Chemically Synthesized Deoxypolynucleotides as Templates for Ribonucleic Acid Polymerase*

Arturo Falaschi, Julius Adler, and H. G. Khorana

From the Departments of Biochemistry and Genetics and the Institute for Enzyme Research, the University of Wisconsin, Madison 6, Wisconsin

(Received for publication, April 25, 1963)

The enzymatic synthesis of ribonucleic acid from the four ribonucleoside 5'-triphosphates in the presence of deoxyribonucleic acid has been documented in a number of laboratories (1-11). The reaction is catalyzed by an enzyme called RNA polymerase and the DNA determines the composition and the nucleic acid has been documented in a number of laboratories (16) was tested previously by Hurwitz et al. (7, 11) and ribonucleoside 5'-triphosphates in the presence of deoxyribonucleotides of known size and sequence have been used to study further the mechanism of action of RNA polymerase. The results reported herein show that deoxyoligonucleotides as small as the pentanucleotide dT₁ can serve as templates for the synthesis of ribopolyadenylate. The rate of the synthetic reaction increases with an increase in the size of the deoxypolythymidylate until a chain length of 14 is reached, and this polymer was actually more active than DNA. The product synthesized from deoxypolythymidylate of various sizes was invariably very much larger than the size of the template. Several lines of evidence showed that the enzyme initiated the synthesis of new chains, rather than causing esterification to the 3'-hydroxyl end of the deoxythymidylate. The rate of the synthetic reaction increases with an increase in the size of the deoxypolythymidylate until a chain length of 14 is reached, and this polymer was actually more active than DNA. The product synthesized from deoxypolythymidylate of various sizes was invariably very much larger than the size of the template. Several lines of evidence showed that the enzyme initiated the synthesis of new chains, rather than causing esterification to the 3'-hydroxyl end of the deoxythymidylate chains. Finally, in these simpler systems, the incorporation of the ribonucleoside 5'-triphosphates again followed the Watson-Crick base-pairing principle, although some exceptions were noted. A brief report of these findings has already appeared (17).

**EXPERIMENTAL PROCEDURE**

RNA polymerase was purified from *Escherichia coli* according to the procedure described by Chamberlin and Berg (10). *E. coli* phosphodiesterase (18) was a gift from Dr. I. R. Lehman. C¹⁴ Ribonucleoside 5'-triphosphates were purchased from Schwarz BioResearch, Inc.

Deoxyribopolynucleotides—Deoxyctydine, deoxyadenosine, and deoxyguanosine oligonucleotides were prepared by published procedures (19-21). The homologous deoxythymidine polynucleotides dT₁ to dT₁₄ were prepared as described previously (16, 22), and C¹⁴-labeled thymidine polynucleotides were prepared by Mr. W. J. Connors by adaptation (23) of these published procedures (16, 22).

Pure thymidine polynucleotides dT₁ to dT₁₄, and a fraction containing members higher than dT₁₄ were prepared from the 1M triethylammonium bicarbonate fraction obtained in an experiment described previously (Khorana and Vizsolyi, Table I, and Fig. I (16)). The latter fraction, as mentioned earlier (16), was a complex mixture of polynucleotides containing apparently a high proportion of oligonucleotides linked together by pyrophosphate bridges between the terminal 5'-phosphomonoester groups, with general structure

$$\text{O} \quad \text{pT(pT)}_{n-1} \text{pT}$$

The mixture (110 optical density units at 267 µm) was treated in dry pyridine (2 ml) with 0.5 ml of acetic anhydride for 3 days in order to selectively cleave the pyrophosphate bonds, and the reaction mixture was worked up as described earlier (22). Chromatography on a DEAE-cellulose (carboxylate form) column (50 x 1.2 cm) gave a series of peaks which were processed by the standard method developed earlier (16). As a result of cleavage of the pyrophosphate bonds, more than 50% of the ultraviolet-absorbing material was present as a mixture of oligonucleotides smaller than dT₁₄. The higher homologues were then applied to paper along with previously characterized dT₁₁ as marker. The dT₁₁, dT₁₂, and dT₁₄ traveled with progressively decreasing *Rₚ* values when chromatographed for 2 weeks in descending n-propyl alcohol-concentrated ammonium-water (55:10:35).

