served in these experiments. It seems that there is a difference in the mode of biosynthesis between these four carbons and the other carbons (carbons 11-16).

The investigation dealing with the metabolism of low-molecular-weight fatty acids using ^{13}C NMR is not yet numerous.

References

Achenbach, H., and Grisebach, H. (1964), *Z. Naturforsch. B 19,* 561.

Adams, R., and Kamm, R. M. (1964), Organic Syntheses, Collect. Vol. I, 2nd ed, Blatt, A. H., Ed., New York, N.Y., Wiley, p 250.

Bentley, R. (1962), *Annu. Rev. Biochem. 31,* 606.

Birch, A. J., Djerassi, C., Dutcher, J. D., Majer, J., Perlman, D., Pride, E., Rickards, R. W., and Thompson, P. J. (1964), *J. Chem. Soc.,* 5274.

Friedman, S. M., Kaneda, T., and Corcoran, J. W. (1964), *J. Biol. Chem. 239,* 2386.

Furumai, T., Takeda, K., and Suzuki, M. (1975), *J. Antibiot. 28,* 789.

Gastambide-Odier, M., Delaumeny, J. M., and Lederer, E. (1963), *Biochim. Biophys. Acta 70,* 670.

Gilman, H., and Kirby, R. H. (1964), Organic Syntheses, Collect. Vol. I, 2nd ed, Blatt, A. H., Ed., New York, N.Y., Wiley, p. 361.

Grisebach H., and Achenbach, H. (1962a), *Z. Naturforsch. B 17,* 6.

Grisebach, H., and Achenbach, H. (1962b), *Tetrahedron Lett.,* 565.

Grisebach, H., Achenbach, H., and Hofheinz, W. (1961), *Tetrahedron Lett.,* 234.

Grisebach, H., and Weber-Schilling, C. A. (1968), *Z. Naturforsch. B 23,* 655.

Jones, A. J., Grant, D. M., and Kuhlman, K. F. (1969), *J. Am. Chem. Soc. 91,* 5013.

Kaneda, T., Butte, J. C., Taubman, S. B., and Corcoran, J. W. (1962), *J. Biol. Chem. 237,* 322.

Ōmura, S., Nakagawa, A., Neszmelyi, A., Gero, S. D., Sepulchre, A. M., Piriou, F., and Lukacs, G. (1975b), *J. Am. Chem. Soc. 97,* 4001.

Ōmura, S., Nakagawa, A., Takeshima, H., Atsumi, K., Miyazawa, J., and Lukacs, G. (1975a), *J. Am. Chem. Soc. 97,* 6600.

Ōmura, S., Nakagawa, A., Takeshima, H., Miyazawa, J., Kitao, Pirious, F., and Lukacs, G. (1975c), *Tetrahedron Lett.* 4503.

Ōmura, S., Takeshima, H., Nakagawa, A., Kanemoto, N., and Lukacs, G. (1976), *Bioorg. Chem. 5,* 451.

Srinivasan, D., and Srinivasan, P. R. (1967), *Biochemistry 6,* 3111.

Vaněk, Z., Puzá, M., and Doležílová L. (1961), *Folia Microbiol. 6,* 408.

Westley, J. W., Priess, D. L., and Pitcher, R. G. (1972), *J. Chem. Soc.,* 161.

White, R. J., Martinelli, E., Gallo, G. G., Lancini, G., and Beynon, P. (1973), *Nature (London) 243,* 273.

DNA Polymerase γ of Human Lymphoblasts†

Marjorie Robert-Guroff, Anthony W. Schrecker, Bonnie J. Brinkman, and Robert C. Gallo*

ABSTRACT: DNA polymerase γ, a component of normal cells, exhibits several properties similar to those of the DNA polymerase of RNA tumor viruses (reverse transcriptase). We have purified DNA polymerase γ more than 9000-fold from a human lymphoblast cell line (NC37) and have reexamined the biochemical nature of the enzyme, its distinguishing features with regard to reverse transcriptase, and its possible relationship to the viral enzyme. The final enzyme preparation is demonstrably free of other DNA polymerase activities by immunological and biochemical criteria. Only one form of the enzyme was detected. It has a molecular weight of 120 000. The enzyme is moderately sensitive to *N*-ethylmaleimide, exhibits a broad pH optimum around 7.4 in imidazole buffer, and is stimulated by ammonium sulfate. Like reverse transcriptase, DNA polymerase γ prefers $(dT)_{12-18}\cdot(A)_n$ to $(dT)_{12-18}\cdot(dA)_n$ as template with either Mg^{2+} or Mn^{2+} present. However, unlike the viral enzyme its activity with $(dG)_{12-18}\cdot(C)_n$ is low, and it does not transcribe the heteropolymeric portions of natural RNA templates. It has been reported that $(dG)_{12-18}$·poly(2′-*O*-methylcytidylate) is a specific primer template for viral reverse transcriptase (Gerard, G. F. (1975), *Biochem. Biophys. Res. Commun. 63,* 706). The observation that DNA polymerase γ does not use this template was confirmed with the NC37 enzyme. However, it was shown that the reverse transcriptases of avian, murine, and primate RNA tumor viruses do not use it with very great efficiency. The same reverse transcriptases do not transcribe RNA-primed DNA templates, in contrast to DNA polymerase γ which exhibits good activity with these templates. The problems of distinguishing reverse transcriptase and DNA polymerase γ in cells are discussed with respect to these properties.

DNA polymerase γ (Weissbach et al., 1975), variously called R-DNA polymerase, DNA polymerase III, DNA polymerase A, or "synthetic" RNA-dependent DNA polymerase, has been studied in a number of cell types from several animal species. These studies have shown that the enzyme is a component of normal cells and is distinct from other cellular DNA polymerases, as well as from RNA-dependent DNA polymerase (reverse transcriptase) of RNA tumor viruses (Weissbach, 1975; Lewis et al., 1974a,b; Spadari and Weiss-

† From the Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014. *Received November 17, 1976.* A preliminary report of these findings has been published (Robert-Guroff and Gallo, 1976).

bach, 1974; Gerard, 1975; Matsukage et al., 1975). Nevertheless, both the precise biochemical nature of the enzyme and the biological function it performs remain unclear. DNA polymerase γ activities isolated from both mouse and human cell systems have been well characterized; the results of these investigations show that several important differences exist between the enzymes. The murine enzyme has a molecular weight of over 230 000 and has not been dissociated into smaller subunits by treatment with high salt concentrations, nucleases, or detergent (Matsukage et al., 1975). Additionally, murine cells may contain more than one DNA polymerase γ like enzyme activity (Matsukage et al., 1974). In HeLa cells, DNA polymerase γ activity is initially resolved into two forms, one being located in the cytoplasm and the other being found in both the nucleus and the cytoplasm (Spadari and Weissbach, 1974). Both forms were reported to have molecular weights of 110 000. This value was in agreement with that reported for DNA polymerase γ isolated from human lymphoblasts (Lewis et al., 1974b). However, in these latter cells, only one form of the enzyme was detected. Recently, following more extensive purification, only one DNA polymerase γ activity was found in HeLa cells (Knopf et al., 1976). While exhibiting many similar properties to the lymphoblast enzyme described here, it differs notably in its molecular weight of 160 000 to 330 000, depending on the method of analysis.

