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Hamster Leukemia Virus: Lack of Endogenous DNA Synthesis and Unique Structure of Its DNA Polymerase

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Infectious hamster leukemia virus (HaLV) contains a DNA polymerase different from those of murine and avian viruses. No endogenous reaction directed by the 60 to 70*S* RNA of HaLV could be demonstrated in detergent-treated HaLV virions, nor could the purified DNA polymerase copy added viral RNA. The virion RNA could, however, act as template for added avian myeloblastosis virus DNA polymerase and the HaLV DNA polymerase could efficiently utilize homopolymers as templates. The HaLV enzyme was like other reverse transcriptases in that certain ribohomopolymers were much better templates than the homologous deoxyribohomopolymers. No ribonuclease H activity could be shown in the HaLV enzyme, but neither could activity be found in the murine leukemia virus DNA polymerase. The hamster enzyme was unique in that poly(A)·oligo(dT) was a poor template, and globin mRNA primed with oligo(dT) was totally inactive as a template. Its uniqueness was also indicated by its subunit composition; electrophoresis of the HaLV DNA polymerase in sodium dodecyl sulfate-containing polyacrylamide gels revealed equimolar amounts of two polypeptides of molecular weight 68,000 and 53,000. The sedimentation rate of the enzyme in glycerol gradients was consistent with a structure containing one each of the two polypeptides. The enzyme thus appears to be structurally distinct from other known virion DNA polymerases. Its inability to carry out an endogenous reaction in vitro might result from an inability to utilize certain primers.

C-type particles have been isolated from hamster cells under a variety of conditions (6, 11, 13, 20, 27). They contain a unique group-specific antigen (12, 19, 21, 24) and some, at least, are infectious for hamster cells (11, 13, 20). They are referred to as hamster leukemia virus (HaLV) although leukemogenesis by the viruses has only rarely been demonstrated (13).

Most infectious RNA tumor virus preparations will carry out an endogenous DNA polymerization reaction in which the 60 to 70*S* virion RNA acts as template and a DNA polymerase can be purified from such preparations which has a number of characteristic properties (28). We have found that preparations of hamster leukemia virus (HaLV) are exceptional in that no endogenous reaction is demonstrable although a fairly standard "reverse transcriptase" can be purified from the virions. The inability of the virions to carry out an endogenous reaction in vitro appears to be a characteristic of the DNA polymerase because both the 60 to 70*S* RNA in disrupted HaLV virions and purified HaLV 60 to 70*S* RNA will act as templates for DNA synthesis by the avian myeloblastosis virus (AMV) DNA polymerase.

MATERIALS AND METHODS

Materials. Tritium-labeled and unlabeled deoxyribonucleoside triphosphates were obtained from New England Nuclear Corp. and Schwarz-Mann. Polynucleotides were obtained from Miles Laboratories or P-L Biochemicals. Oligonucleotides were from 8 to 18 in length and were obtained from Collaborative Research. AMV in plasma and purified Rauscher murine leukemia virus (R-MuLV) were provided under contract from the Virus Cancer Program of the National Cancer Institute.

Preparations of virions. GLOH$^-$ cells were kindly provided by R. Gilden at passage 29 and grown in Joklik-modified Eagle medium supplemented with 7% fetal calf serum and nonessential amino acids. These cells are hamster embryo fibroblasts that were productively infected with HaLV which was derived from a productive murine sarcoma virus-induced hamster tumor by dilution beyond the end point of

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transforming virus (11, 19). Tissue culture supernatant fluids were harvested on day 4 after passaging the cells and virus was then purified as described for MuLV by Fan and Baltimore (8). The virus banded in sucrose at a density of 1.15 g/ml and contained 60 to 70S RNA. Hamster sarcoma virus (HaSV) was obtained from hamster embryo fibroblasts (NiL cells) infected with HaSV from B-34 cells (6, 35) (provided by P. Robbins of M.I.T.).

Purified Moloney murine leukemia virus (M-MuLV) was obtained as described by Fan and Baltimore (8). AMV was purified from chicken serum as previously described (30).

