RNA-Dependent DNA Polymerase Activity in Preparations of a Mutant of Newcastle Disease Virus Arising from Persistently Infected L Cells

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RNA-dependent DNA polymerase activity was found in preparations of a mutant of Newcastle disease virus. The enzyme activity was not found in wild-type virus preparations.

A persistent infection of mouse L cells was established with the Herts strain of Newcastle disease virus (NDV) by Thacore and Youngner (17). These cultures, during the 5 years they have been carried, continually produce from 10^2 to 10^4 PFU/ml of a mutant virus (NDV_{pi}). They also produce 10 to 30 U of interferon per culture continually and are not susceptible to superinfection by vesicular stomatitis virus.

NDV_{pi} was shown by Thacore and Youngner to differ from the wild type (NDV_o) in that NDV_o was stable at 48 C for 1 h whereas NDV_{pi} was inactivated by 99.99%; NDV_{pi} forms smaller plaques than the wild type on chicken embryo (CE) fibroblast monolayer cultures (<1 mm versus 2-3 mm). NDV_{pi} was also about 20-fold more sensitive to interferon than the wild type when tested in normal L-cell cultures.

These properties raise the question: why does this persistent infection not cure itself? The amount of interferon produced in the persistently infected cultures is more than sufficient to completely inhibit the mutant virus present, when tested in normal L cells (8).

The demonstration of RNA-dependent DNA polymerase (RDDP) in certain viruses (1, 11, 14, 16) suggested the possibility to us that NDV_{pi} might contain a similar enzyme, the action of which could account for both the mechanism of maintenance of the infection and the apparent resistance of NDV_{pi} to the action of interferon in L_{pi} cells. The RNA-dependent DNA polymerase and the DNA it could produce suggested a possible mechanism whereby the persistent infection might be maintained in the presence of sufficient interferon which would otherwise cure it. This report describes the presence of

RNA-directed DNA polymerase activity in purified preparations of the mutant virus, NDV_{pi}, and an absence of the enzyme activity in those of the wild type, NDV_o. (Part of this work was taken from the Ph.D. thesis of P. A. Furman).

MATERIALS AND METHODS

Cell cultures. Persistently infected L cells (L_{pi}) were obtained from J. S. Youngner, University of Pittsburgh Medical School, and subcultured as described previously (17).

Viruses. The Herts strain of Newcastle disease virus used here (NDV_o) was propagated in the allantoic cavity of 10-day-old embryonated eggs or in CE cell cultures and harvested after 48 h (17). The mutant virus, NDV_{pi}, was obtained from persistently infected L-cell cultures initiated by infecting with NDV_o. To increase its titer, NDV_{pi} was propagated in an identical manner to NDV_o, but the progeny virus was harvested after 72 h rather than 48 h. When specific pathogen-free eggs (Spafas, Inc., Norwich, Conn.) were used to prepare the CE cultures from which both NDV_o and NDV_{pi} pools were obtained, no differences were noted in the observed activity of RDDP for either virus compared to results obtained with our usual embryonated eggs (Pan American Hatcheries, Hammond, La.).

Viral purification. All procedures were carried out at 4 C. The virus harvests (approximately 5 liters) were clarified by centrifugation at $12,000 \times g$ for 15 min. The supernatant fluid was then centrifuged at $89,000 \times g$ for 3 h. The pellet containing the virus was resuspended in phosphate-buffered saline, pH 7.2 (PBS, 0.01 M), and subjected to two additional cycles of differential centrifugation as described above. After the third cycle, the pellet was resuspended in 5.0 ml of PBS. The virus suspension (0.5 ml) was placed on a 10 to 70% potassium tartrate gradient and centrifuged at $134,000 \times g$ for 3 h in an SW39 rotor. The fractions containing the virus (at a density of 1.18 to 1.25 g/cm³,

measured by weighing 10- μ liter volumes of each fraction) were collected and dialyzed overnight against PBS. The dialysate was concentrated and further purified by two similar cycles of differential centrifugation. The final pellet was resuspended in 1 ml of 0.15 M NaCl-0.01 M Tris-hydrochloride buffer (pH 7.5).

Characterization of NDV_{pi}. The mutant virus, NDV_{pi}, was distinguished from the wild-type virus on the basis of thermal stability and plaque size as described by Thacore and Youngner (17). In each case the concentrated virus had completely retained its characteristic genetic markers during propagation and purification.