Some differences reported concerning the nature of DNA polymerase γ might result from residual DNA polymerase α (Weissbach et al., 1975) activity in the enzyme preparations. Therefore, using a human lymphoblast DNA polymerase γ which has been more highly purified, we have reexamined the above differences. In addition, we have used the highly purified enzyme to examine several criteria by which DNA polymerase γ may be distinguished from viral reverse transcriptase. These two enzymes have a number of similar properties, the most important similarity being the ability of both to transcribe synthetic RNA templates. In view of the current search by many investigators for viral-like components in human cells, it has become increasingly important to be able to clearly determine whether an enzyme activity is attributable to DNA polymerase γ or to reverse transcriptase. Thus, we have examined the recent report of Gerard (1975) concerning the specificity of reverse transcriptase for $(dG)_{12-18}$·poly(2′-*O*-methylcytidylate) ($(dG)_{12-18}$·$(Cm)_n$)[1] and the inability of DNA polymerase γ to use this template. Our data indicate that while this template is an important one for differentiating among purified DNA polymerases it should not be the only one used when screening cells for the presence of reverse transcriptase. Studies on the use of synthetic DNA-RNA hybrid templates, including RNA-primed DNA templates, by these enzymes were also carried out and contribute two additional biochemical criteria for distinguishing the enzyme activities.

Experimental Procedure

Materials

NC37 cells, from a line of normal human lymphoblasts (Gallo and Pestka, 1970), were supplied as frozen cell pellets by Associated Biomedic Systems, Inc., Buffalo, N.Y. Fibrous (DE23) and microgranular (DE52) celluloses and phosphocellulose (P11) were all products of Whatman, Inc., Clifton, N.J. Hydroxylapatite (Bio-Gel HTP) was obtained from Bio-Rad Laboratories, Richmond, Calif. SW40 cellulose was purchased from Brown Co., Berlin, N.H. Collaborative Research, Inc., Waltham, Mass., supplied $(dT)_{12-18}$·$(dA)_n$, $(dG)_{12-18}$·$(C)_n$, $(dG)_{12-18}$, $(Cm)_n$, and $(A)_{13}$. PL-Biochemicals, Milwaukee, Wisc., supplied $(dT)_{12-18}$·$(A)_n$ and $(dT)_n$. [^{3}H]dTTP was a product of New England Nuclear, Boston, Mass. [^{3}H]dGTP and [^{3}H]dATP were obtained from Schwarz/Mann, Orangeburg, N.Y. Unlabeled deoxynucleoside triphosphates were purchased from Sigma Chemical Co., St. Louis, Mo. BPA was obtained from Armour Pharmaceutical Co., Chicago, Ill., and aldolase from Pharmacia Fine Chemicals, Inc., Piscataway, N.J. DNase was a product of Worthington Biochemical Corp., Freehold, N.J. AMV was provided by Dr. Joseph Beard, Life Sciences, Inc., Gulfport, Florida. RLV and SSV were purchased from Electro-Nucleonics Laboratories, Inc., Bethesda, Md. *N*-Ethylmaleimide was a product of Eastman Kodak Co., Rochester, N.Y.

Methods

Purification of DNA Polymerase γ. Approximately 60 g of frozen NC37 cells was quickly thawed at 37 °C. Thereafter, all procedures were carried out at 4 °C. The initial extraction, nucleic acid removal on fibrous DEAE-cellulose, and chromatography on microgranular DEAE-cellulose were performed as described by Lewis et al. (1974b), except that ethylene glycol was substituted for glycerol in the buffers. The 0.3 M KCl eluate of the microgranular DEAE-cellulose column was dialyzed against 50 mM Tris-HCl, pH 7.9, containing 1 mM DTT, 50 mM KCl, and 20% ethylene glycol, and slowly applied to a 2.5 × 37 cm column of phosphocellulose equilibrated in the same buffer. Unbound material was removed by washing with 1 column volume of equilibration buffer. DNA polymerase activity was eluted with the same buffer containing 0.6 M KCl. The 0.6 M KCl eluate was concentrated by dialysis against 30% polyethylene glycol in 40 mM potassium phosphate buffer, pH 7.2, containing 1 mM DTT, 20% ethylene glycol, and 1 mM EDTA (buffer C), and then further dialyzed against 40 mM potassium phosphate buffer, pH 7.2, containing 1 mM DTT and 20% ethylene glycol (buffer P).

This fraction was then applied to a 1 × 30 cm column of hydroxylapatite equilibrated in buffer P. The hydroxylapatite was washed with 1 column volume of 0.15 M potassium phosphate buffer, pH 7.2, containing 1 mM DTT and 20% ethylene glycol and then eluted with a 300-mL linear gradient from 0.15 to 0.3 M potassium phosphate. Fractions containing enzyme activity were pooled and concentrated as before by dialysis against buffer C. Following further dialysis against buffer P, the sample was applied at a flow rate of 7.5 mL/h to a 2.5 × 12 cm column of DNA cellulose, equilibrated in buffer P. The DNA cellulose was washed with 1 column volume of buffer P, and a 600-mL linear gradient of 0 to 0.5 M KCl in buffer P was applied. The column was eluted at a flow rate of 30 mL/h. Fractions containing enzyme activity were pooled and dialyzed against buffer C. Following further dialysis against buffer P, the DNA polymerase γ was divided into small portions and stored at −70 °C. Stored in this fashion, the enzyme preparation has retained complete activity for several months.

Purification of Other DNA Polymerases. DNA polymerases α and β (Weissbach et al., 1975) of NC37 cells were purified simultaneously with DNA polymerase γ. After pre-

[1] Abbreviations used are: $(Cm)_n$, poly(2′-*O*-methylcytidylate); BPA, bovine plasma albumin; AMV, avian myeloblastosis virus; RLV, Rauscher murine leukemia virus; SSV, woolly monkey (simian) sarcoma virus; DTT, dithiothreitol; SSA, activated salmon sperm DNA; DNase, deoxyribonuclease; NEM, *N*-ethylmaleimide; DEAE, diethylaminoethyl; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; EDTA, (ethylenedinitrilo)-tetraacetic acid.

