Endogenous RNA-Directed DNA Polymerase Activity in Uninfected Chicken Embryos

(RNA tumor viruses)

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ABSTRACT Early chicken embryos that are either positive or negative for group-specific antigens of avian leukosis viruses contained endogenous RNA-directed DNA polymerase activity. This endogenous DNA polymerase activity was not increased after mixture of soluble DNA polymerases isolated from chicken embryos with disrupted chicken embryo cells. The endogenous activity was resistant to treatment with deoxyribonuclease, and the initial rate of DNA synthesis was partially resistant to actinomycin D. In contrast, over 90% of the endogenous polymerase activity was destroyed by ribonuclease in medium with high salt concentration. The DNA product of the endogenous DNA polymerase activity from chicken embryos did not hybridize with RNA of Rous sarcoma virus or reticuloendotheliosis virus, whereas about 40% of this DNA product hybridized with the RNA from the same chicken-cell fraction. Antibody against DNA polymerase of avian myeloblastosis virus did not neutralize the chicken endogenous DNA polymerase activity. These results demonstrate that uninfected chicken embryo cells contain endogenous RNA-directed DNA polymerase activity that is not derived from avian leukosis or reticuloendotheliosis viruses.

Endogenous RNA-directed DNA polymerase activity has been found in the virions of all RNA tumor viruses and in a few other RNA-containing viruses (1–3). For several years, we have discussed the hypothesis that RNA-directed DNA synthesis may have important roles in normal cell development (4–6) for such functions as differentiation, antibody formation, and gene amplification. Crippa and Tocchini–Valentini (7) have suggested a similar mechanism for the amplification of the genes for ribosomal RNA. Coffin and Temin (8) recently reported the isolation of an endogenous ribonuclease-sensitive DNA polymerase activity from rat cells infected with B77 virus and from uninfected rat cells. The template for the endogenous DNA polymerase activity from rat cells infected with B77 virus was not related to RNA of B77 virus or to RNA of a rat C-type virus. Here we report the isolation and characterization of endogenous RNA-directed DNA polymerase activity from uninfected chicken embryos.

MATERIALS AND METHODS

General experimental procedures performed in our laboratory were described (8–11).

Primary cultures of chicken fibroblasts were prepared from individual 12-day-old white Leghorn embryos (Sunnyside Hatchery Co., Oregon, Wis.) and were grown in modified Eagle's medium (9) with 20% tryptose phosphate broth and

Abbreviations: gs, group specific; ALV, avian leukosis virus.

5% fetal-calf serum. The cells were tested by standard methods for the presence of contaminating avian leukosis viruses (ALV) and for ALV group-specific (gs) antigens (12, 13). Two of 30 embryos had a contaminating ALV and 20% were ALV gs-antigen positive. B77 strain of avian sarcoma virus and strain T of reticuloendotheliosis virus (14), a kind gift of Dr. A. Levine, Terre Haute, Ind., were grown in cultures of chicken embryo cells negative for ALV gs-antigen, and concentrated and purified as described (11).

Labeled DNA products were prepared by standard polymerase reactions of Temin and Mizutani (2), performed in a total volume of 1.25–6.25 ml containing both [³H]TTP and [³H]dCTP (see Table 1). Both [³H]TTP and [³H]dCTP were incorporated into the product DNA. Nucleic acids from polymerase reactions and purified virus were extracted with diethyl pyrocarbonate (Baycovin; Bayer Ltd., Leverkusen, Germany) and sodium dodecyl sulfate as described (11).

