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RNA-Directed DNA Polymerase from Particles Released by Normal Goose Cells

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Cells from a goose embryo were shown to release particle-associated RNA-directed DNA polymerase and RNase H activities that required the presence of Nonidet P-40 for detection. The particles were not infectious and did not have endogenous DNA synthesis. The goose particle DNA polymerase was related to the DNA polymerase of spleen necrosis virus with respect to size and was inhibited by immunoglobulin G to spleen necrosis virus DNA polymerase. However, goose cells producing DNA polymerase-containing particles did not contain reticuloendotheliosis virus-related nucleotide sequences in their DNA.

RNA-directed DNA polymerase (reverse transcriptase) is an essential constituent of retrovirus virions. This DNA polymerase is found in the virion core and catalyzes the synthesis of viral DNA.

An RNA-directed DNA polymerase has been isolated from A-particles, noninfectious entities found in some tumor cells and embryonic tissues (12, 16). An RNA-directed DNA polymerase activity has also been described in a fraction from normal embryonic chicken cells (4).

More recently, an RNA-directed DNA polymerase has been isolated from the allantoic fluid of normal chicken eggs (2, 3) and from the medium of primary chicken embryo cells (1a). This enzyme is not the product of the expression of the endogenous avian leukosis virus DNA polymerase gene and, therefore, must be coded by a second, different chicken DNA polymerase gene.

With the exception of the viral DNA polymerases, the physiological role of these enzymes remains unclear. However, an involvement of RNA-directed DNA polymerase activity in development has been suggested by Temin in the protovirus hypothesis (14).

In this paper, we describe a particle-associated RNA-directed DNA polymerase that was released spontaneously from the cells of a goose embryo in culture. The particles were of defined density, did not show infectivity in cells of several species, and had no heteropolymeric RNA associated with the DNA polymerase activity. The DNA polymerase activity contained in the particles was characterized as an RNA-directed DNA polymerase activity by its ability to transcribe homopolymeric and heteropolymeric RNA into DNA and by the presence of RNase H activity. The DNA polymerase was shown to be serologically related to the DNA polymerase of reticuloendotheliosis viruses. However, neither particle-producing nor other goose cells contained detectable reticuloendotheliosis virus-related nucleotide sequences in their DNA.

MATERIALS AND METHODS

Nucleic acids. Homopolymers such as $(C)_n \cdot (dG)_{12-18}$, $(A)_n \cdot (dT)_{12}$, $(dA)_n \cdot (dT)_{12}$, and $(dT)_{12}$ were obtained from P. L. Biochemicals, Milwaukee, Wis. Calf thymus DNA was obtained from Calbiochem and was activated by DNase treatment (2.5 mg of calf thymus DNA per ml in 10 mM Tris-hydrochloride, pH 7.5-0.5 mM EDTA-100 mM NaCl-10 mM $MgCl_2$ was treated with 400 ng of DNase I per ml for 20 min at 37°C and then for 10 min at 60°C).

Calf thymus DNA primer was prepared by the method of Taylor et al. (13).

Rabbit globin mRNA was a generous gift of A. Kinniburgh, McArdle Laboratory, Madison, Wis.

Culture of cells. Cells were cultured by standard techniques and were grown at 37°C in a humidified CO_2 atmosphere in Temin-modified Eagle minimal essential medium containing 20% tryptose phosphate broth and supplemented with 2% calf serum and 2% fetal bovine serum.

Viruses. Purified Rous-associated virus-61 (RAV-61) was a generous gift from S. Mizutani of our laboratory. Amherst pheasant virus was a kind gift of H. Hanafusa, Rockefeller University.

Spleen necrosis virus (SNV) was grown and purified as described previously (9).

Determination of cell number. Cells were separated from the plates and each other by trypsin treatment and counted in a Coulter Counter.

Test for infectivity. Materials to be tested were applied in 0.2-ml amounts to cells at 12 to 24 h after passage, in the presence of 15 μg of polybrene per ml. The cells were incubated at 37°C for 40 min with agitation. Then medium was added, and after several days the medium was concentrated by ultracentrifugation and tested for DNA polymerase activity by

using the standard DNA polymerase assay in the presence of 0.05% Nonidet P-40 (NP-40).

Purification of particles from goose cell culture medium. The purification of particles by ultrafiltration and ultracentrifugation is described in the legend to Fig. 1.