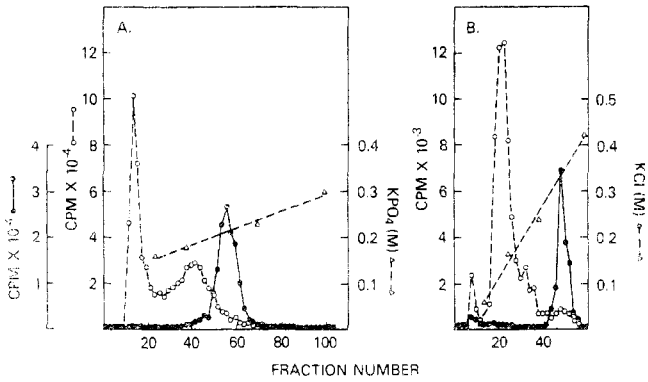

FIGURE 1: Elution of phosphocellulose purified DNA polymerases α and γ from (A) hydroxylapatite and (B) DNA-cellulose. Ten-microliter aliquots of column fractions were assayed for activity with either SSA (○) or $(dT)_{12-18}\cdot(A)_n$ (●). Salt concentration (△) was determined on selected fractions by use of a conductivity meter.

liminary purification on fibrous and microgranular DEAE-cellulose and phosphocellulose, DNA polymerase α was resolved from DNA polymerase γ by hydroxylapatite chromatography. One peak of DNA polymerase α activity did not bind to hydroxylapatite and a second peak of activity eluted at less than 0.2 M potassium phosphate. DNA polymerase γ was eluted at higher potassium phosphate concentrations than either of these two activities. DNA polymerase α was also obtained in flow-through fractions of the DNA-cellulose chromatography step. DNA polymerase α fractions were dialyzed first against buffer C, then against buffer P, divided into small portions, and stored at −70 °C.

NC37 DNA polymerase β was obtained in the 0.05 M KCl eluate of the microgranular DEAE-cellulose column and further purified by phosphocellulose chromatography as described previously (Lewis et al., 1974b).

Viral DNA polymerases from concentrated suspensions of purified RLV and SSV were purified through DEAE-cellulose and phosphocellulose as described by Abrell and Gallo (1973). AMV was purified from chicken plasma as previously described (Robert et al., 1972). The AMV reverse transcriptase was purified through DEAE-cellulose and phosphocellulose, and additionally purified by sedimentation on a glycerol gradient as described by Kacian et al. (1971).

Assays of DNA Polymerase Activities. DNA polymerase assays were carried out in the following standard systems unless otherwise indicated. *DNA polymerase* γ was assayed at 30 °C in a 50-μL reaction mixture containing 50 mM imidazole, pH 7.4; 1 mM DTT; 60 mM $(NH_4)_2SO_4$; 0.2 mM $MnCl_2$; 6 μM [^{3}H]dTTP (15–20 Ci/mmol); 80 μM dATP; and 25 μg/mL $(dT)_{12-18}\cdot(A)_n$. *DNA polymerase* α was assayed at 37 °C in a 50-μL reaction mixture containing 50 mM Tris-HCl, pH 7.9; 1 mM DTT; 10 mM KCl; 0.5 mM $MgCl_2$; 6 μM [^{3}H]dTTP (15–20 Ci/mmol); 80 μM each dATP, dCTP, dGTP; and 75 μg/mL SSA, activated as described below. *DNA polymerase* β was similarly assayed at 30 °C in a 50-μL reaction mixture containing 50 mM Tris-HCl, pH 7.9; 1 mM DTT; 60 mM KCl; 1 mM $MnCl_2$; 6 μM [^{3}H]dTTP (15–20 Ci/mmol); 80 μM dATP, and 25 μg/mL $(dT)_{12-18}\cdot(dA)_n$. *Viral DNA polymerases* were assayed with their preferred cation, Mg^{2+} or Mn^{2+}, at 37 or 30 °C, respectively. These 50-μL reaction mixtures contained 50 mM Tris-HCl, pH 7.9; 1 mM DTT; 60 mM KCl; 10 mM $MgCl_2$ or 1 mM $MnCl_2$; 6 μM [^{3}H]dTTP (15–20 Ci/mmol); 80 μM dATP; and 25 μg/mL $(dT)_{12-18}\cdot(A)_n$. AMV reverse transcriptase was assayed with Mg^{2+}; SSV and RLV reverse transcriptases were assayed using Mn^{2+}. Reactions were stopped by adding 50 μg of carrier *Escherichia coli* tRNA and approximately 2 mL of 10% trichloroacetic acid containing 0.2% sodium pyrophosphate and placing the tubes in ice. The acid precipitates were filtered through Millipore filters (Type HA, 0.45 μm) and washed with approximately 30 mL of 5% trichloroacetic acid containing 0.2% sodium pyrophosphate. The filters were then washed with 2 mL of 75% ethanol, dried, and counted in a scintillation counter in a standard toluene-based cocktail (Yorktown LSC complete).

Preparation of Column Materials. DEAE-celluloses and phosphocellulose were precycled as recommended by the manufacturer. DE52 and P11 celluloses were then stirred with a BPA solution (1 mg/mL) and subsequently washed with 1 M KCl to remove unbound protein before equilibrating.

DNA cellulose was prepared from acid-washed SW40 cellulose and native calf thymus DNA, as described by Litman (1968), and stored in a desiccator at room temperature. Just before use, it was activated by mild treatment with DNase (Matsukage et al., 1975).

Immunologic Methods. The IgG fraction of a rat antiserum to DNA polymerase α was purified (Watson et al., 1972) and utilized in triplicate enzyme neutralization assays essentially as previously described (Smith et al., 1975). The dilutions of immune IgG were made in control IgG, in order to have a constant amount of IgG present in each reaction. The enzyme and antibody were preincubated 16–20 h at 4 °C before assays for inhibition of enzyme activity were performed. Acid precipitates of reaction products were filtered through Reeve Angel glass fiber filters (934AH).

Additional Methods. Native salmon sperm DNA was activated by limited digestion with pancreatic DNase I according to the method of Schlabach et al. (1971). Protein was measured by the method of Lowry et al. (1951). The pH of all Tris buffers was adjusted at 4 °C.

Results

Hydroxylapatite Chromatography of DNA Polymerase γ. DNA polymerase γ was purified sequentially through fibrous and microgranular DEAE-celluloses and then through phosphocellulose as described under Methods. A further purification was obtained by hydroxylapatite chromatography (Figure 1A). The use of a very shallow phosphate gradient resulted in partial separation of DNA polymerase α from DNA polymerase γ. The DNA polymerase α activity, assayed with activated DNA as template, eluted as two peaks, one appearing below 0.15 M potassium phosphate and another at 0.18 M potassium phosphate. These peaks were not in equilibrium with each other, since, when pooled separately and reapplied to hydroxylapatite, they eluted at the same potassium phosphate concentrations. DNA polymerase γ was eluted by 0.21 M potassium phosphate. It was, however, not free of contaminating DNA polymerase α activity, indicated by the overlapping activities with SSA and $(dT)_{12-18}\cdot(A)_n$ templates. Purified DNA polymerase α does not utilize the latter template efficiently (Lewis et al., 1974a; Sedwick et al., 1972, 1975; Bollum, 1975). The reverse has also been demonstrated: highly purified mouse DNA polymerase γ transcribes activated DNA poorly (Matsukage et al., 1975).