Polymerase assay. The conditions for the DNA polymerase assay were similar to those described by Baltimore (1). A standard reaction mixture contained, in a volume of 0.1 ml: Tris-hydrochloride (pH 8.3), 10 μ mol; magnesium acetate, 1.2 μ mol; NaCl, 12 μ mol; dithiothreitol, 4.0 μ mol; Triton X-100, 0.004%; unlabeled deoxyribonucleotide triphosphates (dATP, dGTP, dCTP), 0.1 μ mol; unlabeled thymidine triphosphate and ³H-labeled thymidine triphosphate (New England Nuclear) at specific activities as indicated in the legends to the figures. When poly A was used as the synthetic template with oligo dT₍₁₂₋₁₄₎ as primer (2) (Collaborative Research, Inc., Waltham, Mass.), the deoxyribonucleotide triphosphates, dATP, dGTP and dCTP, were omitted. The reaction mixtures were incubated at 37 C for intervals as indicated in the text and chilled in an ice bath, and the acid-insoluble radioactivity was determined by the addition of 0.1 M sodium pyrophosphate, carrier yeast RNA (100 μ g), and trichloroacetic acid followed by filtration through Selectron membrane filters (Schleicher and Schuell, Inc.) and counted in a Beckman liquid scintillation spectrometer.

Extraction and purification of viral RNA. Viral RNA was extracted from both NDV_o and NDV_{pi} by the method of Duesberg and Robinson (4). The virus pellet, obtained by differential centrifugation after banding in a potassium tartrate gradient, was suspended and diluted in a buffer containing 0.01 M Tris-hydrochloride (pH 8.5), 0.1 M NaCl, and 0.001 M EDTA. Mercaptoethanol and sodium dodecyl sulfate (SDS) were added to make a 1% solution of each. The solution was then extracted three times with freshly distilled phenol which had been saturated with the buffer. The nucleic acid was precipitated twice by adding 0.1 vol of 16% sodium acetate and 3 vol of cold ethanol to the aqueous phase.

Complementarity of DNA product to virion RNA. Complementarity was established using the technique described by Spiegelman et al. (15). The DNA product was extracted from the reaction mixture with freshly distilled phenol and treated with 0.4 M NaOH at 37 C for 24 h to remove completely any RNA. After neutralizing the NaOH, the mixture was added to purified viral RNA; then formamide was added to 50% (vol/vol) concentration. This solution was incubated at 37 C for 23 h, at which time the reaction mixture was chilled to 4 C, placed on a Cs₂SO₄ gradient (density = 1.550) and centrifuged at 192,000 $\times$ g for 60 h at 20 C. Fractions were collected

by bottom puncture, precipitated, and counted as described above.

Immunodiffusion assays. Immunodiffusion assays were carried out as previously described (7, 13) to detect the presence of certain RNA tumor viruses that might be present in the purified virus preparations. Samples of NDV_o and NDV_{pi}, concentrated and purified as described above and which in the case of NDV_{pi} showed RDDP activity, were sent to Louis Potash of Flow Laboratories, Inc. of Rockville, Md. Immunodiffusion assays were carried out on both viruses by using the following nine antisera: anti-VSW (viper), anti-avian myeloblastosis virus (chick), anti 74-C (feline), group specific anti-feline leukemia virus (GS-3), anti-murine leukemia virus (MuLV) (mouse), group-specific anti-MuLV (GS-3), anti-MSB (rat), anti-FL (hamster leukemia virus, hamster) and anti-RD114 (feline, putative human). The properties of these antisera have been described by Gilden and Oroszlan (7) and by Oroszlan et al. (13). No test for the reticuloendotheliosis virus group was made. The sensitivity of this test was found by Flow Laboratories, Inc. to require 20 μ liters of a suspension containing 50 μ g of viral protein per ml or 2.5×10^{10} viral particles/ml in each well. The samples submitted to Flow Laboratories for this immunodiffusion assay contained 3.2×10^{10} and 5.0×10^{10} PFU/ml for NDV_o and NDV_{pi}, respectively. The protein content for both virus preparations was the same, namely, 0.5 mg/ml. However, if a contaminating virus which was capable of a high degree of DNA polymerase activity was present in the preparations this test would not be sufficiently sensitive to detect it.