Standard DNA polymerase assay. The volume of the standard reaction was 100 μl. The reaction mixture contained 10 mM $MgCl_2$, 20 mM KCl, 20 mM Tris-hydrochloride (pH 8.3), 3 mM dithiothreitol, 0.3 mM EDTA, 250 μM each dATP, dCTP, and dGTP, and 5 μCi of [^{3}H]dTTP (18 Ci/mmol). Unless otherwise indicated, activated calf thymus DNA (12.5 μg/100-μl assay) was used as template-primer. When complete viruses or particles were tested, the assays contained 0.05% NP-40. When purified DNA polymerases were tested, the reaction mixtures contained 50 μg of bovine serum albumin per 100 μl. Incubation was at 37°C, and incorporation was linear for more than 2 h in the case of partially purified DNA polymerases.

Test for RNase H activity. Reaction mixtures contained 10 mM $MgCl_2$, 20 mM KCl, 20 mM Tris-hydrochloride (pH 8.3), 3 mM dithiothreitol, 0.3 mM EDTA, and 10,000 cpm of DNA-RNA hybrid with the label in the RNA moiety ([^{3}H]UMP; 22 Ci/mmol). Preparation of the hybrid by using heat-denatured calf thymus DNA and *Escherichia coli* RNA polymerase, as well as the test for RNase H activity, were as previously described (9).

Purification of DNA polymerases. RNA-directed DNA polymerases were purified from RAV-61 or goose particles by affinity chromatography on $(C)_n$-agarose as previously described (3), following the method of Markus et al. (8). Purified SNV DNA polymerase was a generous gift of S. Mizutani and has been described previously (9).

Protein determination. The methods of Lowry et al. (7) or Schaffner and Weissmann (11) were used, as indicated below. If the method of Lowry et al. was applied for determination of marker protein in glycerol gradients containing KCl, low-speed centrifugation was applied before reading the optical density.

IgG inhibition test. Immunoglobulin G (IgG) directed against the DNA polymerases of Rous sarcoma virus-Rous-associated virus 0 (RSV-RAV 0) or SNV and normal IgG were kind gifts of S. Mizutani. The IgG's directed against DNA polymerases were purified from the blood of rabbits that had been injected with electrophoretically pure enzyme preparations. The IgG inhibition test was performed essentially by the method of Watson et al. (15). Briefly, partially purified enzymes were preincubated with the indicated amounts of IgG in the presence of 150 mM KCl-10 mM Tris (pH 8.0)-1 mM dithiothreitol for 30 min at room temperature. The mixtures were then brought to standard DNA polymerase conditions, and the DNA polymerase activities were determined. Values of 100% were obtained from assays preincubated without IgG and performed in duplicate.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The method of Laemmli (6) was used.

Nucleic acid hybridization. SNV [^{125}I]RNA (specific activity, 1.22×10^7 cpm/μg) was a generous gift of E. Keshet of our laboratory. The RNA had been purified from SNV, denatured, selected for polyadenylic acid-containing RNA on oligodeoxythymidylic acid-cellulose, and sized. Material of 35S was iodinated. It has been shown to hybridize to all restriction fragments of SNV DNA produced by several restriction enzymes (E. Keshet, personal communication). DNAs from normal chicken cells, chicken cells chronically infected with SNV, normal goose liver, and cells of goose embryo no. 1 were prepared as described by Kang and Temin (5). Briefly, preparations of nuclei from the different cells were treated with sodium dodecyl sulfate and Pronase, and the material was then extracted once with phenol and twice with chloroform-isoamyl alcohol (24:1). Three volumes of absolute ethanol was added to the combined aqueous phases, and the DNA was spooled on a pipette before hybridization. The DNAs were made approximately 7S by sonic treatment. Nucleic acid hybridization was carried out in sealed capillary pipettes at 63°C under the conditions previously described (5).

RESULTS

Release of sedimentable DNA polymerase activity from goose cells. We routinely screen our cell cultures for sedimentable DNA polymerase activity. Screening secondary cultures prepared from eight single goose embryos, we found one of them positive for sedimentable DNA polymerase activity. This embryo, positive for sedimentable DNA polymerase activity in the culture medium, is referred to as goose embryo no. 1.

After cell passage and addition of serum, the amount of enzyme activity present per number of cells increased in the first 3 days and then remained constant. After the next passage, the same pattern was observed (data not shown).

