Viral and Cellular DNA Polymerase: Comparison of Activities with Synthetic and Natural RNA Templates

Abstract. *Two DNA polymerases purified from normal human lymphocytes are distinguishable from the viral reverse transcriptases of avian myeloblastosis virus and Mason-Pfizer monkey virus by their relative affinity for select templates. In this respect, the activity of the two normal human lymphocyte polymerases closely resembles the activity of* Escherichia coli *DNA polymerase I. The viral and cellular DNA polymerases are equally active with the nonspecific template, poly(rA) • poly(dT). Criteria for distinguishing the activity of viral reverse transcriptase are discussed.*

The initial characterizations of RNA-dependent DNA polymerase activity (reverse transcriptase) in RNA tumor viruses (*1*) were based on the sensitivity of the DNA polymerase activity to ribonuclease, and the requirement of the reaction for all four deoxyribonucleotides. Further, the activity of the reverse transcriptase was stimulated by the addition of exogenous, synthetic, DNA-RNA hybrid templates (*2*). The nonspecificity of such templates, as poly(dT) • poly(rA) (*3*) and poly(dC) • poly(rG) (*4*), has, however, created some confusion regarding the uniqueness of the viral reverse transcriptase. It has recently been reported that reverse transcriptase of avian myeloblastosis virus (AMV) can be distinguished from bacterial DNA polymerases (*5–7*) and calf thymus DNA polymerase (*6*) by its preference for certain synthetic oligomer-homopolymer nucleotides, and by its ability to utilize purified single-stranded RNA as template (*6*).

Based on these distinguishing criteria, we have compared the activities of purified DNA polymerases from AMV, Mason-Pfizer monkey virus (M-PMV), *Escherichia coli*, and normal human lymphocytes (NHL). Reports of reverse transcriptase in normal cells have primarily been based on a response to the nonspecific, synthetic template, poly(rA)•poly(dT), or to RNA not shown to be free of DNA (*8*). We report here that two purified DNA polymerases from NHL can be distinguished from the reverse transcriptase of AMV and M-PMV, and that they more closely resemble the DNA polymerase I of *E. coli*. In addition, it is emphasized that poly(rA) • poly(dT) is an equally effective template for both viral and cellular DNA polymerases, and therefore cannot be used to distinguish reverse transcriptase activity from that of other DNA polymerases.

