Isolation and Characterisation of the L Cell Virion
DNA Polymerase

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Summary
The DNA polymerase of the L-cell virion (LCV) was partially purified by chromatography on DEAE cellulose. This enzyme transcribed poly(A)·oligo (dT), poly(C)·oligo(dG), and poly(Cm)·oligo(dG), had a molecular weight of 77,000 daltons and reacted like other murine viral RNA directed DNA polymerases to anti reverse transcriptase specific IgG preparations indicating that it was probably a typical murine viral reverse transcriptase. In addition, like other partially purified mammalian viral reverse transcriptases the LCV DNA polymerase exhibited template independent, primer stimulated DNA synthesis. The significance of these results to the unusual endogenous activity of the LCV is discussed.

Introduction
The demonstration of a ribonuclease-sensitive endogenous DNA synthesis reaction is considered to be a diagnostic feature of the presence of retrovirus-associated reverse transcriptase (9, 11, 22, 27). We have examined the L-cell virion, an apparently endogenous retrovirus-like particle produced by many strains of the mouse L-cell line (7, 12, 13, 19) for such an endogenous reaction. We have found (10) that with this virus the endogenous activity was enhanced, rather than inhibited, by ribonuclease pre-treatment of disrupted virions. It was thought (10) that this finding might be a result of either unusual DNA polymerase, nucleic acid components, or to some combination of these factors in the LCV. In this communication, we describe the isolation and characteristics of the deoxynucleotide polymerising enzymes from the LCV and show that there is a single DNA polymerase with the properties of a normal reverse transcriptase associated with these particles.

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Materials and Methods

Materials

Radioactive deoxynucleoside triphosphates (dNTP's) were obtained from ICN Chemical and Radiisootope Division, Irvine, California or from Amersham Corporation, Oakville, Ont. Unlabelled dNTP’s, dithiothreitol (DTT), ATP, N-ethyl maleimide (NEM), and ribonucleases were from Sigma Chemical Company, St. Louis, Mo. Synthetic template-primers and primers were purchased from PL Biochemicals Inc. Milwaukee, Wis. Aquaeide was from Calbiochem. Inc. San Diego, Ca. Purified AMV reverse transcriptase and terminal transferase (TdT) were from Boehringer Mannheim Inc., Ville St. Laurent, Que. Microgranular DE-52 DEAE cellulose and 3 MM filter discs were obtained from Whatman Biochemicals Ltd., Maidstone, U.K. Tissue culture reagents were from Flow Laboratories, Irvine, Ca.

Anti-reverse transcriptase antisera and various double density gradient purified retroviruses were obtained through the courtesy of Dr. J. Gruber, Office of Program Resources and Logistics, N.I.H. The following viruses and antisera were used in this study: Rauscher murine leukaemia virus (R-MuLV), Lot Number 916-3-111; Moloney murine leukemia virus (Mo-MuLV), Lot Number 5006-32-57; feline endogenous virus RD-114 (RD-114), Lot Number 13—77; baboon endogenous virus M7 (M7), Lot Number 861; simian sarcoma virus (SSV-1), Lot Number 2460; goat anti-AMV reverse transcriptase serum Lot Number 6S-463; goat anti-RMuLV reverse transcriptase serum, Lot Number 7S-121; goat anti-SSV-1 reverse transcriptase serum, Lot Number 77S-122.

LCV Production and Purification

LCV was produced by mouse L-929 cells (originally obtained from Dr. R. Stewart, Queen’s University, Kingston, Ontario), grown in monolayer culture in Eagles Minimal Essential Medium (MEM) containing 10 per cent fetal calf serum. In some experiments the cultures were pre-treated with IUDR and progesterone to enhance virus production. The biochemical properties of the virus from induced or uninduced cultures appeared to be similar.

The virus from (4—6) l quantities of spent culture fluid was purified by differential and sucrose density gradient centrifugation, as described (10). In some experiments crude pelleted virus, prior to the density gradient step, was used for isolation of the DNA polymerase with no apparent differences.

DNA Polymerase Isolation

The virus associated DNA polymerase activity from LCV, Mo-MuLV and R-MuLV was solubilized and partially purified by DEAE-cellulose chromatography essentially as described by Abrell and Gallo (1), except that the initial extraction time was reduced to 1 hour. The partially purified preparation was stored in aliquots at −70°C and under these conditions was stable for at least 6 months.

DNA Polymerase Assay

The standard assay mixture for measurement of the DNA polymerase activity of the mammalian viral enzymes contained 50 mM Tris HCl pH 8.0, 60 mM NaCl, 0.2 per cent (v/v) Nonidet—P40 (NP40), 2 mM DTT, 2.5 mM MnCl2 or 6 mM Magnesium acetate, 20 μg/ml synthetic template primer, the appropriate complementary [3H]-deoxynucleoside triphosphate. After incubation at 37°C, the acid precipitable radioactivity in aliquots of the reaction mixture on 3 MM filters was determined, as previously described (10). In assays of AMV reverse transcriptase a similar assay mixture was used containing 6 mM Mg2+, [3H]-dGTP and poly(C)·oligo(dG) and in addition 0.5 mg/ml bovine albumin. The terminal transferase standard assay mixture similarly contained 2.5 mM Mn2+, [3H]-dGTP, oligo(dA) and 0.5 mg/ml bovine albumin. These reaction conditions were specific for AMV reverse transcriptase and TdT, respectively and enabled the reliable assay of the individual enzymes in a mixture of the two.