Acetylation of 3'-Hydroxyl End Groups in Pentadeoxyribonucleotides—An aqueous solution of ammonium salt of the polynucleotide (2 µmoles of thymidine) was passed through a column (1 x 2 cm) of Dowex 50 ion exchange resin (H⁺) and
the total effluent and washings evaporated after addition of pyridine (1 ml). To the residue was added triethylamine (0.05 ml) and pyridine (2 ml) and the solution was re-evaporated with vacuum from an oil pump. The residue was rendered anhydrous by repetition of evaporation after addition of dry pyridine. Finally, dry pyridine (0.5 ml) and acetic anhydride (0.2 ml) were added and the sealed reaction mixture kept in the dark at room temperature for 4 hours. Water (2 ml) was then added and the total solution kept for 2 hours at room temperature. It was then evaporated under reduced pressure to an oil which was extracted with dry ethyl ether several times. The insoluble polynucleotide material was obtained as a fine solid deposit on the wall of the round bottom flask. It was dissolved in water and the aqueous solution was lyophilized. The solid residue was made up to 0.2 ml with water.

Resistance of Polynucleotides Bearing Terminal 3'-O-Acetyl Groups to E. coli Phosphodiesterase—An exonuclease purified from E. coli has been shown by Lehman (18) to degrade deoxyribonucleotides in a stepwise manner from the end bearing a 3'-phosphoryl group. The reaction produces deoxyribonucleoside 3'-phosphates until the chain length is reduced to the dinucleotide (18). In a control experiment, 0.17 μmole of the tetranucleotide d-pTpTpTpT was incubated at 37° in a 0.2-ml incubation mixture in the presence of 0.02 ml of 1 M Tris buffer (pH 7.5), 0.02 ml of 0.1 M magnesium chloride, and 100 units (18) of enzyme. Degradation to d pT and d pTP was complete in under 2 hours as determined by paper chromatography of aliquots in descending ethyl alcohol-0.5 M ammonium acetate buffer, pH 8.5 (7.3, v/v). Incubation of the 3'-O-acetyl d-pTP(pTP)pTPT under identical conditions up to 4 hours showed complete resistance of the oligonucleotide to the enzyme. In a second experiment the use of a 3-fold higher concentration of the enzyme preparation under the above conditions showed likewise the absence of any degradation. On the other hand, the degradation of oligonucleotides bearing 3'-hydroxyl groups proceeded normally in the presence of the 3'-O-acetyl derivatives, showing that the latter were not inhibitory.

Assay of RNA Polymerase—The reaction mixture was exactly the same as that described by Chamberlin and Berg (10), except that synthetic deoxypolynucleotides usually replaced DNA. When DNA was used as primer, the C4-ribopolyribonucleotide was neutralized by adding slowly small amounts of dry Dowex 50 (H+) resin; after each addition, about 5 minutes with occasional stirring were allowed before the pH was measured. When the pH reached 7, 5 μl of 1 N NaOH were added in order to avoid any dephosphorylation which could occur if the pH became less than 7 during the subsequent manipulations. The supernatant liquid, combined with 0.1 N ammonium hydroxide washings of the resin, was concentrated by lyophilization and spotted on a strip, 2.5 cm x 57 cm, of DEAE-cellulose paper. Descending chromatography in 0.2 M ammonium formate was carried out for 6 hours until the front reached the end of the paper. This served to separate added (as markers) adenosine, adenosine 3'-phosphate, and adenosine 2'(3'),5'-diphosphate. The dried strip was cut at 1-cm intervals and these pieces were put into scintillation vials and counted as described above. The ratio of radioactivity in adenylic acid to adenosine or in adenylic acid to the adenosine diphosphate was considered the average size of the ribopolyribonucleotide.

**RESULTS**

Deoxypolythymidylylate as Template for Synthesis of Ribopolyadenylate

Effect of Chain Length on Rate of Synthesis—The initial rate of incorporation of adenylate varied with the size of the deoxypolythymidylylate at saturating concentrations of each polymer, as shown in Fig. 1.

With dT3 no activity was observed under the conditions used, and with dT4 there was occasional activity. But dT5 always brought about significant, although small, incorporation of adenylate. With further increase in size the effectiveness of the polymers then rose, at first slowly up to dT7, then with a big leap upward between lengths of 8 and 11. The activity reached
The experimental procedure is described under "Assay of RNA Polymerase." Table I shows that dT14 is actually more active for ribopolyadenylate synthesis than DNA is for the synthesis of RNA in the presence of all four nucleoside triphosphates.