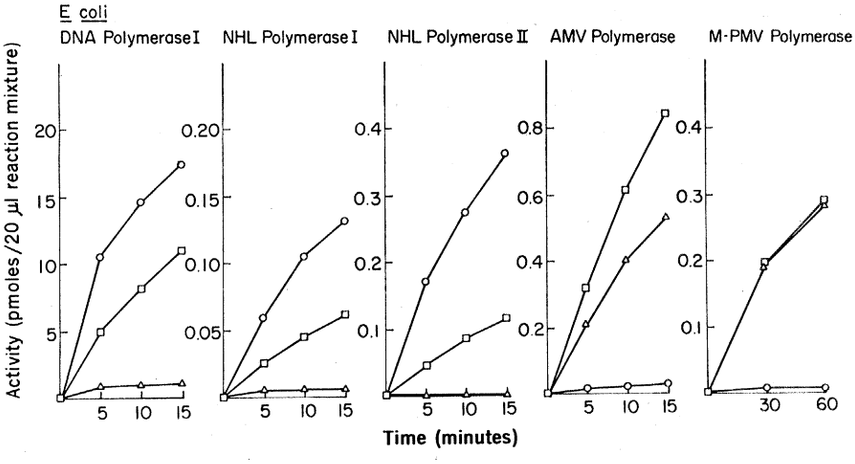

Fig. 1. Kinetics of DNA polymerase activities with synthetic templates. DNA polymerase assays were carried out at 37°C in 100-µl standard reaction mixtures composed of 50 m*M* tris-HCl buffer, *p*H 8.3; 30 m*M* KCl; 10 m*M* $MgCl_2$; 5 m*M* dithiothreitol; 80 µ*M* dATP; 5.6 µ*M* [^{3}H]TTP (15,000 count/min per picomole); and 50 µg/ml template. Aliquots (20 µl) were taken at the indicated times, precipitated, and counted as previously described (*20*). The M-PMV was assayed as above, except that the template concentrations were 20 µg/ml, and the standard assay contained 5 m*M* $MgCl_2$. Results are expressed on the basis of 20 µl of reaction mixture, as the protein concentrations of the purified viral enzymes were not measured. Five microliters of AMV polymerase and 20 µl of M-PMV polymerase were routinely used in their respective reaction mixtures. The *E. coli* DNA polymerase was assayed at 2.8 ng of protein per 20 µl of reaction mixture. The NHL polymerases I and II had protein concentrations of 144 ng/20 µl and 36 ng/20 µl, respectively. The poly(dA)•oligo(dT)$_{12-18}$ and poly(rA)•oligo(dT)$_{12-18}$ were prepared in ratios of 1 : 1 by annealing equimolar amounts of the polymer and oligomer in 0.01*M* tris-HCl, *p*H 7.2; 0.1*M* NaCl, at 70°C for 5 minutes, followed by slow cooling to room temperature over a period of 8 hours. The poly(rA)•poly(dT) was a product of Miles Laboratories. The other polymers and oligomers were obtained from Collaborative Research Inc. □—□, activity with poly(rA)•poly(dT); ○—○, activity with poly(dA)•oligo(dT)$_{12-18}$; △—△, activity with poly(rA)•oligo(dT)$_{12-18}$.

The DNA polymerase of AMV was liberated from the purified virus with a nonionic detergent (Triton X-100) and sonication, and purified in two steps by chromatography on DEAE cellulose and on phosphocellulose. The purified AMV polymerase was free of contaminating nucleic acids, and had no endogenous DNA polymerase reaction. It exhibited two bands on SDS acrylamide gels. An AMV DNA polymerase has recently been purified by Kacian *et al.* (*10*), who also reported two bands on SDS acrylamide gels. Protein in the phosphocellulose fraction was not measured. Due to the presence of Triton X-100 and buffer components which interfered with the protein determination (*11*), enzyme protein had to be precipitated prior to measurement. It was not feasible to precipitate a large volume of the purest enzyme fraction in order to recover the protein quantitatively. Thus, purification was estimated to be greater than 50-fold, as judged by the activity with poly(rA) • oligo(dT)$_{12-18}$ and the minimum detectable level of protein by the method of Lowry *et al.* (*11*).

The DNA polymerase of M-PMV (*12*) was purified similarly to the AMV polymerase. The purified enzyme had

no endogenous DNA polymerase reaction, and showed one band on SDS acrylamide gels. The purification factor was estimated to be greater than tenfold.

Two NHL DNA polymerases were purified from whole cell extracts of normal human lymphocytes that had been stimulated for 72 hours with the mitogenic agent, phytohemagglutinin (*13*). After chromatography on DEAE cellulose and on phosphocellulose, and gel filtration on Sephadex G-200, NHL DNA polymerase I was 300-fold purified and NHL DNA polymerase II approximately 90-fold.

The terminology NHL I and NHL II is a convenient means of designating two enzymes that elute differently from phosphocellulose columns; it should not be confused with *E. coli* terminology. The NHL I elutes early from phosphocellulose, sediments at about 10*S* in sucrose gradients, is most active with native DNA that has been activated by limited digestion with pancreatic deoxyribonuclease, and is sensitive to 3 m*M* NEM. The NHL II elutes late from phosphocellulose, sediments at about 3.3*S*, is most active with synthetic duplex DNA, and is largely resistent to 3 m*M* NEM (*14, 15*).

A highly purified *E. coli* DNA polymerase I (*16*) was also used for comparative purposes.

Kinetic studies of the five purified polymerases with synthetic templates are presented in Fig. 1. The synthetic hybrid template, poly(dT) poly(rA), is an extremely sensitive indicator of DNA polymerase activity. However, as these comparative studies show, its templating ability is also very nonspecific. The viral and cellular DNA polymerases could not be distinguished by reactivity with this template. When we tested synthetic oligomer-homopolymer templates, such as poly(rA) · oligo$(dT)_{12-18}$ and poly(dA) · oligo$(dT)_{12-18}$, the viral reverse transcriptase could clearly be distinguished from the cellular DNA polymerases. The AMV and M-PMV polymerases showed a preference for poly(rA) · oligo$(dT)_{12-18}$, while the *E. coli* polymerase and the NHL polymerases I and II showed a preference for poly(dA) · oligo$(dT)_{12-18}$. The NHL polymerase II had no detectable activity with poly(rA) · oligo$(dT)_{12-18}$.

