POLY(2'-O-METHYLCYTIDYLATE)·OLIGODEOXYGUANYLATE, A TEMPLATE-PRIMER SPECIFIC FOR REVERSE TRANSCRIPTASE, IS NOT UTILIZED BY HELA CELL γ DNA POLYMERASES

Gary F. Gerard
Institute for Molecular Virology
St. Louis University School of Medicine
St. Louis, Missouri 63110

Received February 10,1975

Summary: The ability of γ DNA polymerase activities purified from the cytoplasm and nucleus of HeLa cells (Spadari, S. and Weissbach, A. (1974) J. Biol. Chem. 249, 5809) to copy poly(Cm)·oligo(dG) was tested, and compared with the template activity of poly(C)·oligo(dG). The major HeLa cell nuclear γ DNA polymerase effectively copied poly(C)·oligo(dG), while none of the γ DNA polymerase activities purified from HeLa cells were capable of utilizing poly(Cm)·oligo(dG) as template. These results support the contention that poly(Cm)·oligo(dG), but not poly(C)·oligo(dG), is a specific template-primer for RNA tumor virus RNA-directed DNA polymerases.

Poly(2'-O-methylcytidylate)·oligodeoxyguanylate [poly(Cm)·oligo(dG)] is an effective template-primer for every RNA tumor virus RNA-directed DNA polymerase (reverse transcriptase) thus far tested, including those of primate, feline, murine, and avian origin (1; unpublished results). Poly(Cm)·oligo(dG) appears to be specific for reverse transcriptase, since the three major DNA polymerases in mouse 3T6 cells do not effectively utilize poly(Cm)·oligo(dG) as template (1). A second template-primer combination, polycytidylate·oligodeoxyguanylate [poly(C)·oligo(dG)], was also thought to be specific for viral reverse transcriptase (2-5). Recently, Spadari and Weissbach (6) were able to isolate similar γ DNA polymerase* activities from the cytoplasm and nucleus of HeLa cells that after extensive purification were capable of effectively copying poly(C)·oligo(dG) (6). This finding introduces some doubt as to the specificity of poly(Cm)·oligo(dG) for reverse transcriptase.

But nucleic acid polymerizing enzymes do not necessarily respond with the same specificity to 2'-O-methylated polyribonucleotides as to polyribonucleotides (1,7), and therefore poly(Cm) might not serve as template for an enzyme that copies poly(C). Consequently, I have tested the ability

*Gamma (γ) DNA polymerases, formerly called R-DNA polymerases, are a class of enzymes found in a variety of eukaryotic cells that efficiently copy the synthetic RNA template, poly(A), in poly(A)·$(dT)_{12-18}$ (2,4), but apparently do not copy natural RNA templates as do reverse transcriptases of RNA tumor viruses (2,4,6).

of γ DNA polymerase activities purified from HeLa cells (6) to use poly(Cm)·oligo(dG) as template-primer. I report here that the major nuclear γ DNA polymerase can copy poly(C)·oligo(dG), in agreement with the results of Spadari and Weissbach (6), while none of the γ DNA polymerases from HeLa cells effectively copy poly(Cm)·oligo(dG).

Materials and Methods

Reagents. Commercially available biochemicals were obtained from sources previously described (1). Poly(Cm), with a sedimentation coefficient of 4S (7), was provided by Dr. F. Rottman.

Growth of cells. HeLa S-3 cells were grown in suspension cultures at 37°C in Joklik's modified MEM medium supplemented with 5% horse serum. Cells were harvested by centrifugation, washed with phosphate-buffered saline, and frozen at -70°C.

Purification of HeLa cell cytoplasmic and nuclear γ DNA polymerases. Cells (24 gm) were fractionated into nuclear and cytoplasmic fractions, and the γ DNA polymerases were purified from these fractions as described by Spadari and Weissbach (6) (see Table 1 and 2), with one exception. Hydroxylapatite chromatography was performed with Hypatite C (Clarkson Chemical Co., Inc.) rather than Bio-Gel HT hydroxylapatite (Bio-Rad) to improve column flow rates.

Assay of DNA polymerase activity. The assays for γ DNA polymerase activity were carried out essentially as described (6), with slight modifications. Reaction mixtures (100 µl) contained 50 µg/ml bovine serum albumin, 50 mM Tris-HCl (pH 7.8), 0.5 mM $MnCl_2$, 2 mM dithiothreitol, 50 µM [^{3}H]TTP (1200 cpm/pmole), 50 mM KCl, 50 µM poly(A), and 20 µM $(dT)_{12-18}$. Other synthetic template-primer combinations were also used at 50 µM and 20 µM, respectively. In reaction mixtures with poly(C) and poly(Cm) templates, 30 µM [^{3}H]dGTP (800 cpm/pmole) was substituted for [^{3}H]TTP. The $MnCl_2$ concentration was 0.2 mM with poly(Cm)·$(dG)_{12-18}$. All incubations were carried out at 30°C for 30 min.

