RNA AS A TEMPLATE WITH E. COLI DNA POLYMERASE

Liebe F. Cavalieri and Elizabeth Carroll

Division of Genetics, Sloan-Kettering Institute for Cancer Research
New York, N.Y. 10021

Received October 19, 1970

Summary

5S and ribosomal RNA's from rat liver can serve as primers for DNA synthesis, using DNA polymerase from E. coli. The first product formed behaves like a DNA-RNA hybrid, while later synthesis leads to a DNA-like product.

Some years ago, we presented the first published evidence that the direction of information transfer can be reversed to RNA $\longrightarrow$ DNA; we showed at that time that the synthetic RNA poly(A+U) can serve as a template for the synthesis of the synthetic DNA, poly (dA+T), using DNA polymerase from E. coli (1). In the present report, we show that natural ribonucleic acids, specifically, ribosomal and 5S RNA's from rat liver, can also function in the E. coli DNA polymerase system. The biological significance of this reversal of information flow remains to be established. Two interesting reports (2,3) have recently shown that the RNA of certain animal viruses also serves as a template for the DNA polymerase associated with those viruses. In view of our results, the possibility must be considered that virus-associated DNA polymerase may be a host component included within the virion.

Requirements for the E. coli DNA Polymerase-RNA Template Reaction.

The incorporation of radioactivity from ^{14}C-TTP into an acid-insoluble product occurs under the conditions of the standard DNA polymerase assay (Table I). All four deoxyribonucleoside-5'-triphosphates are required. Neither uridine-5'-triphosphate nor adenosine-5'-triphosphate can substitute for TTP or dATP, in the presence of either Mg^{++} or Mn^{++}. There was no incorporation in the absence of RNA (or DNA) template. Incorporation with ribosomal or 5S RNA in one hour was 10-20% of that obtained with DNA as the

template. This relative efficiency was the same with highly purified DNA polymerase (7) as with the partially-purified polymerase used in the experiments reported here. The DNA polymerase did not show RNA polymerase activity in the presence of DNA and ribonucleoside-5'-triphosphates.

TABLE I

Requirements of the E. coli DNA Polymerase-RNA Template Reaction

	Reaction System with 5S RNA	picomoles of ^{14}C-TMP incorporated *
Complete:	TTP, dATP, dGTP, dCTP, $MgCl_2$, polymerase	200
Without:	dATP, dGTP, dCTP	<10
"	dGTP, dCTP	<10
"	dCTP	<10
"	TTP; plus UTP	<10
"	TTP, $MgCl_2$; plus UTP, $MnCl_2$	<10
"	dATP; plus ATP	<10
"	dATP, $MgCl_2$; plus ATP, $MnCl_2$	<10
	Reaction System with 18 + 28S Ribosomal RNA	
Complete:	TTP, dATP, dGTP, dCTP, $MgCl_2$, polymerase	145
Without:	dCTP	<10
Complete, pretreated with RNase for 1 hr.		<10
" " " DNase " " "		190
" " " " " 2 hrs.		220

* Calculated from cpm, not corrected for counting efficiency. The reaction mixture contained, in 0.5 ml., 25 μmoles Tris, pH 7.8 at 25°, 1 μmole each of magnesium chloride and β-mercaptoethanol, 10 μg of RNA, 75 μg of DNA polymerase [step 4 (4)], 4.5 mμmoles each of dATP, dGTP, dCTP and 7 mμmoles of ^{14}C-TTP, specific activity 17 mc/mMole (Schwarz BioResearch). The reaction mixture was incubated for 1 hr at 37°, chilled in ice-water, and mixed with 0.1 ml of 0.07% albumin followed by 1 ml 10% TCA. The mixture was washed on HA Millipore, dried on planchets and counted. The acid-precipitable radioactivity of an unincubated reaction mixture (< 5%) was subtracted from each value. Equivalent quantities of UTP, ATP and $MnCl_2$ were substituted for TTP, dATP and $MgCl_2$, respectively, wherever indicated.

The 5S RNA and the 18 + 28S mixture of ribosomal RNA's from rat liver were kindly supplied by Dr. Mary L. Petermann and A. Pavlovec. The ribosomal RNA was prepared according to a published procedure (5). The 5S RNA was purified using G-100 Sephadex filtration (6) and showed a single band in acrylamide gel electrophoresis.