RESULTS

Properties of DNA Polymerase-Containing Fraction from Uninfected Chicken Embryo Cells. A previous report (8) from our laboratory has shown that a pellet fraction obtained by high-speed centrifugation of Nonidet P-40-disrupted, uninfected rat cells and B77 virus-infected rat cells contain endogenous DNA polymerase activity that is sensitive to treatment with ribonuclease. To determine whether similar activity could be isolated from uninfected chicken embryo cells, a cytoplasmic extract was prepared from 5-day-old chicken embryos, centrifuged at 45,000 rpm for 1 hr in a Spinco SW50.1 rotor, and tested for endogenous DNA polymerase activity. The pellet, which contained endogenous DNA polymerase activity, was resuspended in Tris · HCl buffer (pH 7.6) and layered on a 10–45% sucrose gradient made in Tris · HCl buffer. The gradient was fractionated after 1 hr of centrifugation at 45,000 rpm in a Spinco SW50.1 rotor, and each fraction was assayed for endogenous and exogenous (calf-thymus DNA-directed) DNA polymerase activities (Fig. 1). The endogenous and exogenous DNA polymerase activities were separated in this gradient. The major endogenous DNA polymerase activity was at a density of 1.05 g/cm³, and the major exogenous DNA polymerase activity was at a density of 1.07 g/cm³. These two DNA polymerase activities could also be separated by velocity sedimentation on a 5–20% sucrose gradient (8). When the 1.05 g/cm³ peak of endogenous DNA polymerase activity was centrifuged at 45,000 rpm for 30 min in a discontinuous gradient of 1.5 ml of 7.5% sucrose and 2.5 ml of 10% sucrose, over 75% of the endogenous activity was recovered from the pellet fraction,

whereas most of the exogenous activity was recovered in the gradient region. Therefore, all preparations containing endogenous DNA polymerase activity were made by a combination of density- and velocity-gradient centrifugation.

To determine whether endogenous DNA polymerase activity was present in cells that were ALV gs-antigen negative or positive, we isolated fractions separately from secondary cells that were prepared separately from each of two gs-antigen negative chicken embryos and separately from each of two gs-antigen positive chicken embryos. Endogenous DNA polymerase activity approximately equal to that found in embryos was present in both ALV gs-antigen negative and positive chicken embryo cells (data not shown).

We attempted to reconstruct endogenous DNA polymerase activity by mixing 0.5 ml of crude soluble DNA polymerases from normal 7-day chicken embryos (kindly supplied by Dr. S. Mizutani) containing 116,000 cpm/hr of 25 µg/ml calf thymus DNA-directed activity with 4.5 ml of the disrupted chicken embryos (Fig. 1) and incubating at room temperature for 30 min. The mixture was centrifuged, and the fraction with endogenous DNA polymerase activity was prepared in 0.5 ml of buffer as described in Fig. 1. The endogenous DNA polymerase activity prepared in the presence of the soluble

TABLE 1. *Requirements for DNA synthesis by chicken endogenous DNA polymerase activity*

Reaction conditions	[³H]TMP incorporated (cpm)
Complete*	8,600
− Mg^{++}	400
− Mg^{++} + Mn^{++}†	3,600
− dCTP	1,700
+ oligo(dC)$_{12-18}$ 500 pmol/reaction	8,750
+ oligo(dT)$_{12-18}$ 500 pmol/reaction	8,200
+ yeast RNA 25 µg/reaction	8,800
+ native calf-thymus DNA (25 µg/reaction)	328,300

Individual components of the DNA polymerase assay (2) were omitted or additions were made as indicated. All assays were incubated for 30 min at 41°. Oligo(dC)$_{12-18}$ and oligo(dT)$_{12-18}$ were purchased from Collaborative Research, Inc., Waltham, Mass.; yeast RNA and calf-thymus DNA were purchased from Worthington Biochemical Corp.

* A complete reaction mixture contained 10 nM dATP, 10 nM dCTP, 10 nM dGTP, 7.5 nM ATP, 10 mM MgCl₂, 20 mM KCl, 0.25 µg phosphoenolpyruvate, 10 µg pyruvate kinase, 2.5 µCi (about 0.2 nM) [³H]TTP in 100 µl of 20 mM Tris·HCl (pH 8.0) containing 4 mM EDTA and 10 mM dithiothreitol. 25 µl of the chicken fraction containing endogenous DNA polymerase activity was added to 100 µl of the reaction mixture.
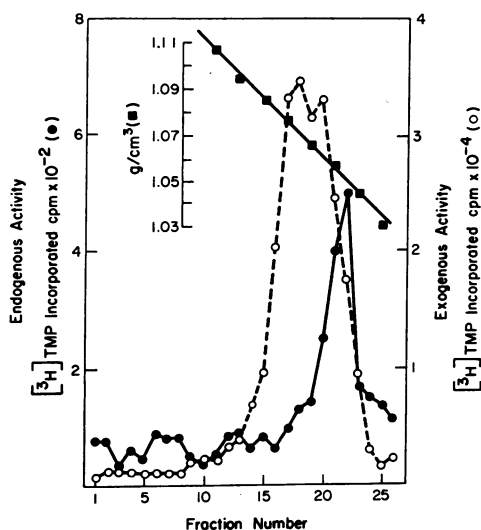
† 1.5 mM MnCl₂ was substituted for the MgCl₂.