Purification of sedimentable DNA polymerase activity. We wanted to find out first whether the sedimentable DNA polymerase activity was contained in structures of defined physical and biochemical properties, that is, in particles. Supernatant medium from goose embryo no. 1 cells was collected, and the sedimentable DNA polymerase activity was concentrated by ultrafiltration and subsequent centrifugation in a fixed-angle rotor. The pellet material was layered on a continuous sucrose density gradient and centrifuged to equilibrium. Material from the peak of DNA polymerase activity was applied to a second continuous sucrose density gradient. As Fig. 1 shows, the material exhibiting DNA polymerase activity banded in this gradient in a symmetrical peak at a density around 1.18 to 1.19 g/cm^3. There was a good correlation between DNA polymerase activity and protein concentration. The DNA polymerase activity, therefore, seemed to be associated with defined particles. Later (Table 1) it is shown that the DNA polymerase activity required NP-40 for detection. This requirement indicates that the

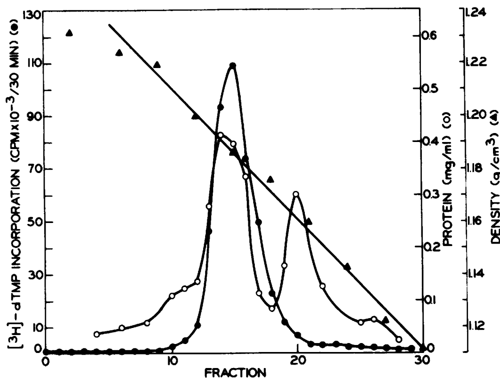

FIG. 1. *Purification from goose embryo cell supernatant of particles containing DNA polymerase activity. A 12-liter amount of supernatant medium of goose embryo no. 1 cells was subjected to low-speed centrifugation to remove cells and cell debris. The supernatant was then filtered through a Millipore membrane concentrator. The material collected on the filter was eluted with Eagle medium and centrifuged in a Beckman 30 rotor at 28,000 rpm for 60 min at 4°C. The pellets were suspended in STE buffer (0.1 M NaCl, 0.01 M Tris-hydrochloride [pH 8.0], 0.001 M EDTA), layered on top of a continuous sucrose density gradient (10 ml of 25 to 50% [wt/wt] sucrose in STE buffer, in an SW27 rotor), and centrifuged at 25,000 rpm for 14 h. The peak of DNA polymerase activity was determined, and material from the peak fractions was diluted with STE buffer and put on a continuous sucrose density gradient (4 ml of 25 to 50% sucrose) in an SW50.1 rotor. After centrifugation for 15 h at 45,000 rpm and 4°C, fractions of 160 μl were collected from the bottom and assayed for DNA polymerase activity, protein concentration, and density.*

DNA polymerase is located inside the particles.

Protein composition of the goose particles. Particles from the density gradient described in the legend to Fig. 1 were analyzed on sodium dodecyl sulfate-polyacrylamide gels (data not shown). A major protein was found at about 30,000 daltons.

Comparison with the proteins of several avian retroviruses showed that the main protein of goose particles was smaller than the p30 of SNV and larger than the p29 of Amherst pheasant virus and the p27 of RAV-61. The goose particles showed further bands at 25,000, 14,000, and 10,000 daltons.

Lack of infectivity of goose particles. To determine whether the goose particles were infectious, the infectivity of fresh harvests of goose particles was tested. Chicken, turkey, pheasant, duck, goose, and mouse cells were inoculated with supernatant medium from goose embryo no. 1 cells. After several days, none of the treated cell cultures showed sedimentable DNA polymerase activity higher than background (300-cpm incorporation per h), in contrast to virus-infected cells (300,000-cpm incorporation per h; data not shown). The particles, therefore, seem to be not infectious.

Lack of endogenous DNA synthesis. Endogenous DNA synthesis is another characteristic property of retrovirus virions. However, the goose particles did not show significant DNA synthesis in the presence of actinomycin D even when primer molecules were added (Tables 1 and 3).