Pretreatment of ribosomal RNA with RNase (Worthington, chromatographically pure beef pancreas RNase A) was carried out for 1 hr at 37° at a concentration of 50γ RNase/ml in 0.05M Tris, pH 7.8. Pretreatment of ribosomal RNA with DNase (beef pancreas, Worthington, 2 x recrystallized) was carried out for 1 hr and for 2 hrs., at a concentration of 10γ/ml. in 0.05M Tris, pH 7.8, 37°. Then the other components of the system were added and synthesis was allowed to proceed for an hour. The nucleases remained present during the synthetic period.

Evidence that RNA is the Template.

When the ribosomal RNA was pretreated with beef pancreas RNase for one hour prior to incubation with DNA polymerase, virtually all template activity was lost (Table I). When the RNase treatment took place in 2m$\underline{M}$ $MgCl_2$, 30% of the template activity remained, a behavior which is characteristic of ribosomal RNA. It is possible, though unlikely, that the products of RNase digestion could be inhibiting the template activity of a small amount of contaminating DNA in the RNA. However, preliminary experiments indicate that this is not the case. Moreover, beef pancreas DNase did not reduce the template activity of the RNA after one or two hours of pretreatment. We, therefore, conclude that the template activity is due to RNA.

It is most probable that single-stranded, rather than helical regions of the RNA constitute the template since brief heating (5 minutes at 60°) of the ribosomal RNA just prior to the reaction, increased the incorporation of TTP by 3-fold in 1 hour.

Nature of the Product Formed.

All four deoxyribonucleoside triphosphates are required in the reaction, and we, therefore, deduce that the synthesis of a polynucleotide chain containing all four nucleotides, rather than simple end addition, is taking place. The ratios of the nucleotides incorporated are given in Table II, where it can be seen that the product of both the ribosomal and 5S RNA's is rich in A+T. The G-C incorporation was not determined with 5S RNA. It is unlikely that the A-T-rich regions arise by a slippage mechanism (i.e. by repeatedly copying an A-U region in the RNA), since synthesis virtually stops in the absence of the other triphosphates. However, it is interesting that Temin and Mizutani (3) find C and G partially dispensible in the reaction using viral RNA and the included DNA polymerase.

To learn whether the first product of the reaction is an RNA-DNA hybrid, we carried out experiments I and II in Table II. In both cases, the synthetic reaction was terminated after one hour by heating to destroy the

TABLE II

Nature of the Products Formed with RNA Templates

	18 + 28S Ribosomal RNA as the Template	picomoles ^{14}C-nucleotide incorporated *
	^{14}C-dATP, TTP, dGTP, dCTP	285
	^{14}C-TTP, dATP, dGTP, dCTP	145
	^{14}C-dGTP, dATP, TTP, dCTP	13.4
	^{14}C-dCTP, dATP, TTP, dGTP	11.7
	^{14}C-TTP, dATP, dGTP, dCTP, 1 hr synthesis	145
	followed by:	
I	RNase treatment (1 hr., 10γ/ml., 0.3M NaCl)	114
I	then DNase treatment (1 hr., 10γ/ml.)	170
	or:	
II	RNase treatment (1 hr., 50γ/ml., no salt)	100
II	then DNase treatment (1 hr., 10γ/ml.)	25
	5S RNA as the Template	
	^{14}C-dATP, TTP, dGTP, dCTP	400
	^{14}C-TTP, dATP, dGTP, dCTP	200

* Calculated from cpm, not corrected for counting efficiency. The synthetic reactions were carried out and the incorporation measured as described in Table I. In each case, the unlabelled triphosphates were present in the amount of 4.5 mμmoles each and the labelled triphosphate in the amount of 7 mμmoles.

Experiments I and II: After one hour, the reaction mixture containing ^{14}C-TTP was heated for 5 minutes at 60° to terminate the reaction. RNase was then added and the mixture incubated at 37° for one hour, followed by the addition of DNase (10γ/ml.) and another hour of incubation. In experiment I, the RNase concentration was 10γ/ml. and NaCl was added along with the RNase to make the reaction mixture 0.3M. In experiment II, the RNase concentration was 50γ/ml. and no NaCl was added.

polymerase; RNase was then added to the reaction mixture for one hour, followed by DNase for one hour. Under the conditions of experiment I (low RNase concentration, high salt), an RNA-DNA hybrid would not be susceptible to attack by the RNase (8); subsequent treatment with DNase should also leave the hybrid intact. In experiment II, at a high RNase concentration and low salt concentration, the RNase should attack both the remaining single-stranded RNA and any RNA-DNA hybrid present, leaving the DNA from such a hybrid susceptible to DNase. Since there was loss of product after

DNase treatment only in experiment II, we conclude that at least some of the product present after one hour of synthesis is in the form of an RNA-DNA hybrid.