FIG. 1. Sucrose density gradient centrifugation of DNA polymerase-containing fractions from uninfected chicken embryos. About ten 5-day-old chicken embryos were washed four times with ice-chilled phosphate-buffered saline (pH 7.2), homogenized with five strokes of a tight-fitting glass Dounce homogenizer, and further disrupted by treatment with 4.5 ml of 0.25% Nonidet P-40 (Shell Chemical Co.) and 0.4% dithiothreitol (Sigma Chemical Co.) in 10 mM Tris·1 mM EDTA buffer (pH 7.6) containing 0.25 M sucrose. The disrupted chicken embryos were then centrifuged at 8000 rpm (7000 × g) for 10 min in a Sorvall SS34 rotor, and the supernatant fluid was further centrifuged at 45,000 rpm (190,000 × g) for 1 hr in a Spinco SW50.1 rotor. The pellet from the high-speed centrifugation was suspended in 0.5 ml of Tris·HCl buffer (pH 7.6) containing 0.4% dithiothreitol and 1 mM EDTA, layered on a 10–45% sucrose gradient made in the same buffer, and centrifuged at 45,000 rpm for 1 hr in a SW50.1 rotor. About 0.2-ml fractions were collected and assayed (2) for endogenous (●) and exogenous calf-thymus DNA (25 µg of native DNA/reaction)-directed (O) DNA polymerase activity. About 5-µl samples of every other fraction were used to determine density in g/cm³ (■) by use of a refractometer (Bausch and Lomb).

DNA polymerases was 3200 cpm/hr, the control was 3700 cpm/hr. This result suggests that the endogenous DNA polymerase activity is not an artifact of cell fractionation.

Requirements for Endogenous DNA Polymerase Activity. Table 1 shows the requirements for DNA synthesis by the chicken endogenous DNA polymerase activity. As previously shown for RNA tumor viruses (3), Mg^{++} and all four deoxyribonucleoside triphosphates were required. The low level of DNA synthesis by the endogenous DNA polymerase activity in the absence of dCTP was probably due to the presence of nucleoside triphosphates in the preparation (15). Addition of oligo(dT)$_{12-18}$ or oligo(dC)$_{12-18}$ did not stimulate DNA synthesis. The fraction with endogenous DNA polymerase activity did not use yeast RNA as a template for DNA synthesis, but calf-thymus DNA was an excellent template.

The concentration of divalent cations necessary for optimal DNA synthesis was different for the endogenous DNA polymerase activities from viruses and cells. 15 mM of Mg^{++} was the optimum divalent cation concentration for the chicken DNA polymerase activity and 1.5 mM of Mn^{++} was the optimum cation concentration for B77 virus DNA polymerase activity.

Characterization of Product. The product of the endogenous activity from chickens was made acid-soluble by treatment with pancreatic deoxyribonuclease (data not shown). In neutral sucrose gradients, the product of the endogenous activity from chickens had sedimentation coefficients between 5 and 30 S; in alkaline gradients, it had a sedimentation coefficient of about 5 S (data not shown). A sedimentation profile identical to the control was obtained after the product was treated with 50 µg/ml of pancreatic ribonuclease in either high

or low salt concentrations (data not shown). The product banded in Cs_2SO_4 density gradients at a density of 1.45 g/cm³ (Fig. 3A). We also tested the product by hydroxyapatite column chromatography and found about 40% of the product was eluted by 0.14 M sodium phosphate buffer (pH 7.0) and 60% by 0.4 M sodium phosphate buffer (pH 7.0) (data not shown). Therefore, we conclude that the product of the endogenous activity from chickens is a mixture of single- and double-stranded DNA; the double-stranded DNA having single-stranded breaks (see ref. 16).