TABLE 1. *Requirements for DNA polymerase activity of goose particles*[a]

Template	Primer	Unlabeled deoxyribonucleoside triphosphates	Reaction conditions	Amt of [^{3}H]-dGMP incorporated per h in 100 μl (cpm)
None	None	None	Complete	200
None	None	dATP, dCTP, dTTP	Complete + actinomycin D	220
None	$(dT)_{12}$	None	Complete	0
None	$(dT)_{12}$	dATP, dCTP, dTTP	Complete + actinomycin D	220
None	$(dG)_{12}$	None	Complete	0
$(C)_n$	None	None	Complete	0
$(C)_n$	$(dG)_{12}$	None	Complete	30,600
$(C)_n$	$(dG)_{12}$	None	−NP-40	0
$(C)_n$	$(dG)_{12}$	None	$-Mg^{2+}$	0
$(C)_n$	$(dG)_{12}$	None	$-Mg^{2+}$, +1 mM Mn^{2+}	33,600
Activated calf thymus DNA	None	dATP, dCTP, dTTP	Complete	32,000
Activated calf thymus DNA	None	dATP, dCTP, dTTP	$-Mg^{2+}$, +1 mM Mn^{2+}	8,200

[a] Standard reactions (100 μl) contained 15 μg of purified goose particles and the indicated templates and primers at the following concentrations (in micrograms per assay): calf thymus DNA, 12.5; $(dT)_{12}$, 1; $(dG)_{12}$, 1; $(C)_n$, 1; $(C)_n(dG)_{12}$, 1. Complete reactions contained 5 μCi of [^{3}H]dGTP (6.5 Ci/mmol) and 0.05% NP-40. The concentration of each unlabeled deoxyribonucleoside triphosphate was 300 μM, and that of actinomycin D was 50 μg/ml. A background of 200 cpm was subtracted from all values.

Addition of $(dT)_{12}$ stimulated incorporation of dTTP, but not of other deoxyribonucleotides (data not shown). This reaction was only slightly inhibited by the absence of the other deoxyribonucleoside triphosphates and was not sensitive to RNase A treatment. Priming by calf thymus DNA primer also gave only a weak reaction. Our conclusion from these experiments is that the goose particles contain some small polyadenylic acid molecules and little heteropolymeric RNA.

Attempts to label heteropolymer RNA with [^{3}H]uridine only led to small amounts of incorporation into particles, not allowing further characterization.

Characterization of the DNA polymerase activity. To prove that an enzyme activity is an RNA-directed DNA polymerase, one has to show the ability of the enzyme activity to use specifically homopolymeric and heteropolymeric RNAs as templates for DNA synthesis. A study of the requirements for DNA polymerase activity (Table 1) showed that the enzyme activity depended on the presence of both template and primer. The stringent requirement for the nonionic detergent NP-40 indicated that the DNA polymerase was located inside the particles. The DNA polymerase activity depended on the presence of Mg^{2+} which could be substituted by Mn^{2+} with the same efficiency in the case of the RNA-directed reaction and with lower efficiency in the case of the DNA-directed reaction. As Table 2 shows, the goose particle DNA polymerase activity could utilize $(A)_n \cdot (dT)_{12}$ and $(C)_n \cdot (dG)_{12-18}$ with 37 or 22% efficiency, respectively, compared with calf thymus DNA-directed DNA synthesis. $(dA)_n \cdot (dT)_{12}$ was used only very inefficiently compared with $(A)_n \cdot (dT)_{12}$. This pattern of utilization of homopolymeric template-primer complexes is characteristic of RNA-directed DNA polymerases (1).

TABLE 3. *Globin mRNA-directed DNA synthesis by goose particle DNA polymerase activity*[a]

Template	Primer	Reaction conditions	Amt of [^{3}H]-dGMP incorporated per h in 100 µl (cpm)
None	None	Complete	220
None	$(dT)_{12}$	Complete	220
Globin mRNA	None	Complete	450
Globin mRNA	$(dT)_{12}$	Complete	14,070
Globin mRNA	$(dT)_{12}$	Complete + RNase A (50 µg/ml)	350
Globin mRNA	$(dT)_{12}$	−dATP	600
SNV 70S RNA	Calf thymus DNA	Complete	7,020
	Calf thymus DNA	Complete	250

[a] Assays (100 µl) contained 15 µg of purified particles, globin mRNA (1 µg/assay), and $(dT)_{12}$ (1 µg/assay) as indicated. Concentrations of unlabeled deoxyribonucleoside triphosphates were 250 µM each. The assays contained 5 µCi of [^{3}H]dGTP (6.5 Ci/mmol). All assays contained 50 µg of actinomycin D per ml and 0.05% NP-40. The concentration of SNV 70S RNA in a parallel experiment was 1 µg/assay, and that of calf thymus DNA primer was 25 µg/ml. Incubation was at 37°C for 1 h. A background of 200 cpm was subtracted from all values.