Hurwitz et al. (7, 11) had already shown that the 1 M triethylammonium bicarbonate fraction referred to above is active for the synthesis of ribopolyadenylate. We now have confirmed the activity of the same 1 M fraction and have found it to be about 60% as active as dT14 (Table I). This lesser activity is probably due to the presence of less active polymers or inhibitors in the mixture. As mentioned above, the 1 M fraction contains polymers of various sizes larger than dT14 and also oligonucleotides linked together by pyrophosphate bridges. It seems very likely that such pyrophosphate compounds are inhibitors for the priming action of polynucleotides.

RNA polymerase will catalyze the synthesis of ribopolyadenylate when DNA is present together with ATP as the only nucleoside triphosphate (10). Denatured DNA is a preferred primer for this activity (24). Table I shows a confirmation of these results and a comparison of DNA with the activity of the 1 M fraction and dT14. For the synthesis of ribopolyadenylate dT14 is the most active polymer.

In order to be sure that the comparison of the results with polynucleotides of different sizes was meaningful, it was necessary to check that the primer did not undergo degradation during the incubation with RNA polymerase (see also below). C14-Labeled dT7 was used in the usual reaction mixture except that ATP was omitted. The mixture was incubated as usual and then put on a DEAE-cellulose carbonate column (0.6 x 27 cm) and eluted with a linear gradient of triethylammonium bicarbonate from 0 to 0.48 M (total volume, 300 ml). More than 99.7% of the radioactivity appeared as a single peak in the position corresponding to dT7. This ruled out the possibility of detectable breakdown of the primer by the enzyme preparation.

Effect of Chain Length on Saturating Concentration of Template—A saturating concentration was determined for each polymer that stimulated the synthesis of ribopolyadenylate in the experiment of Fig. 1. For dT7, dT11, and dT14 the results are plotted in Fig. 2 according to the method that Lineweaver and Burk have used for substrates (25). The polymer concentrations which gave half-maximal rates with dT7, dT11, and dT14 were found to be 50 x 10^-4, 20 x 10^-4, and 2 x 10^-4 M, respectively. Increasing the size of the polymer not only increases the maximal velocity of the reaction (as shown also by Fig. 1) but decreases very strikingly the concentration of polymer required for half-saturation. Apparently the affinity of the deoxypolythymidylate for the enzyme increases markedly with size between dT7 and dT14.

Size of Ribopolyadenylate Formed—The C14-ribopolyadenylate formed in the presence of deoxypolythymidylate of various sizes was hydrolyzed with sodium hydroxide to C14-adenosine, C14-adenosine 2'-3'-phosphate, and a C14-material that resembled adenosine 3',5'-phosphate but was not further characterized. The details are described under "Experimental Procedure." The ratio of radioactivity in adenylic acid to adenosine and in adenylic acid to the adenosine diphosphate was taken to be the average size of the ribopolyadenylate. Table II lists the results for the size of the products formed from dT7, dT8, dT9, dT11, and dT14; for smaller thymidine oligonucleotides too little product was available to provide significant results. The estimate of the chain length of the products is only approximate, owing to the inaccuracy of counting on DEAE paper the small amounts of radioactivity in the adenosine and in the adenosine diphosphate region. The most striking feature of the results is that the product is of a much larger size than the deoxypolythymidylate. No marked differences are apparent between the sizes of the products obtained with different sized deoxypolythymidylates.

Evidence for Noninvolvement of Terminal 3'-Hydroxyl Groups of Polythymidylate in Ribopolyadenylate Formation—During chromatography of the total alkaline hydrolysate of the ribopolyadenylate (see above), no significant amount of radioactivity

\[ \begin{align*}
\text{Table I} \\
\text{Efficiency of DNA and deoxypolythymidylate in stimulating RNA polymerase} \\
\text{The experimental procedure is described under "Assay of RNA Polymerase."} \\
\text{The results for the size of the products formed from dT7, dT8, dT9, dT11, and dT14; for smaller thymidine oligonucleotides too little product was available to provide significant results. The estimate of the chain length of the products is only approximate, owing to the inaccuracy of counting on DEAE paper the small amounts of radioactivity in the adenosine and in the adenosine diphosphate region. The most striking feature of the results is that the product is of a much larger size than the deoxypolythymidylate. No marked differences are apparent between the sizes of the products obtained with different sized deoxypolythymidylates.}
\end{align*} \]