Protein determination. Viral protein was determined by the method of Lowry et al. (12).

RESULTS

Demonstration of RNA-dependent DNA polymerase. To determine if preparations of either the mutant virus (NDV_{pi}) or the wild type (NDV_o) could incorporate thymidine, indicating the activity of RDDP, both viruses were propagated in CE cultures or 10-day-old embryonated eggs, using the same batch of eggs or cultures prepared from eggs of the same batch. Approximately the same volume was harvested for each virus pool, the NDV_{pi} being harvested 72 h postinfection and NDV_o 48 h postinfection. The viruses were purified at the same time, using the methods described in the preceding section, and assayed for RDDP activity. The results shown in Fig. 1 clearly demonstrate that an enzyme capable of incorporating thymidine into acid-insoluble counts was present in the NDV_{pi} preparation but not in the NDV_o preparation.

To establish that these results were due to an enzyme of NDV_{pi}, a series of control experiments were carried out as follows.

FIG. 1. Kinetics of incorporation of ^3H -TTP by NDV_{pi} or NDV_o. NDV_{pi} and NDV_o, grown in CE cultures prepared from eggs of the same batch, were harvested at 72 and 48 h postinfection, respectively, and purified as described. Standard 0.1-ml reaction mixtures were incubated at 37 C. For NDV_{pi} the mixture contained 32.0 μg of viral protein corresponding to 2.0×10^{10} PFU/ml; for NDV_o, 38.4 μg of viral protein in the mixture corresponded to 3.8×10^{10} PFU/ml. The specific activity of ^3H -TTP was 1,500 counts per min per pmol. At the times indicated, samples were chilled and precipitated with 0.5 ml of cold 10% trichloroacetic acid in the presence of 0.5 ml of 0.1 M sodium pyrophosphate and yeast RNA as carrier. The precipitable radioactivity was collected on Selectron membrane filters. Radioactivity was determined in a Beckman liquid scintillation spectrometer using a 2,5-diphenyloxazole-1,4-bis-2-(5-phenyloxazolyl)benzene-toluene scintillation fluid.

(i) Characterization of the assay system.

The enzyme activity of the reaction mixture containing NDV_{pi} was demonstrated to be dependent on the presence of magnesium, dATP, dGTP, dCTP and the detergent, Triton X-100, as shown in Table 1. Furthermore, if RNase was added to the reaction mixture, or to the detergent-disrupted virus and incubated 1.5 h at 37 C before being added to the reaction mixture, a significant diminution of precipitable counts was observed. These results, also shown in Table 1, indicate the requirement of intact viral RNA as a template for the enzyme.

(ii) Characterization of the DNA product of the reaction. The product DNA of the enzyme reaction utilizing the NDV_{pi} viral RNA as template was shown (Table 1) to be completely resistant to RNase (50 $\mu\text{g}/\text{ml}$, 90 min, 37 C) while being sensitive to the action of DNase

(50 $\mu\text{g}/\text{ml}$, 90 min, 37 C). The product DNA was also characterized by density gradient centrifugation (Fig. 2). The density of the product DNA was determined to be 1.460 g/cm^3 . Counts were frequently observed at the bottom of the tube. However, when fractions containing the pellet material were recentrifuged in a similar gradient, the counts found at the bottom of the tube greatly diminished, the greater portion banding at the density of DNA indicating that the pellet was an artifact of centrifugation.

(iii) Other control experiments were carried out to eliminate the possibility that the RDDP activity was due to a passenger virus which might be present in L-cell cultures (5), since the NDV_{pi} was originally isolated from L cells.

The enzyme was shown to be present in a hemagglutinating particle by subjecting the purified NDV_{pi} preparation to three cycles of hemagglutination with chicken erythrocytes. A cycle of hemagglutination was completed as follows: to 0.5 ml of purified NDV_{pi} chilled to 4 C was added 5 ml of a 1% suspension of chicken erythrocytes. After incubation for 1 h at 4 C, the

TABLE 1. Characterization of polymerase assay system and DNA product

Determination	Counts/min
A. Requirements of NDV _{pi} DNA polymerase ^{a, b}	
Complete	2,892
- dATP	254
- dGTP	109
- dCTP	228
- Mg ²⁺	122
- Triton X-100	36
+ RNase ^c	45
B. Characterization of the DNA product ^{a, d}	
Product extracted with phenol + buffer control	2,992
Product extracted with phenol + RNase ^d	2,401
Product extracted with phenol + DNase	193

^a Standard reaction mixtures were incubated at 37 C for 60 min with the substitutions listed and processed as described in Fig. 1.

