DNA AND RNA FROM AVIAN MYELOBLASTOSIS VIRUS AS TEMPLATES FOR VIRAL DNA POLYMERASE*

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1. Introduction

DNA polymerase activity in RNA tumour viruses has been described as RNA-dependent DNA polymerase which transcribes the viral RNA genome into DNA [1–5]. DNA synthesis by detergent-lysed virus particles is sensitive to ribonuclease, but in some studies was not completely depressed by ribonuclease [1, 4, 6]. DNA-dependent DNA polymerase activity has also been detected in RNA tumor virus particles by stimulation of DNA synthesis with a variety of exogenous DNA templates [7–11]. The recent report that avian myeloblastosis virus (AMV) contains DNA as well as RNA [9] suggested the possibility that both viral DNA and viral RNA may function as endogenous templates for DNA synthesis.

We have examined this question directly by isolating the 7 S DNA from AMV particles. The response of DNA polymerase from AMV to added viral DNA as template for DNA synthesis was compared to the template activity of 64 S RNA from AMV.

Our findings show that DNA isolated from purified tumor virus is a more effective template for DNA synthesis than the viral RNA. The results in this communication strongly support the proposition that 7 S viral DNA contributes to synthesis of DNA product in the endogenous reaction where both viral DNA and RNA are present.

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2. Methods and materials

AMV particles were harvested from the plasma of infected, leukemic chicks and purified as previously described [11]. Virus titers were estimated from ATPase activity [12].

Nucleic acids from AMV particles were extracted by a sodium dodecyl sulfate–phenol procedure [13]. High molecular weight RNA was obtained initially by salt fractionation (2 M NaCl, 0° for 1 hr) and 64 S RNA further purified by sedimentation velocity in glycerol gradients. 7 S DNA was obtained from the low molecular weight nucleic acid fraction by preparative polyacrylamide gel electrophoresis [14]. The 7 S DNA appeared as a sharp band well separated from the 4 S RNA (Deeney, unpublished observation) and was further characterized by its resistance to ribonuclease and alkaline hydrolysis, and by its buoyant density in CsCl (1.72 g/cm^3).

DNA polymerase was prepared from AMV particles as before [11] by a modification of the procedure of Temin. Detergent solubilization of virus particles was allowed to proceed at 0° for at least 12 hr prior to assay. Enzyme assays were 0.10 ml in volume and 0.04 M Tris-HCl (pH 8.0), 0.05 M KCl, 0.06 M $MgCl_2$, 3 mM reduced glutathione, 0.2 mM in unlabelled deoxyribonucleoside triphosphates and contained 1 μCi tritiated deoxyribonucleoside triphosphate (^{3}H-TTP, Schwarz BioResearch, 12.4 Ci/mmole; or ^{3}H-dGTP, Amersham/Searle, 9.1 Ci/mmole). Virus particles and templates were added as indicated below. All incubations were carried out at 37°, and the radioactive DNA product determined as previously described [11].

3. Results

Exogenous viral nucleic acid templates stimulated DNA synthesis above the level of the endogenous reaction as shown in fig. 1. Also shown is the effect of ribonuclease on DNA synthesis under each reaction condition. DNA synthesis in the presence of 7 S DNA was not appreciably affected by ribonuclease treatment. However, ribonuclease treatment reduced DNA synthesis to the same level in the endogenous reaction as in the presence of exogenous 64 S RNA, but did not completely depress product synthesis in either instance.

Kinetics of the incorporation of both thymidine and deoxyguanosine into DNA product by AMV–DNA polymerase were examined in assays containing (a) added 7 S DNA, (b) added 64 S RNA, and (c) no added template, and the results are shown in fig. 2. The ratio of thymidine to deoxyguanosine incorporated into product DNA was approximately 1:1 in the endogenous reaction and approximately 1.3:1 in the reac-