The magnitude of these preferences are expressed in Table 1. The viral polymerases of AMV and M-PMV showed 17-fold and 40-fold greater activity, respectively, with poly(rA) · oligo$(dT)_{12-18}$ than with poly(dA) · oligo$(dT)_{12-18}$. The cellular enzymes, *E. coli* polymerase I and NHL polymerase I, had 15-fold and 17-fold greater activity, respectively, with poly(dA) · oligo$(dT)_{12-18}$. The NHL polymerase II was highly active with poly(dA) · oligo$(dT)_{12-18}$. As it did not accept poly(rA) · oligo$(dT)_{12-18}$ at all, the ratio of activities was expressed as greater than 274-fold. Thus, each of the five purified polymerases showed at least a 15-fold greater activity with its preferred template.

Table 1. The DNA polymerase activities with oligomer primer–homopolymer templates.

Polymerase	Incorporation into 20 μl of reaction mixture (pmole/10 min)		Ratio
	(dA)·$(dT)_{12-18}$	(rA)·$(dT)_{12-18}$	(dA)·$(dT)_{12-18}$/ (rA)·$(dT)_{12-18}$
AMV	0.023	0.400	0.058
M-PMV	0.005*	0.190*	0.026
E. coli	14.6	1.00	14.6
NHL I	0.104	0.006	17.3
NHL II	0.274	<0.001	>274

* Incorporation expressed in picomoles per 30 minutes.

The oligomer-homopolymer templates used in these experiments were prepared in both 1 : 1 and 4 : 1 molar ratios of polymer to oligomer, as described in the legend to Fig. 1. Four of the five purified DNA polymerases reacted equally well with their preferred template in both molar ratios. However, NHL DNA polymerase II exhibited a significant decrease in reaction velocity with a 4 : 1 preparation of poly(dA)· oligo$(dT)_{12-18}$ in comparison to a 1 : 1 preparation. Similar differences in reaction rates with 40 : 1 and 1 : 1 preparations of polymer to oligomer have been shown for *Micrococcus luteus* DNA polymerase and AMV polymerase (*7*).

Viral reverse transcriptases can further be distinguished from cellular DNA polymerases by the ability of the viral enzyme to react with single-stranded 70*S* RNA as template. In addition, this reaction can be stimulated by the presence of oligonucleotides (*17*). In our initial experiments, purified AMV polymerase had a low but consistently measurable activity with purified AMV 70*S* RNA. After the addition of a short oligo$(dT)_{10}$ primer, the reaction was stimulated approximately 15-fold (Table 2). The level of activity of the purified M-PMV polymerase was low, so that activity with 70*S* RNA could not be detected. However, after the addition of the primer oligo$(dT)_{10}$, the M-PMV polymerase was stimulated to measurable levels of activity. Activities of the three polymerases with the nonspecific template, poly(rA) · poly(dT), indicate that at the enzyme concentrations used, the *E. coli* enzyme had comparable activity to the AMV enzyme, and yet gave only a trace reaction with 70*S* RNA, with or without the addition of oligo$(dT)_{10}$. While the M-PMV enzyme was 100 times less active with poly(rA) · poly(dT) than was the *E. coli* enzyme, its activity with 70*S* RNA, which was stimulated by oligo$(dT)_{10}$, was 6.7 times greater. Neither NHL I nor NHL II exhibited activity with 70*S* RNA, nor could a reaction be detected with the addition of an oligo$(dT)_{10}$ primer.

The importance of using rigorously purified 70*S* RNA cannot be overemphasized. In early experiments, NHL DNA polymerases I and II showed reactivity with feline leukemia virus (FeLV) 70*S* RNA. However, this ac-

Table 2. Activities of DNA polymerases with AMV 70*S* RNA as template. Assays were carried out in standard reaction mixtures containing, in addition, 80 μM dCTP and 80 μM dGTP with 50 μg/ml 70*S* RNA. Oligo$(dT)_{10}$ was added where indicated at a concentration of 50 μg/ml. The AMV 70*S* RNA was prepared essentially according to Kacian *et al.* (*10*) except that after the phenol-cresol extraction, the RNA was precipitated once with 0.1 volume of 20 percent potassium acetate and two volumes of 95 percent ethanol. The glycerol gradients were centrifuged for 4 hours in the Spinco SW-41 rotor at 41,000 rev/min. Different preparations of purified AMV and NHL I and II polymerases account for higher incorporation values than those seen in Fig. 1.