Purification of virion DNA polymerases. For purification of the HaLV DNA polymerase, purified HaLV virions were lysed with non-ionic detergent, and then DNA polymerase was purified by column chromatography on DEAE-Sephadex followed by phosphocellulose as described before (5, 30), with the following modifications. DEAE-Sephadex (A-25) was equilibrated with buffer A (50 mM Tris-hydrochloride, pH 7.9, 10 mM β-mercaptoethanol, 2 mM magnesium acetate, 10 μM manganese chloride, 0.1% Nonidet P-40, and 25% glycerol) and the enzyme was eluted at about 0.07 M KCl in a gradient from 0 to 0.6 M KCl in buffer A. The phosphocellulose column was equilibrated with buffer B (buffer A without divalent cations) with 0.1 M KCl and the enzyme was eluted at about 0.3 M KCl in a gradient from 0.1 M to 0.6 M KCl in buffer B. Assays for DNA polymerase activity and determination of units of activity were carried out as described before (30). All enzyme reactions were carried out for times during which synthesis was a linear function of time. The purified enzyme was concentrated by dialysis against solid polyethylene glycol and then equilibrated by dialysis with buffer B containing 10% glycerol.

AMV DNA polymerase was purified and assayed as described previously (5, 30). MuLV DNA polymerase was purified in an identical manner except that 0.1% detergent was present in the buffers rather than 0.2%.

RNase H assay. RNase H was assayed by using [^{3}H]poly(A)·poly(dT) as substrate and monitoring the degradation of [^{3}H]poly(A) to acid-soluble material. The reaction mixture in 0.1 ml contained 50 mM Tris-hydrochloride (pH 8.3), 10 mM dithiothreitol, 20 mM magnesium acetate, 30 mM NaCl, 10.7 pmol of [^{3}H]poly(A) (1,000 counts per min per pmol), and 2.8 pmol of poly(dT). The reaction was carried out at 37 C for 60 min (details of the procedure will be published elsewhere in association with S. Drost).

Polyacrylamide gel electrophoresis. Acrylamide, bis-acrylamide gels of 7.5% containing sodium dodecyl sulfate (SDS) were prepared according to the method of Laemmli and Maizel (22, 23). The samples were prepared by precipitation of the protein with 10% trichloroacetic acid. After 15 min at 4 C, the precipitate was recovered by centrifugation in a clinical centrifuge. The precipitate was washed twice with acetone by centrifugation, and the final pellet was dissolved in water. Recovery was 80 to 100%. Approximately 1 to 2 μg of protein was analyzed on a gel. Electrophoresis was carried out for 4 h at 3 A/gel. The gels were stained with Coomassie blue and destained as described (7) and scanned at 550 mμ in a Gilford spectrophotometer. The areas under the peaks were determined by a planimeter. Phosphorylase b, electrophoretically pure bovine serum albumin, and α-amylase (Calbiochem) were used as standards.

RESULTS

DNA polymerase activity in purified virions. The endogenous and exogenously stimulated DNA polymerase activities observed in purified HaLV and HaSV virions after different methods of virion disruption were compared with the DNA polymerase activities of equal amounts of purified M-MuLV and AMV virions (Table 1). Intact HaLV showed a small amount of "endogenous" DNA synthesis and disruption of the virions with detergent or ether increased synthesis only a little. Varying the concentration of Nonidet P-40 from 0.001 to 0.2% did not produce any more endogenous synthesis than shown in Table 1. An amount of disrupted MuLV or AMV virions equal to that of the HaLV exhibited at least 20-fold more endogenous activity than the HaLV (Table 1) and required detergent treatment in order to reveal this activity (1). HaSV lacked a measureable level of endogenous activity.

The HaLV virions did not inhibit the endogenous activity of MuLV virions, and even addition of poly(dT) to disrupted HaLV could not stimulate endogenous activity. When AMV DNA polymerase was added to the disrupted HaLV virions, however, synthesis of DNA was evident (Table 1, final line) and this DNA would hybridize to HaLV 70S RNA (Fan, unpublished observations). Approximately 20% of the HaLV-specific DNA would also hybridize to MuLV-RNA, suggesting a partial homology of these two viruses. The virions of HaLV therefore appear to virtually lack DNA polymerase activity capable of reverse transcribing HaLV RNA in vitro, but the RNA itself is a functional template.