Affinity Chromatography of DNA Polymerase γ *on DNA-Cellulose.* The separation of human lymphoblast DNA polymerase γ from DNA polymerase α on DNA-cellulose has previously been reported from this laboratory (Lewis et al., 1974b). Subsequent preparations of DNA-cellulose made by the method of Alberts and Herrick (1971) did not always ad-

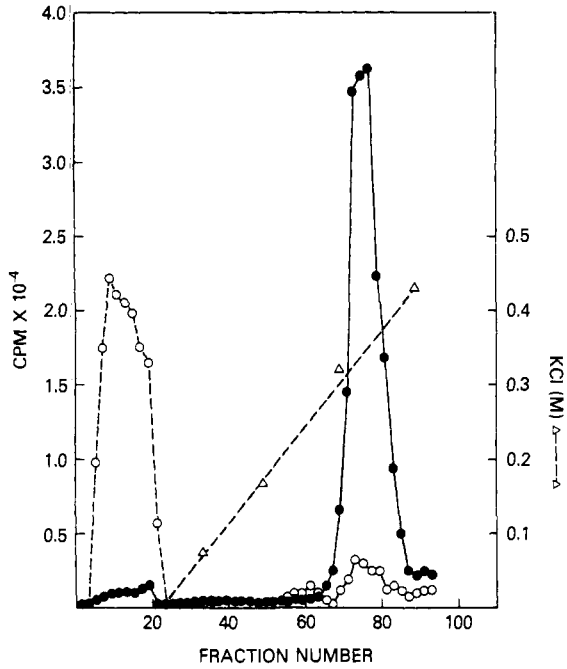

FIGURE 2: Elution of hydroxylapatite-purified DNA polymerases α and γ from DNA-cellulose. Ten-microliter aliquots of column fractions were assayed for activity with SSA (○) and $(dT)_{12-18}\cdot(A)_n$ (●). Salt concentration (△) was determined on selected fractions by use of a conductivity meter.

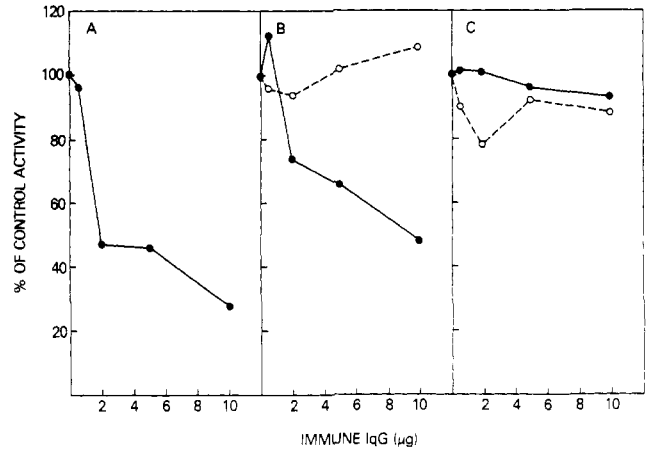

FIGURE 3: Inhibition of DNA polymerases α and γ by antiserum to purified DNA polymerase α. (A) Inhibition of phosphocellulose-purified DNA polymerase α, further purified through the DNA-cellulose chromatography step. (B) Inhibition of DNA polymerase γ purified through the hydroxylapatite chromatography step. (C) Inhibition of final DNA polymerase γ purified through both the hydroxylapatite and DNA-cellulose steps. Symbols represent activity with SSA (●) and $(dT)_{12-18}\cdot(A)_n$ (○). Each reaction mixture contained a total of 10 μg of IgG made up of IgG from a preimmune animal plus the indicated amount of immune IgG. Percent of control activity was calculated assuming 100% for reactions containing 10 μg of preimmune IgG. For all enzymes, 100% activity represents approximately 15 000 cpm of [^{3}H]TMP incorporated.

equately bind DNA polymerase γ activity. Thus, the alternate method of DNA-cellulose preparation reported here was adopted. However, the separation of DNA polymerase α from DNA polymerase γ was not complete on this differently prepared DNA-cellulose. Figure 1B shows the pattern of enzyme activities eluted from DNA-cellulose following chromatography on phosphocellulose. Here again, the overlapping enzyme activities with SSA and $(dT)_{12-18}\cdot(A)_n$ as templates indicate that the peak of DNA polymerase γ activity, eluting at 0.35 M KCl, is not free of contamination by DNA polymerase α. Thus, *both* hydroxylapatite and DNA-cellulose chromatography were found necessary to achieve complete separation of DNA polymerase α from DNA polymerase γ.

Figure 2 shows the pattern of enzyme activities eluting from DNA-cellulose following chromatography on hydroxylapatite. Residual DNA polymerase α activity does not adsorb to the DNA-cellulose, as shown by the peak of activity with activated DNA in the column flow-through fractions. The species of DNA polymerase α which binds to DNA cellulose is removed by prior chromatography on hydroxylapatite. DNA polymerase γ is clearly separated and elutes at 0.35 M KCl. A comparison of Figures 1B and 2 indicates that, based merely on the difference between the activities of the DNA polymerase γ peak with $(dT)_{12-18}\cdot(A)_n$ and SSA as templates, the inclusion of the hydroxylapatite step prior to DNA-cellulose chromatography significantly improved the purification by at least twofold.

The purification of DNA polymerase γ by the methodology described here is summarized in Table I. The final enzyme preparation was purified over 9000-fold with a 7% yield. Other preparations have resulted in yields of up to 15% of the total activity. A large loss results during the DNA-cellulose step, but this step appears necessary in order to obtain a final enzyme preparation with only DNA polymerase γ activity.

Immunological Assessment of Purity of DNA Polymerase γ. The above observations concerning the separation of DNA polymerase α from DNA polymerase γ were confirmed by immunological studies. The relative purity of DNA polymerase γ fractions was assessed using an antibody prepared against a highly purified human DNA polymerase α (Smith et al., 1975). Figure 3A shows the inhibition of NC37 DNA polymerase α by anti-DNA polymerase α IgG. Figure 3B shows the same antibody titrated against DNA polymerase γ purified through only the hydroxylapatite step. That some contaminating DNA polymerase α activity is present is evident by the inhibition of enzyme activity using SSA as template. Figure 3C shows the titration of anti-α against DNA polymerase γ purified through both the hydroxylapatite and DNA-cellulose steps. The inhibition of enzyme activity with activated DNA as template has disappeared, indicating that DNA polymerase α is no longer present. In no case were the activities using

TABLE I: Purification of NC37 DNA Polymerase γ.[a]

Fraction	Total protein (mg)	Total act. (nmol/h)	Sp act. (nmol h^{-1} (mg of protein)$^{-1}$)	Yield (%)
Crude extract	8000	187	0.024	
Fibrous DEAE-cellulose	5304	1198	0.23	100
Microgranular DEAE-cellulose	1808	728	0.40	61
Phosphocellulose	444	342	0.77	29
Hydroxylapatite	99	504	5.09	42
DNA-cellulose	0.38	86	224.4	7

[a] DNA polymerase γ activity was assayed as described under Methods, using $(dT)_{12-18}\cdot(A)_n$ as template. Enzyme activity was linear with respect to time out to 15 min. Calculated values expressed above are based on enzyme activity during a 10-min incubation. Yield was calculated assuming the total activity of the fibrous DEAE-cellulose fraction to be 100%. It was not possible to accurately assess the total activity of the crude extract, as the DNA polymerase γ activity was greatly inhibited.