The Endogenous Template is RNA. Previous observations indicated that the templates for the endogenous DNA polymerase activity in the high speed pellet fractions from normal rat cells and from rat cells infected with B77 virus were RNA (8). In order to characterize the endogenous template of the chicken activity, the effects of ribonuclease, deoxyribonuclease, and actinomycin D were investigated (Fig. 2A). When the chicken fraction was first incubated with 50 μg/ml of pancreatic ribonuclease, over 98% of the endogenous DNA polymerase activity was inhibited during the 5-min initial reaction and more than 85% was inhibited during the first 0.5 hr of incubation. In contrast, when this fraction was first incubated with 50 μg/ml of pancreatic deoxyribonuclease, no inhibition of the endogenous DNA polymerase activity was observed. A control experiment showed that the deoxyribonuclease was active in the presence of the chicken-cell fraction. The initial rate of DNA synthesis was 40% of the control in the presence of 25 μg/ml of actinomycin D. The effects of ribonuclease, deoxyribonuclease, and actinomycin D
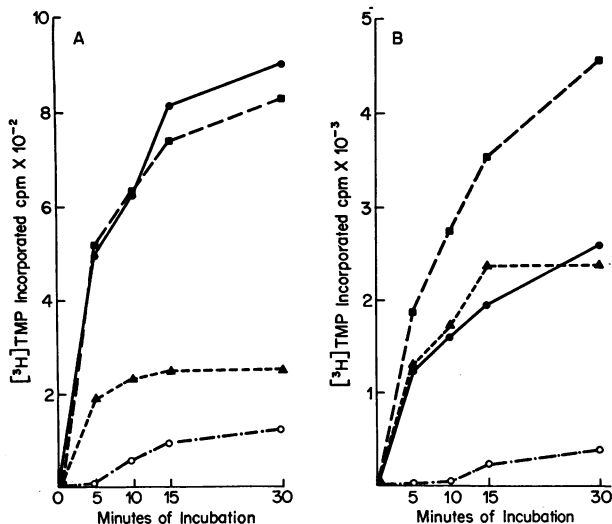

Fig. 2. Effects of nucleases and actinomycin D on endogenous DNA synthesis. The chicken system (A) and disrupted B77 virus (B) were treated with water (■), 50 μg/ml of boiled pancreatic ribonuclease (EC 2.7.7.16) (O) or 50 μg/ml of electrophoretically purified pancreatic deoxyribonuclease (EC 3.1.4.5, Worthington Biochemical Corp.) (●) for 30 min at room temperature (20–23°) in 10 mM Tris·HCl (pH 7.6) containing 0.30 mM NaCl, 30 mM Na-citrate, and 5 mM $MgCl_2$; endogenous DNA polymerase activity was determined by a standard assay procedure (see legend to Table 1). In a parallel experiment, 25 μg of actinomycin D (▲) was added to 1 ml of reaction mixture. 25-μl samples were withdrawn at the indicated times of reaction, and trichloroacetic acid-insoluble radioactivity was determined.

on endogenous DNA synthesis by the chicken system were similar to the effects on the B77 virus endogenous DNA polymerase activity (Fig. 2B). Therefore, the chicken endogenous DNA polymerase activity has the properties of RNA-directed DNA synthesis.

To substantiate the hypothesis that RNA-directed DNA synthesis occurred in the chicken system, nucleic acid hybridization experiments were performed between DNA product from the chicken endogenous DNA polymerase activity and RNA extracted from this fraction. Less than 15% of the DNA product banded at densities greater than 1.53 g/cm³ when 200 μg of the chicken fraction RNA was added per 0.4 ml of annealing mixture (see Fig. 3) (data not shown). However, about 40% of the DNA product of the chicken endogenous activity banded at densities greater than 1.53 g/cm³ in Cs_2SO_4 equilibrium density gradient centrifugation when the [³H]DNA product was annealed with larger amounts (800–1000 μg/0.4 ml) of the purified chicken fraction RNA (Fig. 3C).

Coffin and Temin (17) have shown that 80% of the viral DNA product recovered from hybrid regions of a gradient after annealing with viral RNA hybridized again with viral RNA, whereas only 15% of the viral DNA product recovered from the DNA regions of a gradient after annealing with viral RNA hybridized again with viral RNA. A similar experiment was performed with the DNA product of the chicken endogenous DNA polymerase activity recovered from the gradient shown in Fig. 3C. It was found that about 40% of the DNA product of the chicken endogenous DNA polymerase activity recovered from hybrid regions of a gradient after annealing with chicken fraction RNA hybridized again with 200 μg/0.4 ml of chicken fraction RNA (data not shown). This result suggests that there was a low concentration of template RNA in the chicken fraction.

