COMPARISON OF DNA IN THE CORE COMPONENT FROM SCHMIDT-RUPPIN RSV TRANSFORMING VIRUS AND NON-TRANSFORMING VIRUS*

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SUMMARY

Viral core fractions from transforming Schmidt-Ruppin sarcoma virus (SR-RSV-t) and its transformation-defective derivative (SR-RSV-td) were examined for DNA. The transforming virus contained DNA that was easily detected, but the transformation-defective virus had little or no DNA associated with the core fraction. The buoyant density of the DNA from SR-RSV-t in CsCl was 1.715 g/cm^3.

INTRODUCTION

In addition to RNA it has been reported that DNA was associated with the nucleic acid fraction from avian and murine RNA tumor viruses (1-4). DNA in AMV and MC29 avian leukosis virus has been localized in the inner core structure of the virion (5). Clearly, this demonstrated that the DNA was not a contaminant of the outer membrane structure of the virus. Recently, DNA from murine leukemia virus was found to be of cell origin, but these studies also showed that DNA from the virions was not random host DNA sequences and may be lymphocyte specific (6). In this report we have extended our previous finding on DNA associated with avian tumor virus to show that SR-RSV-t also contains DNA in its viral core fraction, but that SR-RSV-td contains little, if any, DNA.

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Abbreviations:

SR-RSV-t = transforming Schmidt-Ruppin sarcoma virus
SR-RSV-td = transformation-defective Schmidt-Ruppin sarcoma virus
AMV = avian myeloblastosis virus
CEC = chick embryo cells

METHODS

Each strain of SR-RSV (a generous gift from Dr. H. Hanafusa) was grown in CEC. Those cultures receiving SR-RSV-t were well transformed during the virus collection, but cultures receiving the transformation-defective virus showed no transformation. Freshly cloned SR-RSV-t was used for one set of experiments (Fig. 1A) while uncloned virus was used for the other set of experiments (Fig. 1B). Cloned virus was obtained by selecting a single focus of transformed cells grown in agar suspension following the procedure of Duesberg & Vogt (7). Colony-derived stocks of sarcoma virus have been shown to contain mainly transforming virus by the predominance of class *a* RNA and little class *b* RNA (7, 8).

Virus concentrates were made from culture fluids containing transforming or transformation-defective SR-RSV by centrifuging the virus for 2 h at 20,000 rev/min in a Spinco ultracentrifuge, rotor type FA21. The virus concentrate was sedimented two times through a 20% sucrose layer and the final pellet suspended in TNE buffer (0.01 M-tris, 0.1 M-NaCl and 0.001 M-EDTA). The virion suspensions were layered over a discontinuous sucrose gradient (1.5 ml, 20% sucrose; 1.0 ml, 40% sucrose; and 1.0 ml, 70% sucrose prepared in TNE) and centrifuged in a Spinco ultracentrifuge at 50,000 rev/min for 30 min in a SW50.1 rotor (2°C). The band of virus at the interface between 20% and 40% sucrose was collected and pelleted by centrifuging 20 min at 50,000 rev/min in a SW50.1 rotor. Viral cores were prepared from purified virus using Sterox-SL detergent following a procedure described by Stromberg (9). The core fractions from both virus preparations were isolated on a discontinuous gradient as described above, but since cores are more dense than the virus, they collected at the 40-70% sucrose interface. Nucleic acid was isolated from the core fraction as previously described by Weber *et al.* (2). The viral nucleic acids were mixed with CsCl (final density of 1.70 g/cm^3) and centrifuged 42,000 rev/min in a Spinco SW50 rotor for 48 h at 20°C. Under these conditions the DNA formed a band near the center of the CsCl gradient and RNA was located at the bottom of the centrifuge tube. DNA was detected by measuring its priming activity for DNA synthesis in fractions collected from the CsCl gradient using primer-free reverse transcriptase enzyme (10) and [^{3}H]-dGTP with unlabeled dATP, dCTP, dTTP as substrates. Synthesis of the labeled DNA product required all four deoxyriboside triphosphates. Details of the assay procedure have been published elsewhere (2). DNA preparations from AMV similar to those reported in this paper have been identified by UV spectra, sensitivity to DNase I, alkali stability and buoyant density in Cs_2SO_4 and CsCl.