^b 960 counts per min per pmol of ^3H -TTP and 27.1 μg of viral protein.

^c Incubated in the presence of 50 μg of crystalline bovine pancreatic RNase per ml. The salt concentration was that of the reaction mixture.

^d The product from the reaction mixture incubated at 37 C for 90 min with either RNase or DNase at concentrations of 50 $\mu\text{g}/\text{ml}$ in a buffer of 0.15 M NaCl and 0.01 M Tris-hydrochloride, pH 7.5, and then processed as described in Fig. 1.

FIG. 2. Density of product synthesized by NDV_{PI} polymerase. A 0.5-ml amount of a standard reaction mixture containing 50 μ g of viral protein and 0.01 mM 3 H-TTP at 1,500 counts per min per pmol was incubated for 12 h at 37 C. The product was purified as described in Materials and Methods, mixed with saturated Cs₂SO₄ to a density of 1.550 g/cm³ and centrifuged at 192,000 $\times$ g at 20 C for 60 h in a Beckman SW50.1 rotor. Fractions were collected from the bottom and processed as described in Fig. 1. Densities were determined by weighing 10- μ liter vol of each fraction.

erythrocytes were pelleted by centrifugation. The supernatant fluid remaining after three cycles of hemagglutination at 4 C was subjected to conditions which would pellet any remaining virus and reconstituted to its original 0.5 ml vol. This suspension no longer showed the ability to incorporate thymidine, whereas the untreated virus preparation was fully active. The activity present after elution from the erythrocytes was not determined due to the difficulty in eluting NDV_{PI}. Thacore and Youngner (17) have reported the resistance of this virus to elution from chicken erythrocytes.

Additional evidence that a passenger virus originating in L cells was not responsible for the observed RDDP activity was obtained by collecting the supernatant fluid from normal L cell cultures, concentrating it and purifying it in the same manner as described for the NDV_{PI} virus, and subjecting this concentrated, "purified" fluid to a RDDP assay after passage in CE cells, as if it had contained NDV_{PI}. As in all of the RDDP assays reported in this paper, the NDV₀ and NDV_{PI} were passed in cells from the same eggs. No significant RDDP activity was observed in the absence of an exogenous template. However, as shown in Table 2, this L-cell preparation was found to be capable of utilizing

the synthetic template, poly rA-oligo dT. The activity of the preparation from normal L cells, although significant, was only about 4% of that obtained utilizing NDV_{PI} with the synthetic template.

If the "reverse transcriptase" activity observed in preparations of NDV_{PI} was due to the presence of passenger virus which was capable of hemagglutinating chicken erythrocytes, it would be expected that this virus would be present in large enough quantities to permit its detection by immunodiffusion. Consequently, purified, concentrated samples of both NDV_{PI} and NDV₀ which had previously been assayed for RDDP activity were tested using this method. These experiments were carried out by Louis Potash of Flow Laboratories, Inc. of Rockville, Md. The immunodiffusion experiments utilized nine different antisera (see Materials and Methods). Negative results were obtained in all cases, reading the slides at 24, 48, and 72 h, indicating that if antigens of these types were present in the preparations, they were present in concentrations too low to be detected by immunodiffusion. However, it should be emphasized that the immunodiffusion experiments alone are inconclusive, since a contaminant virus which had a high degree of polymerase activity could fail to be detected by this means. The above results when taken together argue against the possibility that the RDDP activity observed with NDV_{PI} is dependent upon the presence of a passenger virus.

To demonstrate that the RDDP activity was associated with infective NDV_{PI} virions but not with NDV₀ virions, an experiment was carried out in which virus samples, purified as de-

TABLE 2. Control experiment^a to determine the contribution to the observed RDDP activity by possible adventitious viruses in L-cell cultures

Determination	Counts/min ^b	
	With synthetic template ^c	No added template
Normal L-cell fluid ^d	395	100
NDV _{PI} ^e	9,300	5,200

^a The polymerase assay was performed as described in Materials and Methods.

^b Counts per minute after 120 min of incubation with reaction mixture at 37 C.

^c Poly-A with oligo dT (12-14) as described in Materials and Methods.