\[ \begin{align*}
\text{Efficiency of DNA and deoxypolythymidylate in stimulating RNA polymerase} \\
\text{The experimental procedure is described under "Assay of RNA Polymerase."} \\
\text{The results for the size of the products formed from dT7, dT8, dT9, dT11, and dT14; for smaller thymidine oligonucleotides too little product was available to provide significant results. The estimate of the chain length of the products is only approximate, owing to the inaccuracy of counting on DEAE paper the small amounts of radioactivity in the adenosine and in the adenosine diphosphate region. The most striking feature of the results is that the product is of a much larger size than the deoxypolythymidylate. No marked differences are apparent between the sizes of the products obtained with different sized deoxypolythymidylates.}
\end{align*} \]

\[ \begin{align*}
\text{Efficiency of DNA and deoxypolythymidylate in stimulating RNA polymerase} \\
\text{The experimental procedure is described under "Assay of RNA Polymerase."} \\
\text{The results for the size of the products formed from dT7, dT8, dT9, dT11, and dT14; for smaller thymidine oligonucleotides too little product was available to provide significant results. The estimate of the chain length of the products is only approximate, owing to the inaccuracy of counting on DEAE paper the small amounts of radioactivity in the adenosine and in the adenosine diphosphate region. The most striking feature of the results is that the product is of a much larger size than the deoxypolythymidylate. No marked differences are apparent between the sizes of the products obtained with different sized deoxypolythymidylates.}
\end{align*} \]
remained at the origin of the chromatogram where the deoxyribo-
olymidylate remains adsorbed. This result indicated that the
ribopolyadenylate synthesis was not initiated by esterification of
the terminal 3'-hydroxyl group in deoxypolythymidylate to form
a phosphodiester linkage. Such a linkage would have been
resistant to alkaline hydrolysis and a product of the general
structure (Diagram I) would have resulted.

To test further whether the 3'-hydroxyl group is essential in
the synthesis of ribonucleotides, 3'-O-acetyl-dT11 was pre-
pared for testing as a primer. Table III (Lines 1 and 3) shows
that 3'-O-acetyl-dT11 is about as active as dT11. The concen-
tration of 3'-O-acetyl-dT11 required to half-saturate the enzyme
reaction depended on the size of the deoxypolycytidylate
and 3'-O-acetyl-dT11 was the active component in the 3'-O-acetyl-dT11 preparation. Furthermore, 3'-O-acetylthymidylate was found to be
stable when incubated with the RNA polymerase preparation;
this served to exclude any acylase activity.

In order to ensure that none of the unacylated dT11 was
present, the preparation of the acetylated polynucleotide was
preincubated with the E. coli phosphodiesterase. Acetylation of
the 3'-hydroxyl group in thymidine oligonucleotides confers
resistance toward this enzyme (see above). Table III (Lines 2
and 4) compares dT11 and 3'-O-acetyl-dT11 after treatment with
phosphodiesterase. This enzyme abolishes nearly all of the
activity of dT11 whereas most of the activity of 3'-O acetyl-dT11
remains resistant to phosphodiesterase.

It may be concluded that 3'-O-acetyl-dT11 is active for the
synthesis of ribopolyadenylate, and that a free 3'-hydroxyl group
end of deoxypolythymidylate is not essential for ribopolyadenylate
synthesis to occur. It follows that addition of adenylate to the
3'-hydroxyl end of deoxypolythymidylate is not a necessary part
of ribopolyadenylate synthesis.

Deoxypolythymidylate as template for synthesis
of ribopolyadenylate

The experimental procedure is described under "Assay of RNA
Polymerase."