TABLE II: NC37 DNA Polymerase Activities with Various Primer Templates.

DNA polymerase	Primer templates			
	SSA	$(dT)_{12-18}\cdot(A)_n$	$(dT)_{12-18}\cdot(dA)_n$	$(dG)_{12-18}\cdot(C)_n$
α	100[a] (16 824)	6 (1002)	2 (282)	0.2 (30)
β	8 (3450)	1 (450)	100 (43 062)	0.1 (42)
γ	7 (14 830)	100 (224 400)	4 (8620)	1 (1724)

[a] Activity is expressed as the percentage of the preferred template activity. The numbers in parentheses represent actual specific activities (pmol h^{-1} (mg of protein)$^{-1}$). Enzyme assay conditions were those described under Methods; enzyme activities were assayed for 10 min, during which time the activity was linear. Values above were calculated from this value.

TABLE III: DNA Polymerase Activities with Viral 70S RNA as Template.[a]

Template	Act. (pmol of [^{3}H]dGMP incorp)	
	NC37 γ	AMV
RLV 70S RNA	0.032	1.31
RLV 70S RNA + $(dT)_{12-18}$	0.045	6.07
$(dT)_{12-18}$ alone	0.014	0.019
RLV 70S RNA + $(dT)_{12-18}$	0.39[b]	10.5[b]

[a] Enzyme assays were carried out at 37 °C in 50-μL reaction mixtures containing 50 mM Tris-HCl, pH 7.4; 1 mM DTT; 60 mM KCl; 1 mM $MnCl_2$ (for NC37 γ) or 10 mM $MgCl_2$ (for AMV reverse transcriptase); 6 μM [^{3}H]dGTP (10 Ci/mmol); 80 μM each dATP, dCTP, dTTP; and, where indicated, 25 μg/mL RLV 70S RNA and 25 μg/mL $(dT)_{12-18}$. The pmols were calculated from values obtained following 10-min incubations. [b] [^{3}H]dTTP was substituted for [^{3}H]dGTP, and dGTP for dTTP in reaction mixtures.

$(dT)_{12-18}\cdot(A)_n$ inhibited by the antibody, suggesting that in these fractions such activity was due only to DNA polymerase γ. DNA polymerase α does not utilize $(dT)_{12-18}\cdot(A)_n$ (Lewis et al., 1974a; Sedwick et al., 1972, 1975; Bollum, 1975). Activity with $(dT)_{12-18}\cdot(dA)_n$ was not tested, as the utilization of this template by DNA polymerase α is at most 5 to 10% of its activity, with SSA (Sedwick et al., 1975). The anti-DNA polymerase α used in these experiments is specific for the homologous enzyme only at low IgG concentrations (Smith et al., 1975). Thus, greater amounts of IgG were not used in an attempt to detect a small amount of DNA polymerase α in the DNA polymerase γ preparation. However, additional immunological evidence suggests that the preparation was not contaminated by DNA polymerase α. An antibody prepared against the highly purified DNA polymerase γ dose not recognize DNA polymerase α purified from NC37 cells (Robert-Guroff and Gallo, 1977).

Properties of Human Lymphoblast DNA Polymerase γ. The activity of the purified DNA polymerase γ with various primer templates is shown in Table II. NC37 DNA polymerases α and β are included for comparison. The assay conditions were not optimized for DNA polymerase γ. Instead, conditions were used for assaying a battery of standard enzymes with preferred primer templates, as would be done when attempting to distinguish a number of DNA polymerase activities. Thus, reaction conditions with SSA as template were optimized for NC37 DNA polymerase α, $(dT)_{12-18}\cdot(A)_n$ conditions were optimized for NC37 DNA polymerase γ, $(dT)_{12-18}\cdot(dA)_n$ conditions were optimized for DNA polymerase β, and $(dG)_{12-18}\cdot(C)_n$ conditions were optimized for reverse transcriptase. Under these conditions, DNA polymerase γ clearly prefers $(dT)_{12-18}\cdot(A)_n$ as template, and is easily distinguished from DNA polymerases α and β. It should be pointed out that under appropriate conditions DNA polymerase β will utilize $(dT)_{12-18}\cdot(A)_n$ quite efficiently (Bollum, 1975; Chang and Bollum, 1972; Wang et al., 1975). The activity of DNA polymerase γ with $(dG)_{12-18}\cdot(C)_n$ is low but significant as reported previously (Spadari and Weissbach, 1974). The activity with the DNA templates can be improved by adjusting reaction conditions. The reaction catalyzed by DNA polymerase γ with synthetic RNA templates ($(dT)_{12-18}\cdot(A)_n$ or $(dG)_{12-18}\cdot(C)_n$) proceeds optimally with Mn^{2+} present at a concentration of 0.2 to 0.5 mM. Mg^{2+} can substitute at a higher concentration (10 mM) in the $(dT)_{12-18}\cdot(A)_n$ reaction, but the activity obtained is only one-third that obtained with Mn^{2+}. On the contrary, when natural or synthetic DNAs serve as primer templates, reactions catalyzed by DNA polymerase γ proceed optimally with Mg^{2+} present. The optimal Mg^{2+} concentration with activated DNA is 12.5 mM and with $(dT)_{12-18}\cdot(dA)_n$ is 10 mM. In the initial studies on DNA polymerase γ, it was reported that the enzyme copied only poly(A) and not other homoribopolymers (Fridlender et al., 1972). Following more extensive purification, the form II enzyme of HeLa cells was shown to copy all homoribopolymers (Spadari and Weissbach, 1974). Because of this change in properties with greater purification, we believed it important to test the ability of the highly purified DNA polymerase γ to transcribe a natural RNA template. Previous reports have failed to show such an ability (Lewis et al., 1974a; Spadari and Weissbach, 1974; Matsukage et al., 1975; Knopf et al., 1976). This was confirmed in our studies using 70S RNA of RLV as template (Table III). DNA polymerase γ does transcribe the poly(A) portion of the 70S RNA when $(dT)_{12-18}$ is provided as primer.

