are in accord with their findings. Oligo dT fractions containing as few as 6–7 base residues per chain were of sufficient length to stimulate the synthesis of poly A with messenger activity for C^{14}-lysine incorporation. Almost optimal stimulation was obtained with fractions containing 7–8 residues per chain.

Effect of inhibitors upon oligo dT and calf thymus DNA-dependent poly A and polylysine synthesis: Chamberlain and Berg[16] have reported DNA-dependent synthesis of poly A in the presence of only ATP. As shown in Table 2, experiment 2, the poly A product of this reaction stimulates the formation of polylysine. Experiment 1 shows that oligo dT did not stimulate poly A or polylysine synthesis in the absence of RNA polymerase. The data of Table 2 also show

TABLE 1

RELATION BETWEEN OLIGO dT CHAIN LENGTH AND ACTIVITY

Addition	C^{14}-AMP incorporation (mμmoles)	C^{14}-lysine incorporation (mμmoles)
None	0.1	0.075
17 mμmoles oligo dT_{6-7}	1.5	0.237
17 mμmoles oligo dT_{7-8}	6.8	0.387
17 mμmoles oligo dT_{9-11}	13.0	0.406

Components of the reaction mixtures and conditions are described under *Materials and Methods*. C^{14}-AMP incorporation was determined after incubating RNA polymerase (stage I) reaction mixtures containing C^{14}-ATP at 37° for 15 min. C^{14}-lysine incorporation was determined in companion reaction mixtures containing C^{12}- instead of C^{14}-ATP. Stage I and II mixtures were incubated at 37° for 15 and 30 min, respectively.

TABLE 2

EFFECT OF VARIOUS INHIBITORS UPON OLIGO dT AND CALF THYMUS DNA-DEPENDENT POLY A AND POLYLYSINE SYNTHESIS

Expt. no.	Additions	C^{14}-AMP incorporation (mμmoles)	C^{14}-lysine incorporation (mμmoles)
1.	None	0.18	0.045
	+ Oligo dT	4.4	0.232
	" " ", minus RNA polymerase	0.10	0.045
	" " ", + 15 μg DNase	2.0	0.171
	" " ", + 5 μg RNase	4.2	0.059
	" " ", + 20 mμmoles puromycin	2.9	0.079
	" " ", + 50 mμmoles puromycin	2.9	0.062
	" " ", + 90 mμmoles chloramphenicol	4.1	0.161
	" " ", + 180 mμmoles chloramphenicol	4.0	0.150
	" " ", 25 mμmoles actinomycin D	3.3	0.241
2.	None	0.16	0.044
	+ Calf thymus DNA	8.8	0.352
	" " " ", 15 μg DNase	0.34	0.038
	" " " ", 5 μg RNase	8.6	0.028
	" " " ", 60 mμmoles chloramphenicol	8.0	0.352
	" " " ", 20 mμmoles puromycin	8.5	0.063
	" " " ", 25 mμmoles actinomycin D	4.1	0.267

The components of the reaction mixtures and conditions are described in the *Materials and Methods* section. All inhibitors were added to stage I reaction mixtures. RNase and DNase were preincubated at 37° for 20 min with stage I components before the reactions were initiated by the addition of RNA polymerase. Stage I and II mixtures were incubated at 37° for 15 and 30 min, respectively. C^{14}-AMP incorporation was determined after incubating separate RNA polymerase (stage I) reaction mixtures containing C^{14}-ATP. 1.2 mμmoles of (pdT) in oligo dT_{13-14}, or 20 mμmoles of base residues in DNA were added where specified.

the effects of inhibitors upon both incorporations. Paper chromatography did not reveal free (pdT) residues after incubation of oligo dT_{13-14} with pancreatic DNase in stage I reaction mixtures, and preincubation of oligo dT_{13-14} and calf thymus DNA with this enzyme inhibited only moderately the synthesis of C^{14}-poly A and C^{14}-polylysine. C^{14}-Polylysine synthesis was inhibited, in order of decreasing effectiveness, by RNase, puromycin, chloramphenicol, and DNase.