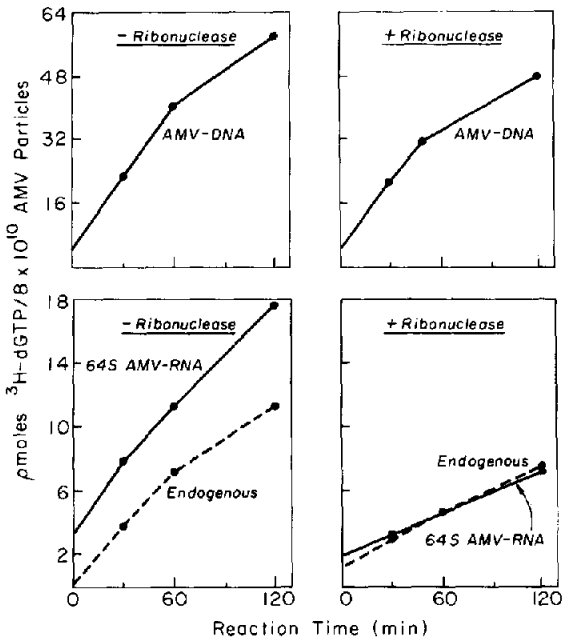

Fig. 1. The effect of ribonuclease on DNA synthesis by DNA polymerase from AMV. Assays were performed as described in Methods. 7 S DNA was added at a level of 0.4 μg per assay and 64 S RNA was added at a level of 3 μg per assay. Specific activity of ^{3}H-dGTP was 1000 cpm/pmole. Ribonuclease was added to a final concentration of 200 μg/ml of reaction volume.

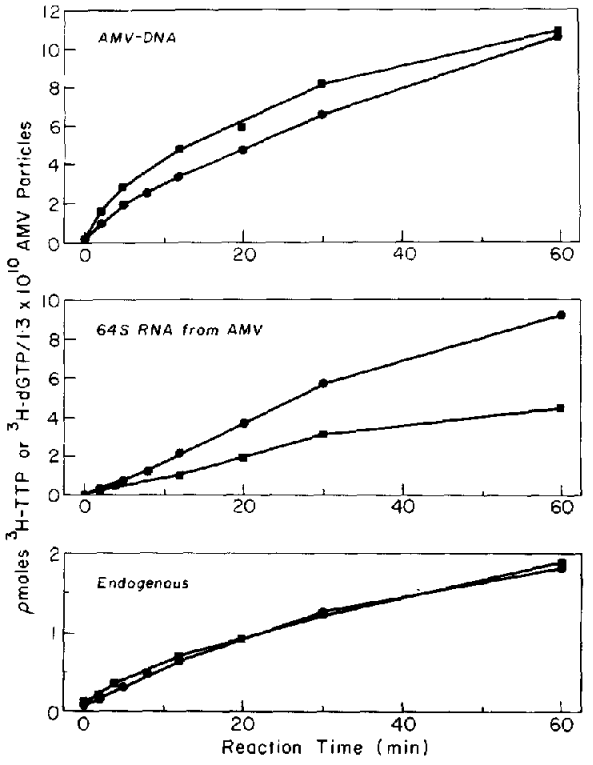

Fig. 2. Kinetics of incorporation of thymidine and guanosine into DNA polymerase from AMV. Assays (0.1 ml) contained 1.3×10^{11} AMV particles and were otherwise as described in Methods. 7 S DNA was added at a level of 5 μg per ml and 64 S RNA was added at a level of 50 μg per ml. At the indicated times 0.10 ml of the incubation mixture was removed for determination of radioactivity as described in Methods. Specific activity of ^{3}H-dGTP (●—●) and ^{3}H-TTP (■—■) was 1000 cpm/pmole.

Table 1
Apparent maximum velocities of DNA synthesis stimulated by AMV–RNA and AMV–DNA.

Template	V_{app}*
64 S AMV–RNA	7
7 S AMV–DNA	40

* V_{app} is expressed in units of pmoles dGMP/10^{10} AMV virus/30 min. Assays were performed as described in Methods using DNA polymerase from 1×10^{10} virus in each assay.

tion containing added AMV–DNA. Conversely, the T:dG ratio of product synthesized in the presence of 64 S RNA from AMV was approximately 1:2.