The nature of the RNA-directed DNA polymerase activity was studied further (Table 3). In the presence of $(dT)_{12}$ primer and a complete reaction mixture, globin mRNA was used efficiently as template. The use of [^{3}H]dGTP insured that reverse transcription of the heteropolymeric part of globin mRNA, rather than just the polyadenylic acid tail, was measured. The nature of the reaction as DNA synthesis directed by heteropolymeric RNA was further substantiated by the result that DNA synthesis did not

TABLE 2. *Utilization ratios of different template-primer complexes by goose particle DNA polymerase*[a]

Template	Primer	Labeled deoxyribonucleoside triphosphate	Unlabeled deoxyribonucleoside triphosphates	Amt of [^{3}H]-dNMP incorporated per h in 100 µl (cpm)	Ratio (%)
$(C)_n$	$(dG)_{12}$	[^{3}H]dGTP	None	36,000	22
Activated calf thymus DNA		[^{3}H]dGTP	dATP, dCTP, dTTP	40,000	100
$(A)_n$	$(dT)_{12}$	[^{3}H]dTTP	None	328,200	37
$(dA)_n$	$(dT)_{12}$	[^{3}H]dTTP	None	14,500	1.5
Activated calf thymus DNA		[^{3}H]dTTP	dATP, dCTP, dGTP	236,500	100

[a] Standard assays (100 µl) contained 15 µg of purified goose particles and the indicated templates and primers. Concentrations of the template-primers were the following (in micrograms per assay): calf thymus DNA, 12.5; $(C)_n \cdot (dG)_{12-18}$, 2; $(A)_n \cdot (dT)_{11}$, 2; $(dA)_n \cdot (dT)_{12}$, 2. The concentrations of the unlabeled deoxyribonucleoside triphosphates were 300 µM each. The specific activities of [^{3}H]dGTP and [^{3}H]dTTP were 6.5 and 58 Ci/mmol, respectively. The assays (100 µl) contained 5 µCi of [^{3}H]dGTP or 20 µCi of [^{3}H]dTTP. For the calculation of ratios in the case of the calf thymus DNA-directed reaction, the values of incorporation were multiplied by four to correct for the unlabeled deoxyribonucleotides incorporated.

occur in the absence of dATP or in the presence of RNase A. Goose particle DNA polymerase activity could also use SNV RNA as template for RNA-directed DNA synthesis.

DNA synthesis directed by a heteropolymeric RNA is regarded as the characteristic activity of an RNA-directed DNA polymerase. The enzyme activity contained in the goose particles clearly, therefore, can be classified as an RNA-directed DNA polymerase activity.

In addition to RNA-directed and DNA-directed DNA polymerase activities, reverse transcriptases exhibit RNase H activity (10). As Table 4 shows, purified goose particles exhibit RNase H activity, which degrades the RNA moiety of RNA-DNA hybrids.

In all of the experiments described in Tables 1 through 4, purified concentrated goose particles had to be used to have sufficient enzyme activity for the relatively insensitive heteropolymeric RNA-directed DNA polymerase and RNase H assays. Therefore, it is not clear whether the same protein has the DNA polymerase activity and the RNase H activity.

Size of the DNA polymerase. The DNA polymerase from goose particles was partially purified by affinity chromatography and compared with the DNA polymerases of SNV (a member of the reticuloendotheliosis virus species) and RAV-61 (a member of the avian leukosis-sarcoma virus species). Comparison of the sedimentation of the purified DNA polymerases in glycerol gradients (in the presence of 0.5 M KCl) showed that the goose particle DNA polymerase sedimented at a slower rate than RAV-61 DNA polymerase and had the same sedimentation rate as SNV DNA polymerase or bovine serum albumin, that is, around 4S (Fig. 2 A through C).