When HaLV virions were assayed for DNA polymerase activity stimulated by an exogenous template, a different picture emerged. Table 1 shows data on the stimulation of dGMP incorporation by poly(C)·oligo(dG). This template·primer will only be copied efficiently by DNA polymerases from RNA tumor viruses and not by enzymes from uninfected cells (3, 25, 28). Using a level of 0.1 of the virion protein concentration used to study the endogenous reaction, undisrupted HaLV virions showed no exogenous activity but detergent treatment revealed a level of dGMP incorporation almost equal to that of detergent-treated MuLV virions (Table 1). HaSV virions showed extensive exogenous

TABLE 1. *DNA polymerase activity of HaLV with endogenous and exogenous templates*[a]

Samples	[^{3}H]dGMP incorporated (pmol)		
	Endogenous reaction	Exogenous reaction	Ratio of exogenous to endogenous
HaLV	0.16	0.1	1.6
HaLV plus Nonidet P-40	0.30	216	>1,800
HaLV plus ether	0.21		
HaLV plus poly(dT) plus Nonidet P-40	0.23		
HaSV plus Nonidet P-40	<0.10	150	>3,750
M-MuLV plus Nonidet P-40	8.03	340	105
M-MuLV plus HaLV plus Nonidet P-40	8.16	482	150
AMV plus Nonidet P-40	10	680	170
AMV plus ether	11		
HaLV plus purified AMV DNA polymerase plus 0.2% Nonidet P-40	4.1		

[a] Endogenous reaction: The complete reaction mix consisted of the following in 0.1 ml: 50 mM Tris-hydrochloride (pH 8.3), 10 mM dithiothreitol, 10 μM manganese chloride, 2 mM magnesium acetate, 60 mM NaCl, 60 nmol of dATP, dCTP, and dTTP, 40 μmol of [^{3}H]dGTP (4,000 counts per min per pmol) and 0.01% Nonidet P-40. In the case of AMV, the detergent concentration was 0.2%, no manganese was used and 6 mM magnesium acetate was added. About 20 μg of viral protein was used in each assay, except for HaSV for which the protein content was not determined. The amount of purified AMV DNA polymerase used was 0.5 to 1.0 unit as defined previously (30). The tubes were sealed under N_2 and incubated at 37 C for 90 min. The acid-precipitable material was determined as described before (2). Ether extraction of virions was performed by adding an equal volume of ether to the virion preparation, mixing for 10 min at 4 C and removing the ether by bubbling N_2 through the preparation. Exogenous reaction: The complete reaction mixture in 0.1 ml was the same as described for the endogenous reaction except that 1 μg of poly(C) and 0.5 μg of oligo(dG) was added and 160 μmol of dGTP (50 counts per min per pmol) was the only deoxyribonucleoside triphosphate included. The amount of detergent used was 0.05% for HaLV, HaSV and M-MuLV and 0.2% for AMV. The amount of viral protein used was about 2 μg per reaction for all viruses except HaSV, which was not determined. Ratio of exogenous to endogenous activity: The ratio was determined by multiplying the endogenous activity by 4 (to include incorporation of the unlabeled nucleotides). The exogenous activity was multiplied by 10 because it was determined using 0.1 of the amount of protein used for the endogenous reaction. For the "HaLV plus Nonidet P-40" the ratio is given as >1,800 because the activity seen without detergent is responsible for at least half of the incorporation.

activity also. The ratios of exogenous to endogenous activity, corrected for the incorporation of the three other unlabeled deoxyribonucleoside triphosphates in the endogenous reaction, are tabulated in Table 1. It is evident that the HaLV and HaSV virions contain DNA polymerases which are extremely deficient in endogenous activity compared to their exogenous activity.

HaLV DNA polymerase. From HaLV virions a DNA polymerase was purified by using the same methodology as we have employed previously for other viral DNA polymerases (30). The enzyme was assayed during purification by using poly(C)·oligo(dG) as template·primer, and the purified enzyme maintained a linear rate of synthesis for at least 90 min at 37 C. Utilization of templates by the enzyme required the presence of complementary primers as seen previously (4).