The DNA polymerase γ was further assayed for inhibition by NEM. As expected, DNA polymerase α was extremely sensitive to this sulfhydryl inhibitor and DNA polymerase β was resistant (Smith and Gallo, 1972). DNA polymerase α activity was inhibited more than 50% at a concentration of 0.1 mM NEM, while DNA polymerase β showed no inhibition of activity by inhibitor concentrations as great as 5 mM. DNA polymerase γ was moderately sensitive to NEM as was reported for the murine DNA polymerase γ (Matsukage et al., 1975). Approximately 3 mM NEM was necessary for 50% inhibition of DNA polymerase γ activity.

DNA polymerase γ was found to be approximately twofold more active in imidazole buffers than in Tris buffers. The enzyme exhibited a broad pH optimum in imidazole with the midpoint at pH 7.4.

The effect of various salts on the activity of DNA polymerase γ was investigated. Of those studied, only the use of ammonium sulfate resulted in significantly better enzyme activity when substituted for KCl, the standard salt used in DNA polymerase reactions. The KCl concentration was optimal over a broad range, from 100 to 150 mM. However, at least a twofold increase in activity was observed when $(NH_4)_2SO_4$ was substituted. This stimulation was observed at concentrations of $(NH_4)_2SO_4$ from 40 to 100 mM. This result is comparable to that obtained with HeLa cell form II DNA polymerase γ (Spadari and Weissbach, 1974).

TABLE IV: DNA Polymerase Activities with Synthetic RNA and DNA Primer Templates.[a]

DNA polymerase	Act. with $(dT)_{12-18}\cdot(A)_n$ (pmol)	Act. with $(dT)_{12-18}\cdot(dA)_n$ (pmol)	Ratio of act. $((dT)_{12-18}\cdot(dA)_n/(dT)_{12-18}\cdot(A)_n)$
Cellular			
NC37 α	0.069	1.86	27
NC37 β	0.065	0.58	8.9
NC37 γ	2.60	0.8	0.33
Viral			
SSV	14.7	0.017	0.001
RLV	1.22	0.024	0.02
AMV	7.09	0.024	0.003

[a] All enzymes were assayed at 37 °C in 50-μL reaction mixtures containing 50 mM Tris-HCl, pH 7.9; 1 mM DTT; 60 mM KCl; 5 mM $MgCl_2$; 6 μM [^{3}H]dTTP; 80 μM dATP; and 25 μg/mL of the indicated primer template. The pmoles were calculated from values obtained at 10-min time points.

DNA polymerase γ was consistently found to be intermediate in size between BPA and aldolase markers when sedimented through 5–35% glycerol gradients in the presence of salt and detergent. Based on these two standards, and assuming globular shape, its molecular weight was estimated as 120 000 (Martin and Ames, 1961). If detergent was not present, the enzyme sometimes sedimented in a partially aggregated form. Due to the enzyme's lability, samples were merely diluted in appropriate buffers immediately prior to gradient centrifugation. It is not known whether dialysis against high salt buffer alone would eliminate the aggregation. No evidence was obtained which suggested more than one form of the enzyme. In addition, analysis by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Maizel, 1969) indicated that a protein of 120 000 molecular weight was being purified. It must be noted, however, that the enzyme preparation was not homogeneous. Thus, the 120 000 molecular weight protein could not be positively attributed to DNA polymerase γ.

Reaction with Synthetic RNA and DNA Templates. A common criterion for distinguishing DNA polymerases of uninfected normal mammalian cells and reverse transcriptase from RNA tumor viruses has been based on the relative utilization of certain synthetic RNA and DNA templates, i.e., $(dT)_{12-18}\cdot(A)_n$ and $(dT)_{12-18}\cdot(dA)_n$, in the presence of Mg^{2+} (Robert et al., 1972). This criterion was reinvestigated using the highly purified DNA polymerase γ. Following extensive purification, DNA polymerase γ exhibits greater activity with the synthetic RNA template than with the synthetic DNA template, in contrast to DNA polymerases α and β (Table IV). This difference among the cellular enzymes is apparently the result of DNA polymerase γ being purified free of contaminating DNA polymerase α. This result agrees with that of Knopf et al. (1976) but is in contrast to that of Matsukage et al. (1975), who reported that highly purified murine DNA polymerase γ exhibited greater activity with $(dT)_{12-18}\cdot(dA)_n$ than with $(dT)_{12-18}\cdot(A)_n$ in the presence of Mg^{2+}. This discrepancy cannot be explained. No other information on the relative utilization of these synthetic primer templates in the presence of Mg^{2+} has been published. There is general agreement that in the presence of Mn^{2+} DNA polymerase γ uses $(dT)_{12-18}\cdot(A)_n$ more effeciently than $(dT)_{12-18}\cdot(dA)_n$ (Lewis et al., 1974b; Matsukage et al., 1975; Fridlender et al., 1972; and our unpublished data).

TABLE V: Activity of DNA Polymerases with $(dG)_{12-18}\cdot(Cm)_n$.[a]

DNA polymerases	Standard template	Standard act. (pmol)	$(dG)_{12-18}\cdot(Cm)_n$ act. (pmol)	$(dG)_{12-18}\cdot(Cm)_n$ rel act. (% of standard)
Cellular				
NC37 α	SSA	7.48	0.02	0.24
NC37 β	$(dT)_{12-18}\cdot(dA)_n$	3.65	<0.001	<0.03
NC37 γ	$(dT)_{12-18}\cdot(A)_n$	4.20	0.008	0.19
Viral				
AMV	$(dT)_{12-18}\cdot(A)_n$	5.48	3.62	66
	$(dG)_{12-18}\cdot(C)_n$	21.9	3.62	17
RLV	$(dT)_{12-18}\cdot(A)_n$	2.32	0.42	18
SSV	$(dT)_{12-18}\cdot(A)_n$	4.38	0.64	15

[a] The reaction conditions using standard templates are described under Methods, except AMV reverse transcriptase was assayed additionally with 25 μg/mL $(dG)_{12-18}\cdot(C)_n$ in 50 mM Tris-HCl, pH 7.9; 1 mM DTT; 60 mM KCl; 10 mM $MgCl_2$; 12 μM [^{3}H]dGTP (10 Ci/mmol); and 80 μM dCTP. The reactions using $(dG)_{12-18}\cdot(Cm)_n$ were carried out in 50 mM Tris-HCl, pH 7.9; 1 mM DTT; 60 mM KCl; 24 μM [^{3}H]dGTP (10 Ci/mmol); 80 μM dCTP; 0.5 mM $MnCl_2$; 50 μg/mL $(Cm)_n$; and 35 μg/mL $(dG)_{12-18}$. The values expressed above represent incorporation of radioactive precursors at 10 min, at which time the enzyme reactions were still linear with respect to time.