Immunological studies. IgG's directed specifically against the DNA polymerase of the avian leukosis-sarcoma virus species or against the DNA polymerase of the reticuloendotheliosis virus species were tested for their inhibitory effect on purified goose particle DNA polymerase. As Fig. 3A shows, there was only a relatively weak inhibitory effect of anti-RSV–RAV O polymerase IgG on the goose particle DNA polymerase compared with the RAV-61 DNA polymerase. The effect was in the same range as the effect of the same IgG on SNV DNA polymerase and was not much stronger than the effect of normal IgG on goose particle DNA polymerase activity. This result showed that the goose particle DNA polymerase was different from the avian leukosis-sarcoma virus DNA polymerase.

When IgG directed against SNV DNA polymerase was tested, a relatively strong inhibitory effect on the activity of the goose particle DNA polymerase was observed (Fig. 3B). Compared with the inhibition of the homologous enzyme, an eightfold excess of IgG had to be applied to obtain 50% inhibition. Therefore, the goose particle DNA polymerase and SNV DNA polymerase seem to be related to each other, although they are not identical.

Nucleic acid hybridization studies. We then wanted to see whether the DNA of goose embryo no. 1 contained more reticuloendotheliosis virus-related nucleotide sequences than normal goose DNA. DNA was purified from goose embryo no. 1 cells, normal goose liver cells, chicken cells, and chicken cells chronically infected with SNV. The DNAs were hybridized to SNV [^{125}I]RNA. As Fig. 4 shows, neither normal goose DNA nor goose embryo no. 1 DNA showed positive hybridization. In contrast was the clear hybridization of the DNA from chronically infected chicken cells and a slightly positive hybridization of the DNA from normal chicken cells. This result shows that goose DNA does not contain endogenous reticuloendotheliosis virus nucleotide sequences.

TABLE 4. *RNase H activity of goose particles*[a]

Source of enzyme	Substrate	Amt of [^{3}H]-UMP released per 40 min in 100 μl
Goose particles	DNA-[^{3}H]RNA	5,000
Goose particles	DNA-[^{3}H]RNA, heated	780
None	DNA-[^{3}H]RNA	600

[a] Standard tests for RNase H activity contained 15 μg of purified goose particles per 100 μl in the presence of 0.2% NP-40. DNA-[^{3}H]RNA hybrid was either added untreated or heated at 95°C for 2.5 min and quickly cooled before use.

DISCUSSION

Our data show that goose cells from one embryo (of eight tested) released a particle-associated RNA-directed DNA polymerase activity. The enzyme required the presence of NP-40 for detection. The particles, which banded at a higher density than retrovirus virions usually do, were not infectious for the cells of the several species tested, including mouse cells. When a variety of different conditions were used, no significant endogenous DNA synthesis was found. Only $(dT)_{12}$-primed polydeoxythymidylic acid synthesis was found, indicating the presence of polyadenylic acid in the particles. The proteins of the particles did not correspond to the proteins of virions of the three avian retrovirus species. Therefore, it appears that the particles are not infectious viruses and are not defective viruses of one of the known avian RNA tumor virus species.