To determine whether the template RNA was related to avian leukosis virus RNA, we performed nucleic acid hybridization experiments. ³H-labeled DNA product from the chicken endogenous DNA polymerase activity was annealed for 5 hr with 2 μg of RNA from B77 virus and 100 μg of yeast RNA, and was analyzed by Cs_2SO_4 equilibrium density-gradient centrifugation. About 4% of the total counts banded at densities greater than 1.53 g/cm³ (Fig. 3A). When the same endogenous [³H]DNA product was annealed with 100 μg of yeast RNA alone, the same percentage of counts banded at densities greater than 1.53 g/cm³ (data not shown). In contrast, about 43% of the ³H-labeled DNA product from the endogenous B77 virus reaction banded at densities greater than 1.53 g/cm³ when annealed with 1 μg of B77 virus RNA (Fig. 3B). Thus, the template for the chicken endogenous DNA polymerase activity was not related to the RNA of B77 virus. In similar nucleic acid hybridization experiments, the template for the chicken endogenous DNA polymerase activity was shown not to be related to RNA of reticuloendotheliosis virus (data not shown).

We conclude from these experiments that the template for the endogenous DNA polymerase activity in the system isolated from chicken embryos is RNA and is different from the avian leukosis or reticuloendotheliosis virus genomes.

Nature of DNA Polymerase in Chicken System. Antibody to the DNA polymerase of avian myeloblastosis virus was tested for its ability to inhibit the endogenous DNA polymerase activities of B77 virus and of the chicken cells (Fig. 4A). The antibody to avian myeloblastosis virus polymerase did not
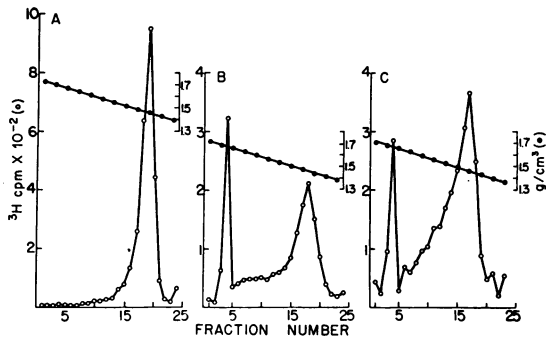
FIG. 3. Hybridization of the product of the chicken endogenous DNA polymerase activity with chicken fraction RNA and B77 virus RNA. RNA-DNA hybridization reactions were performed by a modification of the method described (8). Tritium-labeled DNA product was purified from a 15-min reaction by the chicken endogenous DNA polymerase activity. 50 μl of 10% sodium dodecyl sulfate and 5 μl of Baycovin were added to 1.25 ml of reaction mixture. This mixture was incubated 10 min at 40° and then swirled in a tube under reduced pressure until all the Baycovin was dissolved. 0.25 ml of 2 M KCl was then added, and the mixture was quickly chilled and centrifuged at 12,000 × g for 20 min. The supernatant was poured into 3 ml of cold (−20°) 100% ethanol and left at −20° for 4 hr or more. Nucleic acids were pelleted by centrifugation at 3000 × g for 10 min and suspended again in 0.9 ml of water. 0.1 ml of concentrated (20×) standard saline citrate (0.15 M NaCl–0.015 M sodium citrate, pH 7.0) was added, and the preparation was centrifuged at 12,000 × g for 10 min. The clear supernatant was stored frozen. About 80% of the trichloroacetic acid-precipitable radioactivity from a reaction mixture was finally recovered by this extraction procedure. The DNA preparation was then brought to 0.2 N NaOH and 0.05 M Tris·HCl and incubated for 12 hr at room temperature. After incubation, a small amount of phenol red was added, and the preparation was neutralized with 0.2 N HCl. Appropriate amounts of the labeled DNA product were then distributed in screw-capped vials, and 5 μl of saturated phenol in water, 100 μg of yeast RNA (Worthington Biochemical Corp.), and appropriate amounts of test RNA were added to each vial; all vials were adjusted to the same volume (0.2–0.4 ml) with 0.3 M NaCl–0.03 M sodium citrate. Annealing was performed at 68° for 5 hr. The contents of each vial were then mixed with 0.1 M Tris buffer (pH 7.6) containing 0.01 M EDTA to make 0.7 ml. 20 μl of 5 mg/ml of calf-thymus DNA and 0.6 ml of water were added to 0.7 ml of sample and mixed with 1.75 ml of saturated Cs₂SO₄ (refractive index 1.4015). The refractive index of the final mixture was 1.377. Samples were then analyzed by equilibrium Cs₂SO₄ density-gradient centrifugation at 31,500 rpm for 65 hr in a Spinco SW50.1 rotor, and 23–25 fractions were collected from each gradient through a hole in the bottom of the tube. The refractive index of alternate fractions was measured, and trichloroacetic acid-insoluble radioactivity in a 100-μl portion of each fraction was determined. The amount of hybridization between labeled DNA and RNA was defined as the percent of cpm that banded in such gradients at densities greater than 1.53 g/cm³ (8).