Results

Purification and identification of γ DNA polymerases. The procedures (6) used to purify the γ DNA polymerase activities from the cytoplasm and nucleus of HeLa cells and the results obtained are summarized in Tables 1 and 2. The overall recoveries of enzyme activity after hydroxylapatite chromatography (Tables 1 and 2) were similar to those observed previously (6). Spadari and Weissbach (6) were able to isolate two major γ DNA polymerase activities from the cytoplasm, designated by them as Peak I and

Table 1. Purification of HeLa Cell Cytoplasmic γ DNA Polymerases

Fraction	Volume (ml)	Total Protein (mg)	Activity** (units)	Specific Activity (units/mg)	Purification
Crude Extract	216	823	168	0.20	
DEAE-cellulose chromatography pool	151	94	232	2.5	12.5
Phosphocellulose chromatography pool	41	17.4	354	20.3	101.5
Hydroxylapatite Peak IC	16	-*	147	-	-
Hydroxylapatite Peak IIC	10	-*	17	-	-

* The protein concentration was too low to determine accurately.

** One unit of enzyme activity is the amount of enzyme that catalyzes the incorporation of 1 nmole of [^{3}H]TTP in 30 min at 30°C (6).

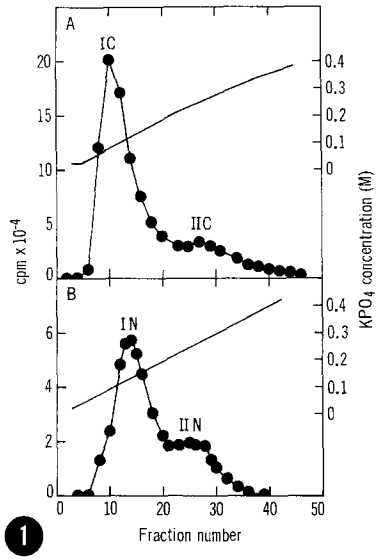
Table 2. Purification of HeLa Cell Nuclear γ DNA Polymerases

Fraction	Volume (ml)	Total Protein (mg)	Activity (units)	Specific Activity (units/mg)	Purification
Crude Extract	81	268	101	0.38	
DEAE-cellulose filtrate pool	156	190	202	1.1	2.8
DEAE-cellulose chromatography pool	185	13.9	86	6.2	16.3
Phosphocellulose chromatography pool	44	9.5	110	11.6	30.5
Hydroxylapatite Peak IN	12	-*	26	-	-
Hydroxylapatite Peak IIN	9	-*	12	-	-

* The protein concentration was too low to determine accurately.

Peak II, and one activity from the nucleus of HeLa cells. Many of the properties of cytoplasmic Peak II and nuclear γ DNA polymerase were similar, including template specificity and sensitivity to $(NH_4)_2SO_4$ and ethanol (6).

I observed that γ DNA polymerase activity from both the cytoplasm (Fig. 1A) and nucleus (Fig. 1B) of HeLa cells eluted from hydroxylapatite as a major peak, designated Peak IC and Peak IN, and a minor shoulder (Peak IIC and Peak IIN). Peak IC and Peak IN eluted at 0.08 M and 0.14 M



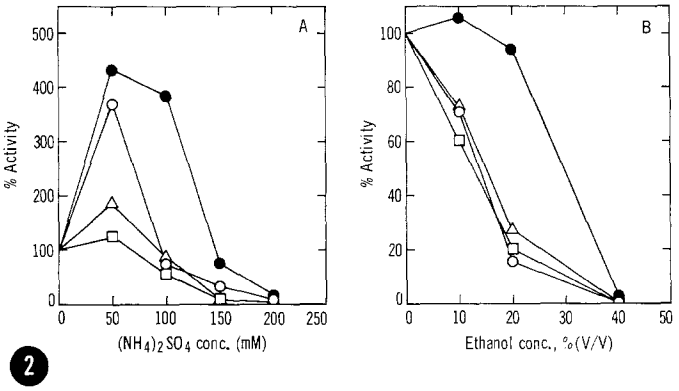


Figure 1. Hydroxylapatite chromatograms of HeLa cell phosphocellulose-purified cytoplasmic (A) and nuclear (B) γ DNA polymerases.

Figure 2. The effect of $(NH_4)_2SO_4$ (A) and ethanol (B) on HeLa cell nuclear [IN, (●); IIN, (□)] and cytoplasmic [IC, (○); IIC, (△)] γ DNA polymerases. In (A), no KCl was present in the reaction mixtures.

potassium phosphate, respectively, while both Peak II activities eluted at approximately 0.25 M salt (Fig. 1). Figure 2 demonstrates that both cytoplasmic (Peak IC and IIC) and the minor nuclear (Peak IIN) γ DNA polymerases obtained from hydroxylapatite were substantially inhibited by the presence in reaction mixtures of 100 mM $(NH_4)_2SO_4$ or 20% ethanol, similar to the cytoplasmic Peak I of Spadari and Weissbach (6). The major nuclear γ DNA polymerase (Peak IN) was relatively insensitive to 100 mM $(NH_4)_2SO_4$ and 20% ethanol (Fig. 2), similar to the nuclear and cytoplasmic Peak II γ DNA polymerases already described (6). Differences between these results and those previously reported (6) in hydroxylapatite elution patterns and cellular localization of HeLa cell γ DNA polymerases can probably be explained by the use of different types of hydroxylapatite (see Materials and Methods) and differences in the efficiency of fractionation of cell nucleus and cytoplasm.