Poly U-directed poly A synthesis: Weiss and his co-workers,[30, 31] and also Krakow and Ochoa[32] have shown that RNA polymerase can catalyze the synthesis of complementary RNA in the presence of either RNA or DNA. Therefore, it seemed feasible to direct poly A synthesis in modified stage I reaction mixtures with RNA (poly U) in place of DNA (oligo dT), and then to determine the activity of the poly A in directing C^{14}-lysine incorporation into protein. As seen in Figure 4,

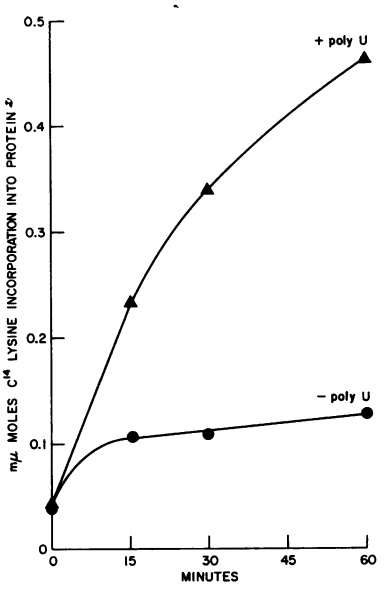

FIG. 4.—Poly U-directed poly A and polylysine synthesis. The components of the stage I reaction mixtures were as follows: 0.04 *M* Tris, pH 7.8; 4×10^{-3} *M* $MgCl_2$; 10^{-3} *M* $MnCl_2$; 2.24×10^{-3} *M* ATP; 6×10^{-3} *M* mercaptoethanol; 20 μg RNA polymerase protein; and 10.2 mμmoles (pU) in poly U where indicated in a total volume of 0.125 ml. After incubation at 37° for 20 min, 0.25 ml of the second stage (protein synthesis) reaction mixture were added to each 0.125 ml stage I reaction mixture. Each 0.25 ml addition contained the following: 0.01 *M* Tris, pH 7.8; 0.014 *M* magnesium acetate; 0.05 *M* KCl; 6×10^{-3} *M* mercaptoethanol; 10^{-3} *M* ATP; 2×10^{-4} *M* each of 19 L-amino acids minus lysine; 2×10^{-5} *M* C^{14}-lysine, and 1.0 mg, preincubated, DNase-treated *E. coli* S-30 protein.[1] Combined 0.375 ml reaction mixtures were incubated at 37° for the stated times.

poly A synthesized from poly U templates stimulated C^{14}-lysine incorporation into protein. In other experiments, no stimulation of C^{14}-lysine incorporation was observed when poly C, rather than poly U, was added to stage I reaction mixtures.

Discussion.—The experiments reported herein have shown that oligo dT_{13-14} directs the synthesis of poly A of average chain-length 60–70 (pA) residues, and the poly A in turn directs the synthesis of polylysine containing 11 or more residues per chain. RNA polymerase is known to catalyze the synthesis of poly A of similar chain length when DNA (preferably denatured) is present and ATP is the only substrate.[16] Our results show that this product also has messenger activity for polylysine synthesis. To account for the chain length of the poly A synthesized in the presence of DNA, Chamberlain and Berg have suggested a "slippage" of poly A along shorter sequences of (pdT) residues in DNA.[16]

Falaschi, Adler, and Khorana[18] have found that oligo dT does not serve as a primer in this system, for (pA) residues were not added to free 3′-hydroxyl ends of

oligo dT chains. Oligo dC was found to stimulate only the incorporation of C^{14}-GMP, whereas oligo dT stimulated C^{14}-AMP incorporation. These results strongly suggest that oligodeoxynucleotides serve as templates rather than as primers.[18] However, exceptions to Watson-Crick base-pairing, such as small stimulations of C^{14}-AMP incorporations by oligo dA, also were reported. Although nucleotide incorporations were almost completely dependent upon the addition of oligo dT under the conditions used in this study, we have detected under other conditions some nucleoside monophosphate incorporations in the absence of oligo dT. The RNA polymerase preparations used in this study were purified 100- to 150-fold, but further purification will be required to determine whether one or more enzymes catalyze the reaction.