The template specificity of purified HaLV DNA polymerase as well as that of the DNA polymerase activity obtained after detergent treatment of purified HaLV virions and that of purified AMV DNA polymerase are shown in Table 2. Like other known DNA polymerases from RNA tumor viruses (4), HaLV DNA polymerase shows a marked preference for certain ribohomopolymers as templates over their homologous deoxyribohomopolymers [compare poly(I) to poly(dI) and poly(A) to poly(dA)]. Like the MuLV DNA polymerase (4), poly(dC)·oligo(dG) is a more efficient template for the HaLV enzyme than poly(C)·oligo(dG). The ability of HaLV polymerase to copy poly(C)·oligo(dG) is a critical finding in support of the idea that the HaLV enzyme is truly a "reverse transcriptase" (3, 25, 28).

The purified HaLV DNA polymerase did not efficiently utilize poly(A)·oligo(dT) as template·primer, whereas poly(A)·poly(dT) was efficiently utilized (Table 2). Most known RNA tumor virus DNA polymerases readily utilize primers of small chain length (4, 28), but purified HaLV DNA polymerase apparently prefers a longer chain length. The disrupted

virions do not show the same preference but, in experiments other than that shown in Table 2, poly(A)·oligo(dT) was poorly utilized even by virions.

It should be noted that the results in Table 2 are comparisons of reaction rates under a given set of conditions and it is possible that a change in the ionic conditions might alter the efficiency of utilization of certain templates.

When HaLV enzyme was compared to AMV enzyme for its ability to copy 60 to 70*S* HaLV RNA and globin mRNA·oligo(dT) (Table 3), no activity was seen with the hamster enzyme, whereas the AMV enzyme was quite active on both templates (33). HaLV enzyme would also not copy globin mRNA·oligo(dG) which is an efficient template for the AMV enzyme. In a similar experiment, R-MuLV DNA polymerase would copy globin mRNA but would copy 60 to 70*S* RNA only poorly (Smoleri and Verma, unpublished observations). The inability of the murine enzyme to copy viral RNA efficiently is in agreement with observations of Wang and Duesberg (34).

RNase H activity of purified HaLV DNA polymerase. RNase H activity in purified HaLV DNA polymerase was assayed by the conversion of radioactivity in a [^{3}H]poly(A)·poly(dT) substrate to acid-soluble form. The RNase H activity of HaLV DNA polymerase was compared with that of purified M-MuLV DNA polymerase and AMV DNA polymerase (Table 4). Under the conditions where the DNA polymerase activity of these enzymes was approximately equal, there was no significant degradation of [^{3}H]poly(A) to acid-soluble products in the presence of HaLV DNA polymerase or M-MuLV DNA polymerase, whereas AMV DNA polymerase degraded 60% of the poly(A). Thus, based on this assay, purified

TABLE 2. *Template specificity of DNA polymerases*[a]

Template·primers	pmoles Incorporated			
	[^{3}H]dNMP incorporated	HaLV virions	Purified HaLV DNA polymerase	Purified AMV DNA polymerase
Poly(C)·oligo(dG)	dGMP	22	22.5	19
Poly(dC)·oligo(dG)	dGMP	90	36	9
Poly(A)·oligo(dT)	dTMP	13.5	0.2	13
Poly(A)·poly(dT)	dTMP	6.7	4.5	6.5
Poly(dA)·oligo(dT)	dTMP	0.18	0.1	0.3
Poly(dA)·poly(dT)	dTMP	0.25	0.1	0.2
Poly(I)·oligo(dC)	dCMP	20.0	6.4	3.5
Poly(dI)·oligo(dC)	dCMP	0.3	<0.1	1.4
Poly(U)·oligo(dA)	dAMP	<0.01	<0.01	<0.01
Poly(dT)·oligo(dA)	dAMP	<0.01	<0.01	<0.01
Poly(dA-dT)	dTMP	3.0	7.0	0.3

[a] The same reaction mixture as described in the legend to Table 1 for the exogenous reaction was used except for addition of appropriate template·primer and complementary deoxyribonucleoside triphosphate. The amount of HaLV virion used was 0.2 μg per reaction. For the purified enzymes, 0.05 units of HaLV DNA polymerase or AMV DNA polymerase were added per reaction. The amounts of template and primer used was 1.0 and 0.5 μg, respectively. The reactions were carried out under N_2 atmosphere for 60 min at 37 C.