About 4000 cpm of the DNA product of the chicken endogenous DNA polymerase activity was annealed with 2 μg of B77 virus RNA plus 100 μg of yeast RNA (A), and about 4000 cpm of the B77 virus endogenous DNA product was annealed with 1 μg of B77 virus plus 100 μg of uninfected rat-cell RNA (B). About 7000 cpm of the product DNA from the chicken endogenous DNA polymerase activity was annealed with 800 μg of chicken fraction RNA and about one-third of the sample was analyzed by Cs₂SO₄ equilibrium density-gradient centrifugation (C). Density in g/cm³ (●) and trichloroacetic acid-insoluble radioactivity (○) were determined.

neutralize the chicken endogenous DNA polymerase activity, whereas the antibody neutralized B77 virus DNA polymerase activity.

We have also studied antibody blocking power in order to determine whether this chicken DNA polymerase cross-reacts with the antibody to avian myloblastosis virus polymerase. As illustrated in Fig. 4B, this chicken DNA polymerase did not block the neutralizing activity of the antibody to avian myloblastosis virus polymerase. In contrast, B77 virus DNA polymerase containing about 6500 cpm of endogenous activity completely blocked the neutralizing activity of the antibody.

As a further control to demonstrate that this chicken polymerase was not that of a contaminating virus, the antibody to avian myloblastosis virus polymerase was shown to inhibit DNA polymerase activity of two avian leukosis viruses isolated by us from normal chicken cells. Reticuloendotheliosis virus polymerase activity was not inhibited by the antibody, but did have blocking activity.
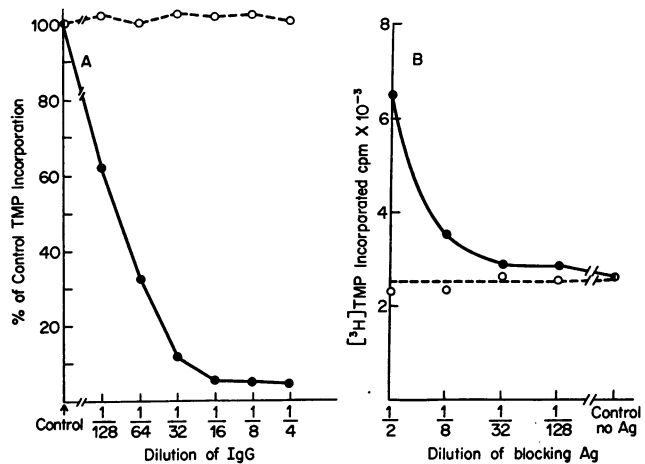

FIG. 4. Neutralization of endogenous DNA polymerase activity by antibody against avian myeloblastosis virus DNA polymerase and the antibody-blocking power of the chicken fraction. IgG against avian myeloblastosis virus DNA polymerase was prepared by R. C. Nowinski, McArdle Laboratories, University of Wisconsin, with polymerase purified by K. Watson, Institute of Cancer Research, Columbia University. (A) An equal volume of either the chicken fraction (○) or disrupted B77 virus (●) was added to 50 μl of appropriately diluted IgG. The mixtures were incubated at 37° for 30 min, and the residual endogenous DNA polymerase activities were determined in a standard polymerase reaction. 100% of B77 virus DNA polymerase activity was 10,500 cpm, and 100% of the chicken DNA polymerase activity was 5600 cpm incorporated. (B) Serial 4-fold dilutions of chicken fraction (○), containing about 4500 cpm endogenous DNA polymerase activity in 25 μl, were made and mixed with equal volumes of a 1/32 dilution of antibody to avian myeloblastosis virus polymerase. The mixtures were incubated at 37° for 30 min and then at 41° for 4 hr to inactivate all endogenous DNA polymerase activity. The residual neutralizing activity of the antibody was tested by addition of 25 μl of disrupted B77 virus containing 6500 cpm of endogenous DNA polymerase activity to each reaction mixture. The residual endogenous DNA polymerase activity in each sample was checked after incubation at 37° for 30 min by a standard DNA polymerase assay. A parallel experiment was performed with disrupted B77 virus (●) containing about 6500 cpm of endogeneous DNA polymerase activity in 25 μl.