In a second round of synthesis, one would expect some free DNA to be formed. If this is so, then some of the product should eventually become sensitive to DNase without prior RNase treatment. This, in fact, was shown to be the case (Table III). Incorporation of TMP increases during two hours of incubation in the standard assay, followed by a decrease in the third hour. The decrease is due to deoxyribonucleases known to be present in the DNA polymerase preparation. This was corroborated by increasing the polymerase concentration 5-fold, which results in maximum incorporation after one hour, followed by a rapid decrease in the second. Moreover, when DNase was added after one hour of synthesis, incorporation continued to increase during the next hour, but the increase was less than would have occurred if no DNase were present. These results (Table III) are consistent with the

TABLE III

Kinetics of Synthesis in the Presence and Absence of DNase, using 18 + 28S Ribosomal RNA as Template

Length of Incubation in Complete System	picomoles ^{14}C-TMP incorporated *
1 hr.	145
2 hrs.	1400
3 hrs.	580
2 hrs., with DNase present during second hr.	435
Length of Incubation in Complete System at 5-fold higher DNA polymerase concentration	
1 hr.	435
2 hrs.	120

* Calculated from cpm, not corrected for counting efficiency. The reaction was carried out as described in Table I, for the lengths of time indicated. The DNase (10γ/ml.) was added directly to the reaction mixture after 1 hr. incubation. The higher polymerase concentration is 5 times that in Table I. Measurement of incorporation was carried out as described in Table I. The amount of synthesis at 2 hours corresponds to about 60% or more of the amount of RNA present.

suggestion than an RNA-DNA hybrid, not susceptible to DNase, is first formed and then serves as a template for DNA synthesis, the latter product being susceptible to DNase.

In summary, we have shown that 5S and ribosomal RNA's from rat liver can serve as templates or primers for DNA synthesis, using DNA polymerase from E. coli, thus reversing the normal direction of information flow. The fact that this bacterial enzyme can function with synthetic polyribonucleotides as well as mammalian RNA's implies that this in vitro reaction is of a general nature. The analogous results of Baltimore (2) and Temin and Mizutani (3), as well as those of others (9,10,11), probably have significance in the replication of viruses, but is has not been ruled out that they are observing an adventiously associated host DNA polymerase.

The authors wish to thank Dr. Mary L. Petermann and A. Pavlovec for generous gifts of 5S and ribosomal RNA's. This work was supported in part by an NCI grant CA 08748 and an Atomic Energy Commission contract AT(30-1)-910.

REFERENCES

1. Lee-Huang, S. and Cavalieri, L.F. Proc. U.S. Nat'l. Acad. Sci. 50: 1116 (1963).
2. Baltimore, D. Nature 226: 1209 (1970).
3. Temin, H. and Mizutani, S. ibid 226: 1211 (1970).
4. Cavalieri, L.F. and Carroll, E. Proc. U.S. Nat'l. Acad. Sci. 59: 951 (1968).
5. Petermann, M.L. and Pavlovec, A. J. Biol. Chem. 238: 3717 (1963).
6. Christman, J.K. and Goldstein, J. Biochim. Biophys. Acta 179: 280 (1969).
7. Cavalieri, L.F. and Carroll, E. Proc. U.S. Nat'l. Acad. Sci. 67: 0000 (1970).
8. Gillespie, D. in Methods in Enzymology XII (B) p. 641, Eds. L. Grossman and K. Moldave, Academic Press, New York, London, 1968.
9. Spiegelman, S., Burny, A., Das, M.R., Keydar, J., Schlom, J., Travnicek, M. and Watson, K. Nature 227: 1029 (1970).
10. Spiegelman, S., Burny, A., Das, M.R., Keydar, J., Schlom, J., Travnicek, M. and Watson, K. Nature 227: 563 (1970).
11. Rokutanda, M., Rokutanda, H., Green, M., Fujinaga, K., Ray, R.K. and Gurgo, C. Nature 227: 1026 (1970).