Table IV indicates that the activity ratios still distinguish purified viral reverse transcriptases from the three cellular enzymes. The effect of the greater utilization by DNA polymerase γ of $(dT)_{12-18}\cdot(A)_n$ with respect to determining the cellular or viral nature of an enzyme activity is discussed below.

Reaction with $(dG)_{12-18}\cdot(Cm)_n$ as Template. It has been reported that $(Cm)_n$ primed with $(dG)_{12-18}$ is a specific template for reverse transcriptases and is not used by mammalian cellular DNA polymerases (Gerard, 1975; Gerard et al., 1974). The data shown in Table V confirm this observation. Use of even greater amounts of cellular DNA polymerases than those shown did not result in significant incorporation of [^{3}H]dGMP with $(dG)_{12-18}\cdot(Cm)_n$ as primer template. The viral reverse transcriptases tested were all active with this primer template; however, incorporation was much less in comparison with that achieved with $(dT)_{12-18}\cdot(A)_n$. AMV reverse transcriptase showed the greatest activity with $(dG)_{12-18}\cdot(Cm)_n$ relative to activity with $(dT)_{12-18}\cdot(A)_n$ (66%). However, when the activity of the AMV enzyme with $(dG)_{12-18}\cdot(Cm)_n$ was compared to the activity with its preferred template, $(dG)_{12-18}\cdot(C)_n$, the relative activity was less than 20%, similarly to the relative activities achieved by the DNA polymerases of RNA tumor viruses of mammalian species.

RNA-Primed DNA Synthesis. It has been reported that cellular and bacterial DNA polymerases efficiently use synthetic RNA primers complexed to DNA templates (Spadari and Weissbach, 1974; Keller, 1972; Tamblyn and Wells, 1975). The AMV reverse transcriptase apparently does not use this template (Keller, 1972; Tamblyn and Wells, 1975). These observations were tested using DNA polymerases α, β, and γ from NC37 cells and reverse transcriptases of avian, murine, and primate RNA tumor viruses (Table VI). The data indicate that the cellular DNA polymerases can utilize oligoribonucleotides to prime synthesis on synthetic DNA templates. In contrast, none of the viral reverse transcriptases tested used the synthetic RNA-primed DNA template.

TABLE VI: Activity of DNA Polymerases with $(A)_{13}\cdot(dT)_n$.[a]

DNA polymerase	Standard template	Standard act. (pmol)	Act. with $(A)_{13}\cdot(dT)_n$ (pmol)	Rel. act. (standard/ $(A)_{13}\cdot(dT)_n$)
Cellular				
NC37 α	SSA	9.84	5.87	1.68
NC37 β	$(dT)_{12-18}\cdot(dA)_n$	1.96	0.21	9.30
NC37 γ	$(dT)_{12-18}\cdot(A)_n$	0.75	0.71	1.06
Viral				
AMV	$(dT)_{12-18}\cdot(A)_n$	3.76	0.006	627
RLV	$(dT)_{12-18}\cdot(A)_n$	19.1	<0.001	19 100
SSV	$(dT)_{12-18}\cdot(A)_n$	5.13	<0.001	5 130

[a] DNA polymerase assays with standard templates were carried out as described under Methods, except the concentration of [^{3}H]-dTTP was increased to 24 μM. Activity with $(A)_{13}\cdot(dT)_n$ was assayed in a 50-μL reaction mixture containing 50 mM Tris-HCl, pH 7.9; 75 mM KCl; 1 mM DTT; 0.5 mM $MnCl_2$; 24 μM [^{3}H]dATP (17.1 Ci/mmol); 80 μM TTP; 40 μM $(A)_{13}$ and 100 μM $(dT)_n$. Values expressed above represent pmol of radioactive precursor incorporated in a 10-min incubation.

Discussion

DNA polymerase γ has several properties which are similar to those of DNA polymerase α and which make resolution of the two activities very difficult by ion-exchange chromatography, gel filtration, or velocity gradient centrifugation. We felt that the variable reports concerning the nature of this enzyme might result from incomplete separation of DNA polymerase α from DNA polymerase γ. The present study was undertaken to demonstrate the complete resolution of DNA polymerase γ from DNA polymerase α and to reassess the properties of the highly purified product.

Based on its size and template responses, the human lymphoblast DNA polymerase γ is similar to the form II enzyme purified from the nucleus and cytoplasm of HeLa cells (Spadari and Weissbach, 1974). However, only one DNA polymerase γ activity has been detected in NC37 cells (Lewis et al., 1974b; this manuscript). Following more extensive purification of the HeLa system, only one polymerase γ activity was reported (Knopf et al., 1976). Except for its molecular weight, its properties resemble those of the human lymphoblast enzyme. Thus, while there seems to be only one form of human polymerase γ, in murine systems additional DNA polymerase γ like activities have been reported (Matsukage et al., 1974). The possibility exists that multiple forms of DNA polymerase γ result from nucleic acid–enzyme complexes, degradation during purification, or from other contaminating enzyme activities. Alternately, DNA polymerase γ, representing only a minor fraction of the total DNA polymerase activity of cells (Bollum, 1975), may not have highly conserved properties. Rather, it may exhibit widely different properties from cell type to cell type and species to species.

The enzyme purified here exhibits quite different properties than the murine enzyme extensively purified by a similar procedure (Matsukage et al., 1975). The murine enzyme was reported to have a molecular weight greater than 230 000. The NC37 enzyme was found to have a molecular weight of 120 000. Differences in relative responses to various template primers are also evident; the most significant is the failure of the murine enzyme to utilize $(dG)_{12-18}\cdot(C)_n$. Whether another form of the murine enzyme which is able to utilize this template exists is not known. Such a result would be analogous to the HeLa system. It is also not known whether the murine enzyme exists as a complex of two or more subunits. No evidence for subunit structure exists for the human DNA polymerase γ.

The observation that $(dG)_{12-18}\cdot(Cm)_n$ is a specific template for reverse transcriptase (Gerard, 1975) was confirmed here. Under several reaction conditions, the highly purified NC37 DNA polymerase γ did not copy this template. The pattern of activities seen with the viral reverse transcriptases is of interest. It has been reported previously that reverse transcriptases of RNA tumor viruses from murine and primate species, as well as from avian species, utilize this template primer (Gerard et al., 1974). However, the data showing activity with $(dG)_{12-18}\cdot(Cm)_n$ relative to activity with preferred template primers was not presented. Although the reverse transcriptases of avian, murine, and primate type-C RNA tumor viruses utilize this template, their usage as shown here is inefficient. This becomes important if one is attempting to detect reverse transcriptase activity in non virus-producing cells, where sensitivity is crucial. Such usage of this template for detection of a reverse transcriptase in human breast tumors has been reported (Gerard et al., 1975). Nevertheless, additional criteria must be considered when examining relatively impure cell systems. It seems likely that without removal of nucleic acids from crude extracts observed activity can not be confidently judged to be due to reverse transcriptase. The possibility that nucleic acid–enzyme complexes are responsible for the activity must be considered. In this regard, more vigorous methods of cell fractionation and enzyme isolation must be employed before such a specific template is used (Sarngadharan et al., 1975).