TABLE 2. *Activity of different template-primers*

	[³H]TMP incorporated (cpm)*						
	Calf thymus DNA (25)†	Poly (dA-dT)· poly (dA-dT) (2.5)	Poly(dA)· (dT)₁₀ (2.5)	Poly(rA)· poly(dT) (2.5)	Poly(rA)· (dT)₁₀ (2.5)	B77 virus RNA (0.1)	Fraction RNA (5)
B77 virus DNA polymerase	31,000	40,800	1,350	124,400	70,700	1080	730
Chicken fraction DNA polymerase	3,000	2,460	58,100	5,450	840	170	0

DNA polymerases from B77 virus and the chicken-cell fraction were solubilized with 1% deoxycholate, 10% Nonidet P-40, and 1 M KCl and partially purified in 20–40% linear glycerol gradients made in 0.01 M Tris·HCl (pH 7.5) containing 1 mM EDTA, 10 mM dithiothreitol, and 50 mM KCl. The gradients were centrifuged at 50,000 rpm for 15 hr in a SW50.1 rotor. 0.2-ml fractions were collected and assayed for calf-thymus DNA (25 μg/reaction; Worthington Biochemical Corp.)-directed DNA polymerase activity. The peak fractions of the DNA polymerase activity were pooled and assayed with the indicated template-primers. The rate of [³H]TMP incorporation was linear during the 30-min incubation period. B77 virus RNA and the fraction RNA templates were prepared as described (11). All synthetic template-primers were purchased from Pabst Laboratories, Milwaukee, Wis.

* Trichloroacetic acid-insoluble radioactivity was determined after 30 min.

† Amounts in parentheses are μg/reaction.

We conclude that the DNA polymerase of the endogenous chicken activity is different from ALV DNA polymerase.

DNA polymerases from RNA tumor viruses can be distinguished from some cellular DNA polymerases by their template preferences (18–21). To compare the template preferences of DNA polymerase from the chicken fraction with DNA polymerase from B77 virus, we solubilized DNA polymerase from virus and chicken fraction with 1% deoxycholate, 10% Nonidet P-40, and 1 M KCl and partially purified the polymerases on linear, 20–40% glycerol gradients containing 0.4% dithiothreitol. The activity of different template-primers with the partially purified polymerases is shown in Table 2. The B77 virus DNA polymerase behaved as described by others (18–21). The chicken fraction polymerase behaved as did other cell polymerases.

DISCUSSION

We have isolated RNA-directed DNA polymerase activity from uninfected chicken embryos and cells in culture. We have shown by chemical and enzymatic tests that the product is DNA and that the template is RNA. We have shown that this activity is not an artifact of cell fractionation, that the DNA polymerase of this activity is not serologically related to the DNA polymerase of ALV or reticuloendotheliosis viruses, and that the RNA template does not have any homology to nucleic acids of Rous sarcoma virus or reticuloendotheliosis virus.

We do not know the origin of this activity, the nature of its DNA polymerase, the nature of its RNA template, or its function. The activity could come from an unknown latent Rous-virus, or be of cellular origin. The DNA polymerase solubilized from the activity did not share the Rous sarcoma virus polymerase preference for poly(rA)·oligo(dT) template-primers over poly(dA)·oligo(dT) template-primers. The polymerase of the chicken-cell RNA-directed DNA polymerase activity may not have been solubilized or may not have template-primer preferences like viral DNA polymerases. The relationship of

this chicken DNA polymerase to other cellular DNA polymerases is under investigation. The RNA template is probably present in very low concentration. Its nature is unknown.

It is tempting to assume that this activity is related to normal cell functions as described in the protovirus hypothesis.

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