Activity of HeLa cell γ DNA polymerases with poly(Cm)·oligo(dG). The response of the cytoplasmic and nuclear γ DNA polymerases to various synthetic templates is summarized in Table 3. The important conclusions that can be drawn from these results are that (i) poly(A)·oligo(dT) is by far the best template for each enzyme and is some 3-16 fold better than poly(dA)·oligo(dT), (ii) poly(C)·oligo(dG) is an effective template for Peak IN and

Table 3. Template Specificities of HeLa Cell Nuclear and Cytoplasmic γ DNA Polymerases

Template	[^{3}H]Substrate	% Activity with Hydroxylapatite Peak Fractions			
		Cytoplasmic		Nuclear	
		I (10)*	II (26)*	I (14)*	II (25)*
Poly(A)·$(dT)_{12-18}$	TTP	100 (18.1)**	100 (2.3)**	100 (4.1)**	100 (1.5)**
Poly(dA)·$(dT)_{12-18}$	TTP	12.8	7.7	36.4	6.3
Poly(C)·$(dG)_{12-18}$	dGTP	1.7	0.2	24.6	0.1
Poly(Cm)·$(dG)_{12-18}$	dGTP	0.1	0.1	0.1	0.2

* Number of peak fraction shown in Figure 1 used to perform assay.

** Amounts (pmoles/μl of enzyme) of incorporation of [^{3}H]TTP with poly(A)·$(dT)_{12-18}$ as template.

to a lesser extent for Peak IC, and (iii) none of the γ DNA polymerases can copy poly(Cm)·oligo(dG) under the assay conditions used. Under the same conditions at 37°C, purified (8) Moloney murine sarcoma-leukemia virus DNA polymerase incorporated 52 and 13 pmoles of [^{3}H]deoxyribonucleotide/μg of protein with poly(A)·oligo(dT) and poly(Cm)·oligo(dG), respectively, as templates.

Results essentially the same as those presented in Tables 1, 2, and 3 and Fig. 1 were obtained with human KB cells.

Discussion

Thus far none of the eukaryotic DNA polymerases tested, including α, β, and γ DNA polymerase from mouse 3T6 (1), KB (9, unpublished data), or HeLa cells copy poly(Cm)·oligo(dG). Purified DNA polymerases from other sources such as human embryo cells and leukemic lymphocytes are being examined for activity. At present, poly(Cm)·$(dG)_{12-18}$ appears to be a specific template-primer for RNA tumor virus reverse transcriptase.

HeLa cell γ DNA polymerases differ from RNA tumor virus reverse transcriptases in apparent molecular weight (6) and in ability to copy natural RNA (6) or poly(Cm)·oligo(dG), and are antigenically unrelated to known primate tumor virus DNA polymerases (6). The function of γ DNA polymerase in the cell is not known. However, a γ DNA polymerase is known to be associated with a DNA-nuclear membrane complex from adenovirus-infected KB cells (10) which synthesizes exclusively adenovirus DNA *in vitro* (11).

Acknowledgments

I thank Dr. F. Rottman for providing the poly(Cm) used in this study, and Drs. D. Grandgenett, M. Arens, M. Green, and J. Boezi for critical evaluation of the manuscript. This work was conducted under contract NO1 CP 70692 within the Virus Cancer Program of the National Cancer Institute, National Institutes of Health, Public Health Service, Bethesda, Maryland.

References

1. Gerard, G.F., Rottman, F., and Green, M. (1974) Biochem. 13, 1632-1641.
2. Fridlender, B., Fox, M., Bolden, A., and Weissbach, A. (1972) Proc. Nat. Acad. Sci. USA 69, 452-455.
3. Weissbach, A., Bolden, A., Muller, R., Hanafusa, H., and Hanafusa, T. (1972) J. Virol. 10, 321-327.
4. Bolden, A., Fox, M., Muller, R., Citarella, R., and Weissbach, A. (1972) Arch. Biochem. Biophys. 153, 26-33.
5. Sarngadharan, M.G., Sarin, P.S., Reitz, M.S., and Gallo, R.C. (1972) Nature New Biol. 240, 67-75.
6. Spadari, S., and Weissbach, A. (1974) J. Biol. Chem. 249, 5809-5815.
7. Gerard, G.F., Rottman, F., and Boezi, J.A. (1972) Biochem. Biophys. Res. Commun. 46, 1095-1101.
8. Gerard, G.F., and Grandgenett, D.P. (1975) J. Virol., in press.
9. Green, M., and Gerard, G.F. (1974) Prog. in Nucleic Acid Res. and Mol. Biol., Vol. 14 (W.E. Cohn, Ed.), pp. 187-334, Acad. Press, New York.
10. Ito, K., Arens, M., and Green, M., in preparation.
11. Yamashita, T., Arens, M., and Green, M., J. Biol. Chem., in press.