Reverse transcriptase is not found in large amounts in cells. Considering the low sensitivity of $(dG)_{12-18}\cdot(Cm)_n$, additional criteria will continue to be necessary to score for the presence of reverse transcriptase. In this regard, the studies reported here suggest an alternative method for distinguishing reverse transcriptase activity from DNA polymerase γ: the ability of the latter to utilize $(A)_{13}\cdot(dT)_n$ as template. DNA polymerase γ copies this template as efficiently as $(dT)_{12-18}\cdot(A)_n$; however, the reverse transcriptases of AMV, RLV, and SSV do not use it at all. Thus, a poly(A)-transcribing enzyme with low activity can be judged as cellular or viral using this template. The combined use of the RNA-primed DNA template and $(dG)_{12-18}\cdot(Cm)_n$ may provide a more definitive criterion for distinguishing cellular DNA polymerases from viral reverse transcriptase than the now standard use of ratios of activity with $(dT)_{12-18}\cdot(A)_n$ to $(dT)_{12-18}\cdot(dA)_n$. The data presented here suggest that the ability to distinguish DNA polymerase γ from viral reverse transcriptase by relative activity with these two templates depends on the degree of purity of the system studied. Both reverse transcriptase and DNA polymerase γ prefer $(dT)_{12-18}\cdot(A)_n$ as template. The difference rests on the very poor ability of reverse transcriptase to use $(dT)_{12-18}\cdot(dA)_n$. Thus, depending on the kind and amount of additional polymerase activities present, DNA polymerase γ may resemble DNA polymerase α or β, and reverse transcriptase may look like DNA polymerase γ.

The most definitive criterion for distinguishing reverse transcriptase and DNA polymerase γ continues to be an immunological assay. To date, based on studies with antisera made against purified viral reverse transcriptases, no relationship of the enzymes has been found (Lewis et al., 1974a; Spadari and Weissbach, 1974; Matsukage et al., 1975). As DNA polymerase γ is not obviously related to viral reverse transcriptase, the question of its function remains unclear. It

is presumably not an expression of some sort of endogenous viral information in cells, nor does it seem to be precursor to reverse transcriptase. The fact that it is a minor activity in adult cells suggests several possibilities. It may play a role during some particular phase of development, or it may be largely confined to a certain tissue or cell type. Alternatively, it may merely reflect an activity present in some former organism which has gradually lost importance with continued evolution. It may also be related to some RNA virus other than the type-C RNA tumor virus. All these possibilities remain to be investigated.

Acknowledgments

We thank Amy Herfort and Marilyn Levy for excellent technical assistance, Dr. M. G. Sarngadharan for critically reviewing the manuscript, and Dr. Arthur Weissbach for sending us a preprint of his unpublished work.

References

Abrell, J. W., and Gallo, R. C. (1973), *J. Virol. 12,* 431.
Alberts, B., and Herrick, G. (1971), *Methods Enzymol. 21,* 198.
Bollum, F. J. (1975), *Prog. Nucleic Acid Res. Mol. Biol. 15,* 109.
Chang, L. M. S., and Bollum, F. J. (1972), *Biochemistry 11,* 1264.
Fridlender, B., Fry, M., Bolden, A., and Weissbach, A. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 452.
Gallo, R. C., and Pestka, S. (1970), *J. Mol. Biol. 52,* 195.
Gerard, G. F. (1975), *Biochem. Biophys. Res. Commun. 63,* 706.
Gerard, G. F., Loewenstein, P. M., Green, M., and Rottman, F. (1975), *Nature (London) 256,* 140.
Gerard, G. F., Rottman, F., and Green, M. (1974), *Biochemistry 13,* 1632.
Kacian, D. L., Watson, K. F., Burny, A., and Spiegelman, S. (1971), *Biochim. Biophys. Acta 246,* 365
Keller, W. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 1560.
Knopf, K. W., Yamada, M., and Weissbach, A. (1976), *Biochemistry 15,* 4540.
Lewis, B. J., Abrell, J. W., Smith, R. G., and Gallo, R. C. (1974a), *Science 183,* 867.
Lewis, B. J., Abrell, J. W., Smith, R. G., and Gallo, R. C. (1974b), *Biochim. Biophys. Acta 349,* 148.
Litman, R. M. (1968), *J. Biol. Chem. 243,* 6222.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 183,* 265.
Maizel, J. V. (1969), in Fundamental Techniques in Virology, Habel, K., and Salzman, N. P , Ed., New York, N.Y., Academic Press, p 334.
Martin, R. G., and Ames, B. N. (1961), *J. Biol. Chem. 236,* 1372.
Matsukage, A., Bohn, E. W., and Wilson, S. H. (1974), *Proc. Natl. Acad. Sci. U.S.A., 71,* 587.
Matsukage, A., Bohn, E. W., and Wilson, S. H. (1975), *Biochemistry 14,* 1006.
Robert, M. S., Smith, R. G., Gallo, R. C., Sarin, P. S., and Abrell, J. W. (1972), *Science 176,* 798.
Robert-Guroff, M., and Gallo, R. C. (1976), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 35,* 1720.
Robert-Guroff, M., and Gallo, R. C. (1977), *Biochemistry 16* (following paper in this issue).
Sarngadharan, M. G., Allaudeen, H. S., and Gallo, R. C. (1975), *Methods Cancer Res. 12,* 3.
Schlabach, A., Fridlender, B., Bolden, A., and Weissbach, A. (1971), *Biochem. Biophys. Res. Commun. 44,* 879.
Sedwick, W. D., Wang, T. S., and Korn, D. (1972), *J. Biol. Chem. 247,* 5026.
Sedwick, W. D., Wang, T. S., and Korn, D. (1975), *J. Biol. Chem. 250,* 7045.
Smith, R. G., Abrell, J. W., Lewis, B. J., and Gallo, R. C. (1975), *J. Biol. Chem. 250,* 1702.
Smith, R. G., and Gallo, R. C. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 2879.
Spadari, S., and Weissbach, A. (1974), *J. Biol. Chem. 249,* 5809.
Tamblyn, T. M., and Wells, R. D. (1975), *Biochemistry 14,* 1412.
Wang, T. S., Sedwick, W. D., and Korn, D. (1975), *J. Biol. Chem. 250,* 7040.
Watson, K. F., Nowinski, R. C., Yaniv, A., and Spiegelman, S. (1972), *J. Virol. 1,* 959.
Weissbach, A. (1975), *Cell 5,* 101.
Weissbach, A., Baltimore, D., Bollum, F., Gallo, R., and Korn, D. (1975), *Science 190,* 401.