RESULTS AND DISCUSSION

Results presented in Fig. 1A and 1B compare the DNA in viral core preparations from transforming and transformation-defective SR-RSV. These experiments show that DNA was found in the core of virions that were transforming but was nearly absent in virions that were transformation-defective. In the experiment described in Fig. 1A, the virus from SR-RSV-t was collected from 1,950 ml of culture fluid and the SR-RSV-td was collected from 3,950 ml of culture fluid. The virion concentration in each preparation was estimated by measuring the reverse transcriptase activity. It

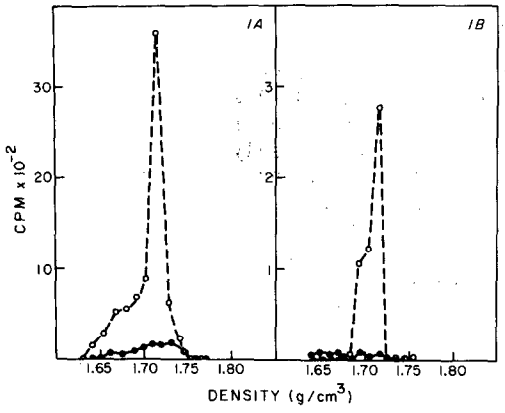

Fig. 1. CsCl denstiy gradient of nucleic acids from core fractions of SR-RSV-t and SR-RSV-td. Two experiments (1A and 1B) showing the DNA band from SR-RSV-t (0) and SR-RSV-td (●) after centrifuging the viral nucleic acid preparations in a CsCl gradient (see text for conditions). The DNA was measured in the gradient by analyzing each fraction for primer activity in the synthesis of [^{3}H]-DNA with primer-free reverse transcriptase (2). Under the conditions of the assays the relationship between DNA primer and [^{3}H]-DNA synthesis was proportional up to about 32,000 cpm of [^{3}H]-dGMP incorporated into DNA.

was found that the yield of virus in the culture fluid of SR-RSV-td was 1.7 times greater than the virus yield in the culture fluids collected from the cells infected with SR-RSV-t because of the difference in culture fluid volume. The results given in Fig. 1A were adjusted for the difference in amount of virus in each preparation by multiplying the observed values obtained from the SR-RSV-t by 1.7, but the data for the transformation-defective virus were the observed measurements. Each preparation providing the data in Fig. 1B was derived from the same volume of culture fluid (1220 ml), but the virus concentration of the transforming virus as estimated by reverse-transcriptase activity was 3 times that of the transformation-defective virus and therefore the reported data for SR-RSV-t in Fig. 1B were adjusted by reducing the observed data by three-fold. The graph depicting the DNA content of SR-RSV-td again represents the observed measurements on each gradient fraction. Using the same techniques

for detecting DNA, culture fluids from CEC that were not infected with RNA tumor virus did not contain DNA and gave results similar to the transformation-defective virus preparation in Fig. 1B (data not shown). The results from SR-RSV-td in Fig. 1A suggest that some DNA may be present in the viral core fraction, however it is judged to be very low relative to the SR-RSV-t and other avian tumor viruses we have tested. The buoyant density of the DNA from SR-RSV-t in the peak fraction from the CsCl gradient was 1.715 g/cm^3 with both preparations. DNA from AMV had previously been found to have a density of 1.70 g/cm^3 and DNA associated with MC29 virus was found to have a density of 1.72 - 1.73 g/cm^3, but after RNase treatment was 1.70 g/cm^3 (2).

It is not clear why DNA is associated with viruses that have RNA as their genetic material. Although we have examined only 4 different RNA tumor viruses for DNA, the transformation-defective derivative of SR-RSV has been the only virus in this group that we have failed to identify the presence of DNA in the viral core fraction. Experiments in this paper suggest that at least for SR-RSV-td, the presence of viral DNA was not required in the viral replication cycle, but this would be true only if a majority of the virions were infectious.

One wonders then why DNA is associated with SR-RSV-t, or the other RNA tumor viruses, AMV and MC29 virus. There are two explanations that may be of consequence in understanding tumor virus expression. One consideration is that DNA may have some role in viral transformation and another possibility is that DNA may appear in the virion as a result of its assembly in a transformed cell. If either of these speculations are valid, then similar results should be found with other transforming virus and their nontransforming varients. Experiments are underway to investigate these two possibilities.

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