TABLE 3. *Polyribonucleotides as templates for DNA polymerases*[a]

Template·primer	dGMP incorporated (pmol)				
	HaLV polymerase			AMV polymerase	
	0.05 unit	0.1 unit	0.2 unit	0.05 unit	0.1 unit
Poly(C)·oligo(dG)	22.5	47	86	19	41
60 to 70*S* HaLV RNA	<0.04	<0.04	<0.04	0.4	0.8
Globin mRNA·oligo(dT)	<0.04	<0.04	<0.04	5	11

[a] The reaction mixtures described in Table 1 were used with [^{3}H]dGTP (5,000 counts per min per pmol for the HaLV enzyme with the two RNAs and 50 counts per min per pmol for the other reactions). The poly(C)·oligo(dG)-stimulated reaction was incubated for 60 min and the others for 90 min at 37 C. The amount of RNA used was 2,800 pmol of nucleotide and 1,400 pmol of nucleotide in $(dT)_{10}$ was used as primer for the globin mRNA.

HaLV DNA polymerase and M-MuLV DNA polymerase do not exhibit any detectable RNase H activity. The results with M-MuLV DNA polymerase confirm those of Wang and Duesberg (34).

Physical analysis of HaLV DNA polymerase. Electrophoresis of purified HaLV DNA polymerase in SDS-containing polyacrylamide gels revealed two polypeptide bands. When compared to standards of known molecular weight, the bands were found to represent polypeptides of 68,000 and 53,000 (Fig. 1). The ratio of the estimated molecular weights of the larger to the smaller of these two polypeptides was approximately 1.3, and quantitation by optical density scanning of stained gels at 550 nm indicated a mass ratio of 1.4. Thus, it appears that the HaLV DNA polymerase preparation contained these two polypeptides in equimolar amounts. In comparison, AMV DNA polymerase showed two bands of 90,000 and 55,000 daltons, whereas R-MuLV viral DNA polymerase has one band of approximately 70,000 daltons (Verma, unpublished results). These results with AMV and R-MuLV polymerases confirm those of Kacian et al. (17), Grandgenett et al. (14), and Tronick et al. (29) except that we find a lower molecular weight for the AMV polymerase subunits than previously reported (25).

TABLE 4. *Relative RNase H activity of DNA polymerases*[a]

Sample	DNA polymerase act (pmol of [^{3}H]dGMP incorporated)	RNase H act-[% poly(A) degraded]
HaLV DNA polymerase	1,100	<1
AMV DNA polymerase	1,160	60
R-MuLV DNA polymerase ..	1,000	<1

[a] DNA polymerase assays used poly(C)·oligo(dG) as indicated for exogenous reaction in Table 1 but were incubated for 15 min. RNase H was determined as described in the Materials and Methods. About 10 units of each of the DNA polymerases were used for both assays.

The AMV, MuLV, and HaLV enzymes were analyzed by glycerol gradient centrifugation (Fig. 2). AMV polymerase sedimented ahead of *Escherichia coli* DNA polymerase I marker (110,000 daltons [16]), indicating a molecular weight significantly in excess of 110,000. MuLV polymerase sedimented behind the marker, whereas HaLV DNA polymerase sedimented just ahead of the marker. The sedimentation rates of the mammalian enzymes were consistent with molecular weights of 70,000 for the MuLV enzyme and 120,000 for the HaLV enzyme. Thus, sedimentation analysis indicates that the HaLV polymerase has a molecular weight consistent with it having one molecule each of the two polypeptides found by gel electrophoresis.

DISCUSSION

Virions of HaLV appear in many ways to be normal C-type viruses. They have a buoyant density in sucrose of 1.15 g/ml and a 60 to 70*S* RNA. They have a DNA polymerase able to copy poly(C)·oligo(dG) and their RNA is able to act as template for the AMV DNA polymerase. However, in disrupted virion preparations the HaLV DNA polymerase will not synthesize