Almost optimal stimulation of polylysine synthesis was obtained with oligo dT fractions containing 7–8 residues per chain. Thus, it seems possible that chemically synthesized oligodeoxynucleotides of known sequence may be useful in the determination of nucleotide sequence and polarity of RNA code-words and in the study of control mechanisms related to DNA-directed protein synthesis.

Summary.—In the presence of chemically synthesized oligodeoxythymidylate of chain length 6–14 and ATP, RNA polymerase catalyzes the synthesis of polyadenylate which, in cell-free *E. coli* extracts, directs the synthesis of polylysine. Thymus DNA and polyuridylate also have been used to direct polyadenylate, and then polylysine synthesis. The possible use of chemically synthesized DNA in studies pertaining to the genetic code is briefly discussed.

* The following abbreviations are used: poly d(AT), poly (deoxyadenylate-deoxythymidylate) copolymer; poly dG, polydeoxyguanylate; poly dC, polydeoxycytidylate; poly A, polyadenylate; poly U, polyuridylate; poly C, polycytidylate; oligo dT, oligodeoxythymidylate; pdT, deoxythymidine-5′-phosphate residues in oligo dT; pA, adenosine-5′-phosphate residues in poly A; AMP, adenosine-5′-phosphate; ATP, adenosine-5′-triphosphate; PP, pyrophosphate; subscript following oligonucleotides (example: oligo dT_{13-14}) refers to the number of nucleotide residues per molecule; tris, tris(hydroxymethyl) aminomethane; PEP, phosphoenol pyruvate, potassium salt; TCA, trichloroacetic acid; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis-2′-(5′-phenyloxazolyl) benzene.

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THE INDEPENDENCE OF THE CONTINUUM HYPOTHESIS

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This is the first of two notes in which we outline a proof of the fact that the Continuum Hypothesis cannot be derived from the other axioms of set theory, including the Axiom of Choice. Since Gödel[3] has shown that the Continuum Hypothesis is consistent with these axioms, the independence of the hypothesis is thus established. We shall work with the usual axioms for Zermelo-Fraenkel set theory,[2] and by Z-F we shall denote these axioms without the Axiom of Choice, (but with the Axiom of Regularity). By a model for Z-F we shall always mean a collection of actual sets with the usual ϵ-relation satisfying Z-F. We use the standard definitions[3] for the set of integers ω, ordinal, and cardinal numbers.

THEOREM 1. *There are models for Z-F in which the following occur:*

(1) *There is a set a, $a \subseteq \omega$ such that a is not constructible in the sense of reference 3, yet the Axiom of Choice and the Generalized Continuum Hypothesis both hold.*

(2) *The continuum (i.e., $\mathcal{P}(\omega)$ where $\mathcal{P}$ means power set) has no well-ordering.*

(3) *The Axiom of Choice holds, but $\aleph_1 \neq 2^{\aleph_0}$.*

(4) *The Axiom of Choice for countable pairs of elements in $\mathcal{P}(\mathcal{P}(\omega))$ fails.*

Only part 3 will be discussed in this paper. In parts 1 and 3 the universe is well-ordered by a single definable relation. Note that 4 implies that there is no simple ordering of $\mathcal{P}(\mathcal{P}(\omega))$. Since the Axiom of Constructibility implies the Generalized Continuum Hypothesis,[3] and the latter implies the Axiom of Choice,[5] Theorem 1 completely settles the question of the relative strength of these axioms.

Before giving details, we sketch the intuitive ideas involved. The starting point is the realization[1, 4] that no formula $\alpha(x)$ can be shown from the axioms of Z-F to have the property that the collection of all x satisfying it form a model for Z-F in which the Axiom of Constructibility ($V = L$,[3]) fails. Thus, to find such models, it seems natural to strengthen Z-F by postulating the existence of a set which is a