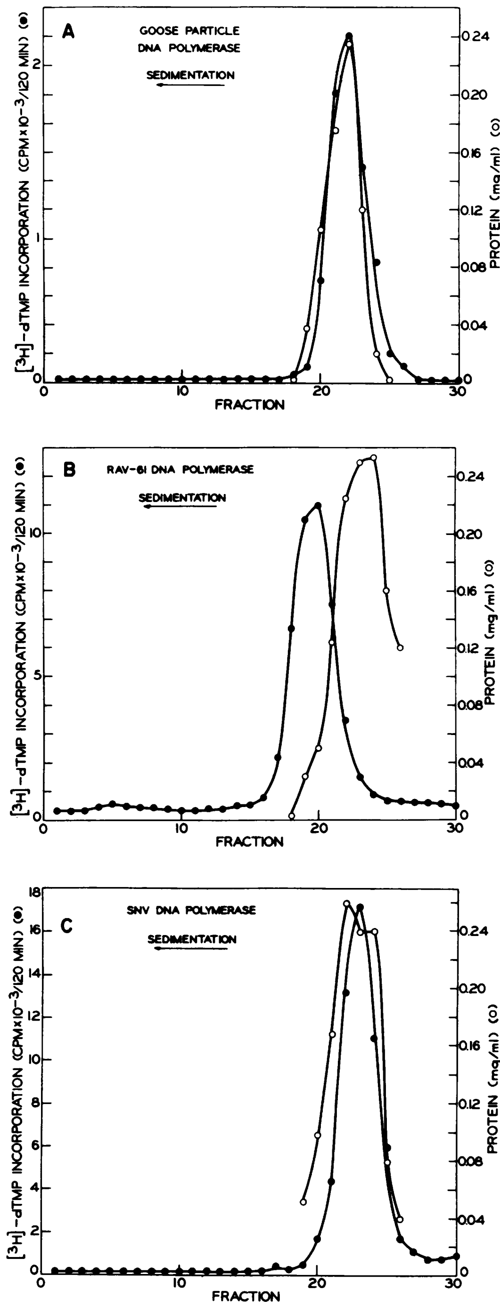

FIG. 2. *Glycerol gradient centrifugation of goose particle, RAV-61, and SNV DNA polymerases. Goose particle DNA polymerase was partially purified by affinity chromatography on (C)$_n$-agarose. After purification, bovine serum albumin was added to a final concentration of 1 mg/ml, and a sample of 200 µl was layered on a linear glycerol gradient (10 mM potassium phosphate [pH 8.0], 10 to 30% glycerol, 0.5 M KCl, 1 mM dithiothreitol). A 200-µl amount of RAV-61 DNA polymerase and 200 µl of SNV DNA polymerase (each sample also containing 200 µg of bovine serum albumin) were layered on top of parallel gradients. Centrifugation was for 16.5 h at 45,000 rpm at 4°C in an SW50.1 rotor. Fractions were collected from the bottom. A 10-µl amount of each fraction was tested in a standard DNA polymerase assay for 120 min. A background of 200 cpm was subtracted from all values. The position of the marker bovine serum albumin was determined by the method of Lowry et al. (7).*

The particles from goose cells contained RNA- and DNA-directed DNA polymerase activity and RNase H activity. The partially purified DNA polymerase from the goose cell particles was related to reticuloendotheliosis virus DNA polymerase immunologically and with respect to its size, but was not related in these ways to the DNA polymerase of the avian leukosis-sarcoma virus species.

We showed, however, that neither goose embryo no. 1 DNA nor normal goose DNA contained reticuloendotheliosis virus nucleotide sequences. This result excludes contamination of goose embryo no. 1 cells by a reticuloendotheliosis virus as a trivial explanation of our results. It also shows that the gene for a reticuloendotheliosis virus-related DNA polymerase in goose cells and the reticuloendotheliosis virus gene for DNA polymerase have different nucleotide sequences, whereas the proteins coded by those sequences have some immunological determinants in common.

There are at least three possible explanations for the origin of the goose gene for the cellular RNA-directed DNA polymerase described. First, a reticuloendotheliosis-like virus might have been introduced into the germ line of geese early in evolution. The genes of this virus were kept, although the genetic information was changed during time. Partial or complete expression of this viral information led to the presence of the DNA polymerase activity detected. Second, a reticuloendotheliosis-like virus introduced into the germ line long ago might have had most of its information deleted. Expression of the remaining defective endogenous viral information gene gave rise to the DNA polymerase activity described in this paper. Third, cellular genes for DNA polymerase might have evolved to a gene specifying an RNA-directed polymerase as discussed in the protovirus hypothesis. The same original gene may have been a precursor of part of the genome of a reticuloendotheliosis virus that evolved from the cell genome and then further evolved after it had become an exogenous virus.