Template	Incorporation into 50 μl of reaction mixture (pmole/hour)				
	AMV	M-PMV	*E. coli*	NHL I	NHL II
Poly(rA)·poly(dT)	236	1.19	123	3.02	3.16
70*S* RNA	4.03	<0.01	0.08	<0.01	<0.01
70*S* RNA + $(dT)_{10}$	59.1	0.40	0.06	<0.01	<0.01
$(dT)_{10}$	0.02	<0.01	<0.01	<0.01	<0.01

tivity was shown to be due to a small amount of contaminating DNA in the FeLV 70*S* RNA preparation. When this DNA was removed by Cs_2SO_4 density-gradient centrifugation, no activity of NHL polymerases with the 70*S* RNA was observed.

In order to show that the failure of cellular DNA polymerases to utilize a 70*S* RNA template was not due to an inhibitor in the purified enzyme preparations, we assayed each of the cellular DNA polymerases in combination with the AMV polymerase. No inhibition of AMV polymerase activity with 70*S* RNA was observed, nor was a decrease in stimulation by oligo$(dT)_{10}$ seen. In fact, when the AMV enzyme was mixed with the cellular enzymes, slightly greater activity was detected. This small stimulation perhaps reflects increased stability of the AMV polymerase during the course of the reaction due to the presence of additional protein. The 70*S* RNA was used in these mixing experiments because of its great sensitivity to ribonuclease. Deoxyribonucleases were not a concern because the enzymes all accept natural DNA or poly[d(A-T)] as template (data not shown).

These experiments confirm and extend other reports that viral and cellular DNA polymerases can be distinguished by their activity with certain oligomer-homopolymer templates, and by the ability of viral reverse transcriptases, but not the cellular DNA polymerases, to react with purified, single-stranded 70*S* RNA templates (*6*). Based on these criteria, the two purified NHL DNA polymerases did not resemble viral reverse transcriptase, but reacted in a manner similar to that of *E. coli* DNA polymerase I.

That poly(rA) • poly(dT) is a nonspecific template which is equally effective with viral and cellular DNA polymerases must be emphasized. Other synthetic templates, as poly(rA) • oligo$(dT)_{12-18}$ and poly(dA)•oligo$(dT)_{12-18}$, used in combination, can provide a sensitive indicator for viral reverse transcriptase activity. However, even these templates only serve as effective indicators when purified DNA polymerases are assayed in the presence of magnesium. For example, at least one cellular DNA polymerase will accept poly(rA) • oligo$(dT)_{12-18}$ more effectively than poly(dA) • oligo$(dT)_{12-18}$ in the presence of manganese, though there is no indication that it is a true reverse transcriptase (*18*). Purified 70*S* RNA, free of contaminating DNA, is a less sensitive, although a biologically more important indicator of reverse transcriptase activity. Acceptable evidence that a DNA polymerase resembles or is identical to a viral reverse transcriptase can be achieved when the product of a DNA polymerase reaction templated by 70*S* RNA is shown to specifically hybridize to the RNA template (*6*). All of these distinguishing criteria will enable more critical determinations to be made as to whether a viral-like reverse transcriptase is associated with neoplastic disease. The RNA-dependent DNA polymerase from human acute leukemic cells satisfies all these criteria for a reverse transcriptase (*15, 19*).

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Cribriform Degeneration (cri): A New Recessive Neurological Mutation in the Mouse

Abstract. *The mutation cribriform degeneration* (cri) *occurred in the DBA/2J strain; it is in linkage group VIII, 31 recombination units from* b. *Homozygotes show severe vacuolar degeneration in white and gray matter of the spinal cord and brainstem, normocytic anemia at birth which decreases in severity with age, and abnormalities of electrolyte distribution.*

Mutant genes with multiple or pleiotropic effects may be useful for revealing relations between apparently diverse developmental processes. Such mutations in laboratory animals can be subjected to experimental analysis, and if they resemble, even in part, human inherited diseases, they may serve as models to aid in the analysis of basic mechanisms. We report here a new pleiotropic mutation in the laboratory mouse, which causes neurological, hematological, and electrolyte abnormalities.

The new autosomal recessive mutation was discovered by the late M. M. Dickie in the DBA/2J strain of mice in