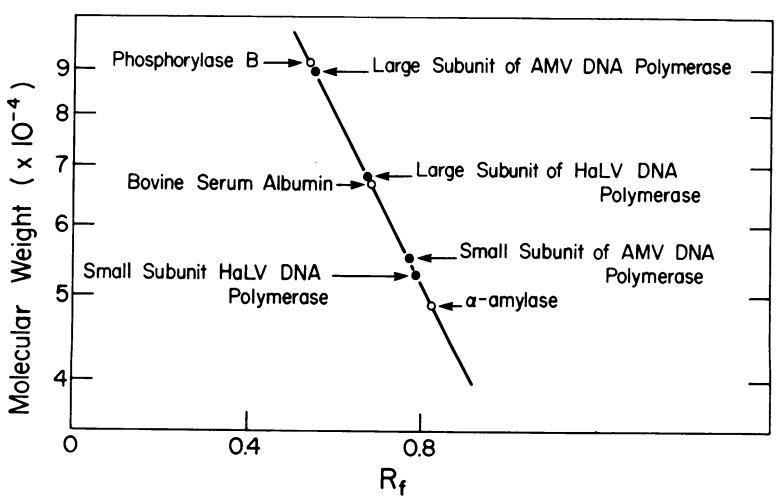

FIG. 1. *Determination of the molecular weights of the subunits of HaLV DNA polymerase on polyacrylamide gels.*

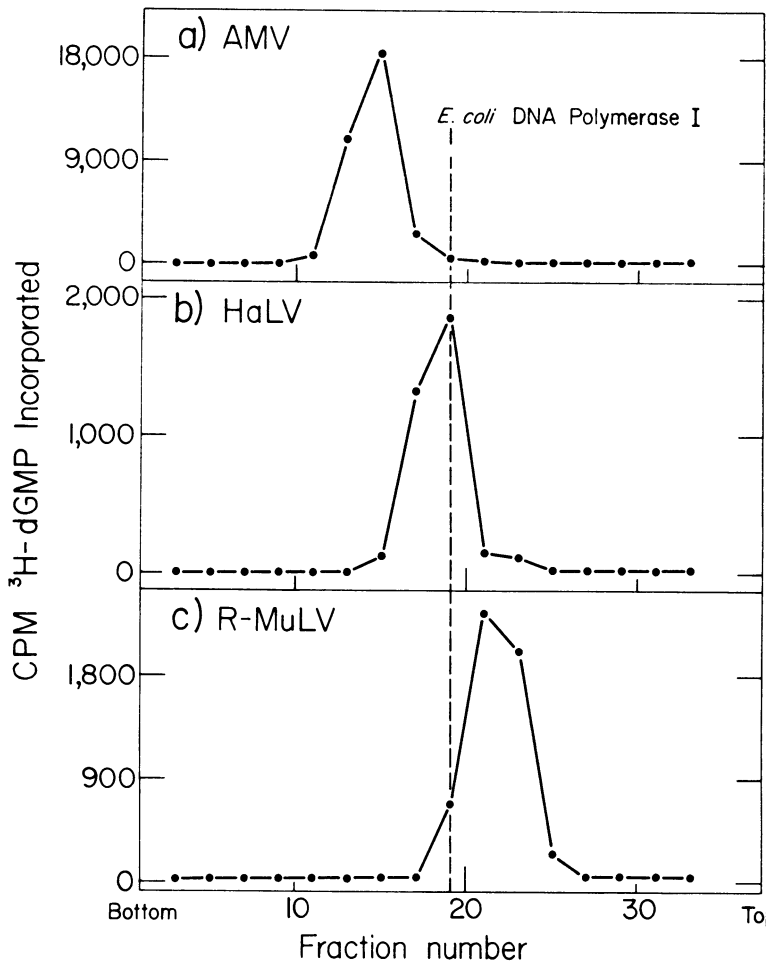

FIG. 2. *Glycerol gradient centrifugation of DNA polymerases. Twenty-two units of AMV DNA polymerase, 15 units of R-MuLV DNA polymerase, and 5 units of HaLV DNA polymerase each contained in 0.4 ml were layered separately on 4.6 ml, 20 to 40% glycerol gradients made in 50 mM Tris-hydrochloride, pH 8.3, 0.1 M β-mercaptoethanol, 0.001 M EDTA, 0.2 M KCl, and 0.2% Nonidet P-40. To each gradient was added 0.24 μg of E. coli DNA polymerase 1 as marker; the peak position of this enzyme is shown. The gradients were centrifuged for 27 h at 45,000 rpm at 4 C in SW 50.1 rotor of a Spinco ultracentrifuge. The gradients were punctured from the bottom, and fractions of 0.13 ml were collected. For assays of viral DNA polymerases 50 μliters of every other fraction was added to a reaction mixture described for exogenous reaction in Table 1, except that the specific activity of [³H]dGTP was 500 counts per min per pmol. The reaction mixture was incubated at 37 C for 60 min. For E. coli DNA polymerase activity 20 μliters of every other fraction was added to a reaction mixture (16) containing 50 mM potassium phosphate, pH 7.5, 6 mM magnesium acetate, 10 nmol of [³H]dTTP (500 counts per min per pmol), 0.5 μg of poly(dA), and 0.05 μg of oligo(dT) to a final volume of 0.1 ml. The reaction mixture was incubated at 37 C for 60 min. The recovery of AMV DNA polymerase was over 90%, whereas HaLV DNA polymerase and R-MuLV DNA polymerase was 5 and 1%, respectively. The figure is drawn by aligning the E. coli DNA polymerase 1 activity of the three gradients.*