In the deoxypolyguanylate series, only dG1 was tested. It
proved to be inactive for the incorporation of nucleotide from
CTP. This may be explained by the finding that even small
homologues of deoxypolyguanylate have a high tendency to
form aggregates of very large molecular weight (28). Chamber-
lin and Berg (27) have reported that deoxypolyguanylate pre-
pared from the deoxypolyolymidylate-deoxypolyguanylate
polymer (29), has been shown by Chamberlin and Berg to be
active for the synthesis of ribopolyguanylate.
incorporation of guanylate disappeared at a time when the
inhame conditions. As the enzyme preparation became older, the
no significant incorporation of CTP or UTP was observed in 10
0.65 mpmole of guanylate from GTP was incorporated under the
tive for the synthesis of ribopolyuridylate.
failed to show significant incorporation of nucleotides from UTP.
fraction that contained a mixture of homologues larger than d&
Poly dT, 1

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Nucleotide incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATP only</td>
</tr>
<tr>
<td>Poly dT, 1 M fraction</td>
<td>8.7</td>
</tr>
<tr>
<td>dC10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Poly dA, 1 M fraction</td>
<td>1.4</td>
</tr>
<tr>
<td>dC4</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Deoxypolyadenylate homologues of sizes up to dA4 and a 1 M fraction that contained a mixture of homologues larger than dA4 failed to show significant incorporation of nucleotides from UTP. Possibly, conditions other than those used so far might be effective for the synthesis of ribopolyuridylate.

Specificity of Incorporation of Nucleotides

In the presence of the 1 M fraction of deoxythymidylate, no significant incorporation of CTP or UTP was observed in 10 minutes, under conditions in which 8.7 mmoles of adenylate from ATP were incorporated (Table V, Line 1). Surprisingly, 0.65 m mole of guanlylate from GTP was incorporated under the same conditions. As the enzyme preparation became older, the incorporation of guanlylate disappeared at a time when the incorporation of adenylate had decreased by only 60%. Furth, Hurwitz, and Anders in one experiment (7) also noted a small incorporation of guanlylate.

Deoxypolyuridylate brought about specific incorporation of guanlylate (Table V, Line 2). With dC10, 0.69 m mole of nucleotide was incorporated from GTP but there was no significant incorporation of nucleotide from ATP, CTP, or UTP.

With the 1 M fraction of deoxypolyadenylate, the incorporation of 1.4 m moles of nucleotide from ATP was unexpectedly observed (Table V, Line 3); in this experiment there was no significant incorporation of nucleotide from UTP, CTP, or GTP.

Discussion

The present work has demonstrated that short chain deoxypolyadenylates serve as templates for the synthesis of ribopolyadenylates in the presence of RNA polymerase. The effectiveness of the homologous members in the reaction increases with an increase in chain length, the maximal rate in the case of the polythymidylate series being reached with dT14. It is noteworthy that the maximal rate here obtained was higher than the rate normally obtained when all the four ribonucleoside triphosphates and DNA are used. The saturating concentrations of the thymidine polyadenylates decreased with an increase in chain length and the enzyme showed high affinity even for the short chain length dT4.

A major point of interest established by the present work has been that the ribopolyadenylate synthesis does not begin by adding nucleotides to the terminal 3'-hydroxyl group of the deoxyribonucleotide. This conclusion is based primarily on two findings: alkaline hydrolysis of the ribopolyadenylate product leaves no detectable adenylate in the deoxythymidylate, and dT14 carrying a 3'-O-acetyl group is still effective in the synthesis of ribopolyadenylate. Throughout this paper we have referred to the deoxypolyadenylates as "templates" for RNA polymerase rather than "primers" because the synthesis of the product involves the formation of new complementary chains instead of elongation of chains. Since addition to the end of the deoxypolyadenylate is not necessary, it may be that replication can begin anywhere along the template; this has significance at the biological level, for it allows the synthesis of messenger RNA from any one of the genes in a DNA molecule without requiring synthesis from all.

Although in the present work the Watson-Crick type of base-pairing was ordinarily observed, the slight incorporation of deoxyguanlylate in the presence of thymidine polyadenylates was noted, an observation which has also been made previously (7). A further noteworthy exception, which merits further study, was the formation of ribopolyadenylate when deoxyribopolyadenylates were used as templates. The reaction resembles the previously documented ribopolyadenylate-primed synthesis of ribopolyadenylate (29), and its biological significance remains unknown. Under the conditions tested no polyuridylicate synthesis occurred when deoxyadenylate was used as template. The failure is perhaps due to the lack of appropriate conditions, since the polyrriboadenylate-dependent polyuridylicate synthesis has already been found to be very sensitive to temperature and to the presence of a critical concentration of manganous ions (30).