^d Normal L-cell fluid, passed in CE cultures, concentrated from 5 liters and purified as described in the Materials and Methods for NDV_{PI}.

^e The sample contained 5.0×10^{10} PFU/ml and 0.50 mg of protein per ml.

scribed above, were simultaneously assayed for density, infectivity, and RDDP enzyme activity. The results of this experiment are summarized in Fig. 3. These results show the coincidence of enzyme activity with infectivity for NDV_{pi} and emphasize the absence of activity of NDV_o. A difference in densities between the two viruses (1.23 g/cm³ for NDV_o, 1.19 g/cm³ for NDV_{pi}) is also shown. However, both densities are in agreement with the range of previous determinations of NDV densities by Blair and Duesberg (1.20 to 1.23 g/cm³) (3) and by Huang, Baltimore, and Bratt (1.19 g/cm³) (10).

Hybridization studies. Viral RNA was extracted from both NDV_o and NDV_{pi} and purified as described above. The viral RNA was extracted with phenol and characterized in a sucrose gradient (5 to 20% in 0.1 M NaCl, 0.01

M Tris-hydrochloride, 0.001 M EDTA at pH 7.4). The NDV_{pi} RNA and the NDV_o RNA were both found to have a value of 57S by centrifuging at 45,000 rpm for 1 h at 4 C in an SW50.1 rotor, using intact R 17 bacteriophage as a marker.

The RNA samples were hybridized by the method of Spiegelman et al. (15) with DNA made by using NDV_{pi} RNA as template. The results of the hybridization (Fig. 4 and 5) demonstrated that the product DNA extracted from the reaction mixture hybridizes with viral RNA extracted from either NDV_o or NDV_{pi}. In Fig. 4, the results of the hybridization of NDV_{pi} RNA with product DNA are shown. The original peak at a density of 1.460 has been shifted to a density of 1.660, indicating the formation of the DNA-RNA hybrid.

FIG. 3. Isopycnic sedimentation in a potassium tartrate gradient showing the location of the NDV_{pi} RDDP activity and the absence of activity in the region of the wild type, NDV_o. Partially purified NDV_{pi} and NDV_o were layered onto linear gradients made from 10 to 70% potassium tartrate. The buffer was phosphate-buffered saline. Centrifugation was in a Beckman SW50.1 rotor at $170,000 \times g$ for 3 h at 4 C. Fractions were collected from the bottom. Infectivity was determined by a plaque assay on CE monolayer cultures. The enzyme assay was carried out as described in Materials and Methods by using ³H-thymidine triphosphate with a specific activity of 980 counts per min per pmol. The densities were determined by weighing 10- μ l portions of each fraction. Symbols: NDV_o infectivity, $\circ$ — $\circ$; NDV_{pi} infectivity, $\square$ — $\square$; NDV_o enzyme assay, $\times$... $\times$; NDV_{pi} enzyme assay, Δ — Δ .

FIG. 4. Hybridization of the DNA product with NDV_{pi} viral RNA. A 0.5-ml amount of a standard reaction mixture was incubated at 37 C for 18 h. The nucleic acids were extracted as described in Materials and Methods and treated with 0.4 M NaOH for 24 h at 37 C to completely hydrolyze RNA. After neutralization the purified product (1,000 counts/min) was mixed with 10 μ g of NDV_{pi} viral RNA in 0.3 ml of 0.4 M NaCl-0.002 M EDTA (pH 7.4)-50% (vol/vol) formamide and incubated at 37 C for 24 h. The nucleic acids were then analyzed in equilibrium density gradients of Cs₂SO₄ as described in Materials and Methods.

In a similar experiment, viral RNA extracted from NDV_o virions was hybridized with the product DNA from the NDV_{pi} RDDP reaction. The results of this experiment (Fig. 5) show that wild-type RNA will also hybridize with the product DNA, causing a shift in density from 1.460 to 1.660 g/cm³. This experiment obviates the problem of a possible passenger virus, since no "reverse transcriptase" activity was observed in NDV_o.

To insure that the hybridization results were due to the alignment of proper base sequences and not to nonspecific base pairing, vesicular stomatitis virus RNA was hybridized with the DNA product. Upon centrifugation in a cesium sulfate gradient as described above, no shift in the position of the labeled DNA product was observed.