None of these hypotheses can be tested with

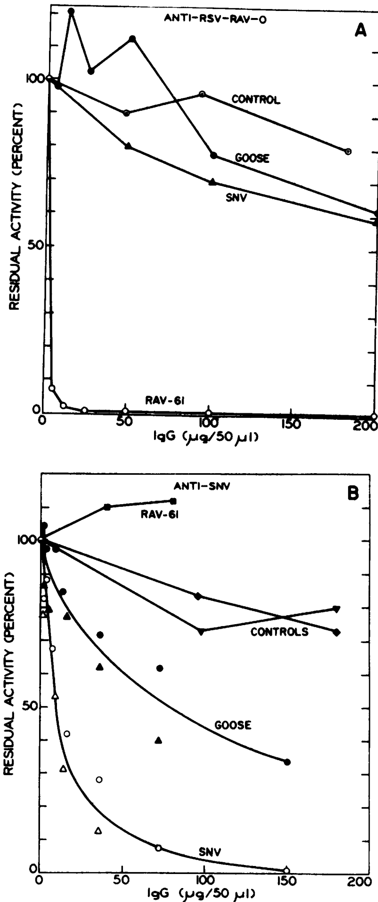

FIG. 3. *Characterization of goose particle DNA polymerase by the IgG inhibition test. A constant amount of each partially purified enzyme was preincubated with the indicated amounts of IgG for 30 min at room temperature, and then the residual activity was determined. A background value of 400 cpm was subtracted from all values. Values of 100% (no IgG added) were obtained from duplicate experiments. (A) Symbols: ○, RAV-61 DNA polymerase and anti-RSV-RAV O DNA polymerase IgG (100% = 59,300 cpm incorporated per h); ●, goose particle DNA polymerase and anti-RSV-RAV O DNA polymerase IgG (100% = 10,000 cpm incorporated per h); ⊙, goose particle DNA polymerase and normal IgG (100% = 10,000 cpm/h); ▲, SNV DNA polymerase and anti-RSV-RAV O DNA polymerase IgG (100% = 26,000 cpm/h). (B) Symbols: ○, SNV DNA polymerase and anti-SNV DNA polymerase IgG (experiment 1; 100% = 26,000 cpm/80 min); △, SNV DNA polymerase and anti-SNV DNA polymerase IgG (experiment 2; 100% = 20,800 cpm/80 min); ●, goose particle DNA polymerase and anti-SNV DNA polymerase IgG (experiment 1; 100% = 19,600 cpm/80 min); ▲, goose particle DNA polymerase and anti-SNV DNA polymerase IgG (experiment 2; 100% = 25,400 cpm/80 min); ▼, SNV DNA polymerase and normal IgG (100% = 25,000 cpm/80 min); ◆, goose particle DNA polymerase and normal IgG (100% = 12,600 cpm/80 min); ■, RAV-61 DNA polymerase and anti-SNV DNA polymerase IgG (100% = 26,000 cpm/80 min).*

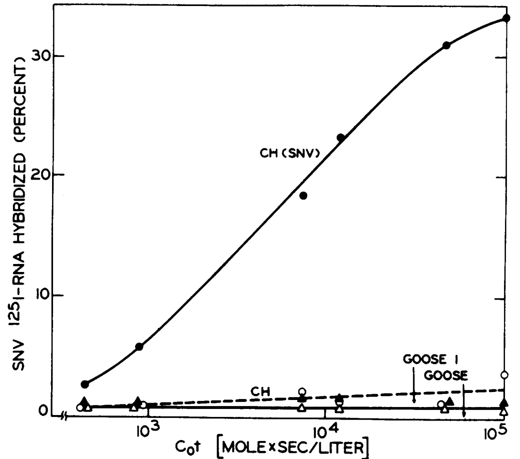

FIG. 4. *Hybridization of SNV [^{125}I]RNA to different DNAs. SNV [^{125}I]RNA (2,300 cpm) was hybridized with 250 µg of the DNAs indicated in 25 µl of hybridization buffer at 63°C in sealed capillary pipettes. At the indicated times samples were withdrawn and kept frozen at −70°C. At the end of the final incubation, the extent of hybridization was determined after RNase A digestion. A background of 75 cpm was subtracted from all values, and the percentage of hybridization was calculated. Symbols: ●, DNA from chicken cells chronically infected with SNV [CH(SNV)]; ○, DNA from normal chicken cells (CH); ▲, DNA from goose embryo no. 1 cells; △, DNA from normal goose liver.*

the present data. The point common to all of them is the presence of a gene for RNA-directed DNA polymerase in goose cells and its occasional expression without transformation or spread of infectious virus. The interesting and open question remains, do such cellular genes for RNA-directed DNA polymerase have functions in physiological processes?

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ADDENDUM IN PROOF

Hunter et al. (Proc. Natl. Acad. Sci. U.S.A. **75**: 2708–2712, 1978) have recently described amino acid sequence homologies between the amino terminus of

the reticuloendotheliosis virus p30 and mammalian retrovirus p30's. In addition, we have detected immunological cross-reactivity between the RNA-directed DNA polymerases of SNV and Moloney murine sarcoma virus (G. Bauer and H. M. Temin, unpublished data). In light of these findings, a fourth explanation for the phenomenon described here may be that goose embryo no. 1 (or its ancestors) might have been infected by a mammalian retrovirus that produces defective particles in goose embryo no. 1.

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