RNA polymerase brings about the synthesis of ribopolyadenylate when DNA is present and ATP is the only substrate (10, 24). The reaction proceeds best when denatured DNA is used (24). The size of the ribopolyadenylate formed has been estimated to be in the range of 60 to 70 (10) and 400 (24). To explain that the expected short run of thymidylicates in DNA could bring about the synthesis of much larger sized ribopolyadenylate, Chamberlin and Berg (10) postulated a "slippage" mechanism whereby a run of -iLIP residues would slip along the DNA, but not necessarily. Such a mechanism would not be surprising in view of the fact that a 3'-O-acetyl group is still effective in the synthesis of ribopolyadenylate. Throughout this paper we have referred to the deoxypolyadenylates as "templates" for RNA polymerase rather than "primers" because the synthesis of the product involves the formation of new complementary chains instead of elongation of chains. Since addition to the end of the deoxypolyadenylate is not necessary, it may be that replication can begin anywhere along the template; this has significance at the biological level, for it allows the synthesis of messenger RNA from any one of the genes in a DNA molecule without requiring synthesis from all.

Although in the present work the Watson-Crick type of base-pairing was ordinarily observed, the slight incorporation of deoxyguanlylate in the presence of thymidine polyadenylates was noted, an observation which has also been made previously (7). A further noteworthy exception, which merits further study, was the formation of ribopolyadenylate when deoxyribopolyadenylates were used as templates. The reaction resembles the previously documented ribopolyadenylate-primed synthesis of ribopolyadenylate (29), and its biological significance remains unknown. Under the conditions tested no polyuridylicate synthesis occurred when deoxyadenylate was used as template. The failure is perhaps due to the lack of appropriate conditions, since the polyrriboadenylate-dependent polyuridylicate synthesis has already been found to be very sensitive to temperature and to the presence of a critical concentration of manganous ions (30).

RNA polymerase brings about the synthesis of ribopolyadenylate when DNA is present and ATP is the only substrate (10, 24). The reaction proceeds best when denatured DNA is used (24). The size of the ribopolyadenylate formed has been estimated to be in the range of 60 to 70 (10) and 400 (24). To explain that the expected short run of thymidylicates in DNA could bring about the synthesis of much larger sized ribopolyadenylate, Chamberlin and Berg (10) postulated a "slippage" mechanism whereby a run of -iLIP residues would slip along the sequence of thymidylicates, leading to an elongation of the polyadenylate chain. The present work indicates that it would take a run of only five to seven thymidylicates in DNA in order to synthesize ribopolyadenylate of a much larger size.

All evidence points to the involvement of a single enzyme for the DNA-dependent synthesis of RNA and of ribopolyadenylate.
Since the synthesis of ribopolynucleotides observed here is so similar to the DNA-dependent polynucleotide synthesis, and since the same purified enzyme (10) was used here, it seems most probable that the syntheses studied in this work are due actually to RNA polymerase rather than some contaminating enzyme.

The assay used in the present work involved separation of the product from the unreacted labeled nucleoside triphosphate by anion exchange chromatography on a DEAE-cellulose paper. Oligonucleotides as short as the tetranucleotide could be separated from the unreacted labeled nucleoside triphosphate by this assay. This assay was developed to detect low molecular weight polynucleotides, which could be missed by the usual assay (10) on anion exchange paper. The product formed from the unreacted labeled nucleoside triphosphate by this assay has in each case an average chain length of 50 to 100. The synthesis of ribopolyadenylate is noted.

Summary

Short chain thymidine polynucleotides serve as templates for the synthesis of ribopolyadenylate in the presence of RNA polymerase. The effectiveness as template increases markedly with activity whereas the maximal activity is reached with tetradecanucleotide, the latter being more active for ribopolyadenylate synthesis than thymus DNB is for ribopolyadenylate or RNA syntheses. Oligonucleotides as short as the tetranucleotide could be separated from the unreacted labeled nucleoside triphosphate by anion exchange chromatography on a DEAE-cellulose paper. Oligonucleotides as short as the tetranucleotide could be separated from the unreacted labeled nucleoside triphosphate by this assay. This assay was developed to detect low molecular weight polynucleotides, which could be missed by the usual assay (10) on anion exchange paper. The product formed from the unreacted labeled nucleoside triphosphate by this assay has in each case an average chain length of 50 to 100. The synthesis of ribopolyadenylate is noted.

Acknowledgment—We wish to acknowledge the technical assistance of Miss Rachel Gettle.

REFERENCES