DISCUSSION

A set of criteria to establish the presence of "reverse transcriptase" in any virus has been suggested by Gallo (6) as follows: (i) demonstration of an endogenous reaction that is RNase sensitive; (ii) requirements for all four deoxynucleoside triphosphates; (iii) proof that the product is DNA; (iv) polymerase activity that bands at the density characteristic of the virus which depends on detergent treatment; (v) complementarity between the RNA template and the DNA product; (vi) demonstration that one

of the two strands is RNA. The results obtained in experiments described above will be discussed in terms of these criteria.

The enzyme present in the NDV_{pi} preparation was shown to be capable of utilizing viral RNA as an endogenous template (see Fig. 1). The results in Table 1 point out that this reaction is RNase sensitive and also show the requirements for the deoxynucleoside triphosphates. That the product was DNA was shown by its resistance to RNase and its susceptibility to DNase (Table 1) and also by its density in Cs₂SO₄ (Fig. 2).

The polymerase activity was shown to band at a density associated with the intact virus (Fig. 3), and the essential detergent activation of the enzyme is shown in Table 1. Complementarity between NDV_{pi} viral RNA and the DNA product is shown in Fig. 4. That one of the strands is RNA is deduced from the density of the hybrid.

However, the degree to which the above criteria are met does not preclude the possibility that these results are artifactual and are due to the presence of a passenger virus which carries the reverse transcriptase. This possibility was minimized by the following control experiments. (i) The enzyme activity of purified virus preparations could be removed by adsorbing the virus to chicken erythrocytes. Further experiments are planned to determine if the enzyme activity is removed when non-hemagglutinating erythrocytes are employed, in order to provide a control for a possible adventitious virus absorbing non-specifically to the erythrocytes. (ii) Normal L-cell fluid taken from cultures of cells arising from the same clone as those in which the persistent infection had been established showed no significant enzyme activity unless a synthetic template was added. Even so, this

FIG. 5. Hybridization of the DNA product to NDV_o viral RNA. The procedure used in this experiment is described in legend to Fig. 4.

activity was much less than if NDV_{pi} were present in the reaction mixture. (iii) The presence of RNA tumor-type antigens could not be demonstrated by immunodiffusion against nine different antisera to agents of this type. Again, it should be mentioned that the immunodiffusion tests alone do not eliminate the possibility that a contaminant virus is responsible for the RDDP activity observed. That these activities are due to polymerases in the uninfected chicken cells is unlikely since the RDDP activity in NDV_o, grown in identical cultures as NDV_{pi}, was negligible. Likewise, if the activity were due to avian leukosis viruses, it would be expected to appear in NDV_o preparations as well as in NDV_{pi}. (iv) Complementarity was demonstrated between the NDV_{pi}-produced DNA and the NDV_o viral RNA. Since the DNA produced by RDDP in the mutant virus preparations hybridized with wild-type RNA and the wild-type virus did not contain RDDP activity, it follows that the DNA was not produced by an adventitious passenger virus.

When these results and their control experiments are taken together, it appears likely that NDV_{pi} carries in its virion assembly the enzyme RNA-dependent DNA polymerase. The significance of this finding does not lie in the observation that a fowl respiratory virus contains this enzyme; rather, our findings raise the more important question of whether the processes which contribute to persistent infection permit a virus to express or acquire reverse transcriptase activity.

Why the mutant virus preparations (but not of the wild type) should contain reverse transcriptase must be regarded as speculative at this time. The presence of the enzyme could represent a recombinational event between either a cellular RNA or an RNA tumor virus and the NDV RNA. Another possible explanation is that the information for RDDP could be contained in the wild-type genome. The mutational event or events could have resulted in a derepression of this information in the mutant.

It remains to be proven if the action of reverse transcriptase in forming a viral coded DNA, which could be integrated into the cell genome, is responsible for the maintenance of this persistent infection in L-cell cultures. On the one hand, such a mechanism could explain the observation that this infection persists in the presence of sufficient interferon to completely inhibit the amount of virus produced (8). On the other hand, it makes it difficult to explain the cure of this persistent infection by interferon (9). If this persistent infection is maintained as

a result of NDV_{pi} RDDP, it would be expected that the product DNA is integrated with cell DNA. Experiments are currently underway in this laboratory to determine if such an association exists between NDV_{pi} DNA and the L_{pi} DNA.

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