detectable DNA by copying the 60 to 70S RNA found in the virions. The purified HaLV enzyme will also not copy 60 to 70S RNA nor will it copy globin mRNA·oligo(dT), and it is very inefficient with poly(A)·oligo(dT). The inability of heteropolymers to act as templates for the HaLV enzyme could be an intrinsic property of the enzyme or it could be that certain primers, such as those associated with 60 to 70S RNA (9, 10, 27, 28, 31, 32) cannot be utilized by the polymerase. Kang and Temin (18) have noted that reticuloendotheliosis virus also contains both a DNA polymerase which demonstrates little endogenous activity and an RNA which

can be copied by a heterologous DNA polymerase.

A small amount of DNA was produced by HaLV virions in the absence of detergent (Table 1). A previous report noted such an activity (15) which was interpreted as an endogenous reaction. However, since no detergent is required to demonstrate this activity, it is probably due to contamination of the virion preparations with cellular DNA polymerase. The amount of potential endogenous reaction in HaLV is therefore only the difference of the detergent-treated and the untreated sample. This activity is not significant.

The MuLV DNA polymerase, although poor in its ability to copy 60 to 70*S* RNA, does synthesize detectable DNA in response to this template (34; Smoler and Verma, unpublished observations). MuLV enzyme can also utilize 60 to 70*S* RNA primed with oligomers (34), globin mRNA·oligo(dT), and poly(A)·oligo(dT). The HaLV enzyme is therefore even less responsive to viral RNAs and oligo(dT)-primed RNAs than the murine enzyme, and both are deficient relative to avian enzymes.

The HaLV enzyme differs from the MuLV enzyme in having two subunits instead of one. In this way it resembles the avian enzyme although the molecular weights of one of the avian and hamster enzyme subunits are quite different. The murine and hamster enzymes are similar in having no detectable RNase H activity, whereas the avian enzyme has RNase H activity in the polypeptide which has DNA polymerase activity (14).

In spite of its lack of detectable endogenous DNA polymerase activity, HaLV is an infectious virus. The cells used in our studies were producing infectious virus originally (19) and were recently rechecked by K. Somers and S. Kit (personal communication) who showed that the released virus would infect hamster embryo fibroblasts and would rescue MSV from nonproducer transformed hamster cells (we are grateful to them for performing these tests). The titers of virus were, however, quite low relative to amount of physical virions produced. Freeman et al. (11) also noted previously the low specific infectivity of HaLV. Whether it might be more infectious in cells other than those derived from hamster requires further study. The low specific infectivity of HaLV, however, suggests that the enzyme we have characterized may be derived from noninfectious particles.

The HaSV used in our studies was also infectious (P. W. Robbins, personal communication). It presumably is really MuSV(HaLV) and its lack of endogenous DNA polymerase activity is probably a result of its DNA polymerase being derived from HaLV. D. Ward (Yale University) has also observed that HaSV demonstrates little or no endogenous DNA synthesis but that a DNA polymerase is present (personal communication).

Somers et al. (26) reported that a line of hamster cells derived from a lymphoma, called D-9, were producing a noninfectious C-type particle which lacked DNA polymerase activity. We were able to confirm the lack of DNA polymerase in D-9 virus by using all of the templates described here (Verma and Paskind, unpublished results). The D-9 virus thus differs from other HaLV strains, and its lack of infectivity is probably due to its lack of DNA polymerase as suggested by Somers et al. (26).

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