The difference in the rates of incorporation of the two deoxyribonucleotides into product under the three assay conditions indicated that the enzyme activity in the endogenous reaction was not instructed singly by either the viral DNA or viral RNA. Of course these results could be explained by a participation of both viral templates in the endogenous reaction or by only portions of the endogenous nucleic acid being available for template function.

A careful comparison of the response of AMV–DNA polymerase to each viral nucleic acid was made. Template saturation studies were performed with each viral nucleic acid and a Lineweaver-Burke plot constructed to accurately determine the velocity of the polymerase reaction under conditions of primer saturation [15]. Although it is difficult to rigorously interpret kinetic constants for the complicated polymerase reaction, the apparent maximum velocities which arose from Lineweaver-Burke plots under identical assay conditions indicated the viral DNA stimulated the DNA polymerase to a synthesis rate 6-fold higher than did the 64 S RNA from the virion (table 1).

4. Discussion

We have observed that some DNA synthesis proceeds in the endogenous reaction of AMV–DNA polymerase in the presence of a high level of ribonuclease. If this were due solely to the ability of the polymerase to use fragmented viral RNA as template, or de novo synthesized product DNA as template, as has been suggested [6], then presumably adding a large excess of 64 S viral RNA to an assay would increase the level of DNA synthesis in the presence of ribonuclease. We did not find this to be the case. The level of ribonuclease-resistant DNA synthesis was the same in the endogenous reaction as in the reaction containing an excess of 64 S RNA. The results show that the endogenous synthesis gives a DNA product having equal amounts of guanosine and thymidine. This equivalence of incorporation of the two deoxyribonucleotides was not observed using either of the nucleic acid templates isolated from the virus. These findings have 3 possible interpretations: (1) the template in the enzyme preparation is partially destroyed, (2) part of the template is unavailable for copy in the "native" state, or (3) DNA products are made by synthesis from both templates available in the virus.

Our results demonstrated RNA-directed DNA synthesis by the viral DNA polymerase, as already shown by many investigators, but in addition DNA polymerase activity was stimulated to a greater extent by DNA from the purified virus.

The function of the viral DNA and its template activity is not presently known. More rigorous analyses of the DNA products synthesized from each of the viral nucleic acid templates are in progress for comparison with the DNA product synthesized during the endogenous reaction. Studies of this nature are necessary to determine what nucleic acid synthesis is directed by the tumor viruses inside the cells after infection.

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References

[1] H.M. Temin and S. Mizutani, Nature 226 (1970) 1211.
[2] D. Baltimore, Nature 226 (1970) 1209.
[3] S. Spiegelman, A. Burny, M.R. Das, J. Keydar, J. Schlom, M. Travnicek and K. Watson, Nature 227 (1970) 563.
[4] M. Green, M. Rokutanda, K. Fujinaga, R.K. Ray, H. Rokutanda and C. Gurgo, Proc. Natl. Acad. Sci. U.S. 67 (1970) 385.
[5] E. Scolnick, E. Rands, S.A. Aaronson and G.J. Todaro, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1789.
[6] J.P. McDonnell, A.C. Garapin, W.E. Levinson, N. Quintrell, L. Fanshier and J.M. Bishop, Nature 228 (1970) 433.
[7] S. Spiegelman, A. Burny, M.R. Das, J. Keydar, J. Schlom, M. Travnicek and K. Watson, Nature 227 (1970) 1029.
[8] S. Mizutani, D. Boettiger and H.M. Temin, Nature 228 (1970) 424.
[9] J. Riman and G.S. Beaudreau, Nature 228 (1970) 427.
[10] K. Fujinaga, J.T. Parsons, J.W. Beard, D. Beard and M. Green, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1432.
[11] G.H. Weber, A.A. Kiessling and G.S. Beaudreau, J. Virol. 7 (1971) 214.

[12] G.S. Beaudreau and C. Becker, J. Nat. Cancer Insit. 20 (1958) 339.
[13] J.W. Carnegie, A.O'C. Deeney, K.C. Olson and G.S. Beaudreau, Biochim. Biophys. Acta 190 (1969) 274.
[14] J.L. Hodnett and H. Busch, J. of Biol. Chem. 243 (1968) 6334.
[15] A.D. Kiessling, thesis, Oregon State University (1971) 93.