spond to societal organization but were crosscut by patterns of ecological adaptation. The notion of "tribe" is not useful either in ethnohistory or prehistory, since it is the classificatory device of outsiders (for example, Han Chinese or Dutch) and does not accurately describe the aboriginal scene at the time of contact, nor does it allow for the indigenous culture contact and change which took place.

JUDITH M. TREISTMAN
Anthropology, City University of New York, Graduate Center, New York 10036

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DNA of Rous Sarcoma Virus: Its Nature and Significance

Abstract. *Purified preparations of Rous sarcoma virus (an avian tumor virus with an RNA genome) contain small amounts of double-stranded DNA. This DNA cannot be hybridized to viral RNA, but will reanneal completely with the DNA of avian cells. Extensive substitution of bromodeoxyuridine for thymidine in "viral" DNA does not photosensitize the biological activity of the virus. These observations indicate that the DNA associated with Rous sarcoma virus is derived from the DNA of the avian host cell, and is probably devoid of any function in the life cycle of the virus.*

The principal nucleic acids of the RNA tumor viruses (oncornaviruses) are a 70*S* single-stranded RNA (the putative viral genome) and two forms of low-molecular-weight RNA (*1*). Recently, however, small amounts of DNA have also been found in purified preparations of avian (*2, 3*) and murine (*4*) oncornaviruses. This observation is of interest in view of the substantial body of evidence indicating that DNA is essential to both the replication of oncornaviruses and the induction of cellular transformation by these viruses (*5*). We now describe experiments designed to ascertain the source and significance of the DNA associated with virions of the Schmidt-Ruppin strain of Rous sarcoma virus (RSV).

Virus was propagated in secondary cultures of chick embryo fibroblasts, labeled with [^{3}H]thymidine (14 c/mmole), [^{14}C]uridine (55 mc/mmole), or [^{32}P]orthophosphate (all from Schwarz/Mann), and purified as described (*2, 6*). Nucleic acids were extracted from purified virus with sodium dodecyl sulfate and pronase (*7*), then fractionated by elution from hydroxyapatite (*8*). DNA comprises approximately 0.5 to 1.0 percent of the total nucleic acids obtained from RSV in this manner. Similar results were obtained with phenol extraction, although the recovery of nucleic acids was reduced (*2*).

The DNA extracted from purified RSV is double helical, as judged by its elution from hydroxyapatite (data not shown). The standards for this analysis were the single-stranded DNA of fd bacteriophage and the double-stranded DNA of avian cells [see (*8*) for details of the procedure and its standardization]. The RSV DNA is completely denatured to the single chain form when boiled in 3 m*M* EDTA for 10 minutes or when treated with 0.4*N* NaOH at 37°C for 1 hour (unpublished observation). These data indicate that virion DNA is composed of double-stranded molecules with no propensity to "snap back" after denaturation.

Virions of oncornaviruses contain an RNA-directed DNA polymerase (*9*) which transcribes the viral RNA genome into double-helical DNA (*8, 10*). It is conceivable that virion DNA represents the product of such transcription occurring within the virion. We tested this possibility by examining the ability of denatured virion DNA to anneal with 70*S* RSV RNA, purified by rate-zonal centrifugation in density gradients of sucrose (*6*). The annealed nucleic acids were analyzed by equilibrium centrifugation in Cs_2SO_4 (*11*). As a control, single-stranded DNA synthesized in vitro by the RNA-directed DNA polymerase of RSV (*7, 8*) was reacted with a large excess of 70*S* viral RNA (Fig. 1a). Virtually all of the DNA hybridizes with viral RNA, and consequently bands at approximately the same density as single-stranded RNA. The nature and specificity of these hybrid structures have been recorded (*11*). No hybrids are formed when denatured virion DNA is reacted with 70*S* viral RNA (Fig. 1b). The breadth of the band of virion DNA is due to its low molecular weight [about 100,000 to 500,000; (*2*)] compared to that of the lambda bacteriophage DNA marker (about 30×10^6).

Failure of the virion DNA to hybridize with viral RNA under the

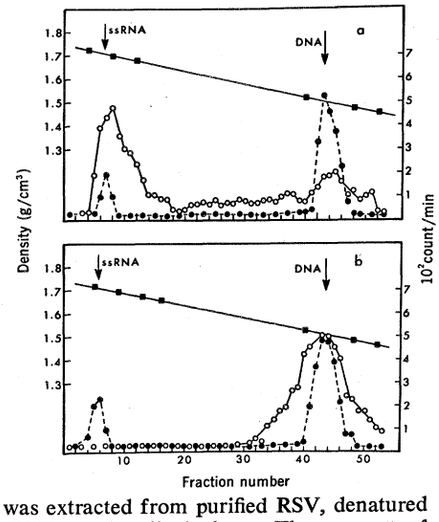

Fig. 1. Hybridization of DNA with RSV RNA. Denatured DNA (<0.1 μg) was incubated with 5 μg of 70*S* RSV RNA in 0.4 ml of annealing solution (*11*) for 24 hours at 37°C. The nucleic acids were then analyzed by equilibrium centrifugation in Cs_2SO_4 (*11*). *Escherichia coli* RNA and lambda phage DNA both labeled with ^{32}P served as density markers. (a) Virus-specific DNA synthesized by RNA-directed DNA polymerase. ^{3}H-Labeled DNA was synthesized with the virion-associated polymerase of RSV (*7, 9*), and single-stranded DNA was isolated from the reaction product as described (*8*). This DNA is entirely complementary to RSV 70*S* RNA, and consequently can be extensively hybridized to viral RNA as illustrated here and reported (*11*). ●—●, ^{32}P, count/min; ○—○, ^{3}H, count/min; ssRNA, single-stranded RNA marker; DNA, DNA marker. (b) Virion DNA. ^{3}H-Labeled DNA was extracted from purified RSV, denatured with NaOH, and incubated with 70*S* RSV RNA as described above. The amount of virion DNA (< 0.1 μg) was estimated on the basis of the specific activity of DNA extracted from the cells used to produce the labeled virus. ●—●, ^{32}P, count/min; ○—○, ^{3}H, count/min; ■, density, g/cm^3.

above conditions could be ascribed to a more rapid interaction of complementary DNA strands to re-form double-helical molecules. We have tested this possibility with negative results. At the conclusion of the annealing reaction, the virion DNA still elutes from hydroxyapatite as single-stranded molecules (unpublished observation).

Having failed to find any complementarity between nucleotide sequences of virion DNA and viral RNA, we examined virion DNA for sequences homologous to those of cellular DNA. This was done by measuring the reassociation kinetics of various DNA's in the manner described by Britten and Kohne (*12*). As noted above, and illustrated further in Fig. 2a, denatured virion DNA does not reassociate at the concentrations now available. The incubation described in Fig. 2a was carried out in the presence of salmon sperm DNA, the reassociation of which proceeded normally. By contrast, denatured virion DNA does reanneal into a double-helical state when incubated in the presence of denatured avian cell DNA (Fig. 2b). Moreover, the extent of reassociation as a function of time is identical to that of the cellular DNA. We conclude that virion DNA of RSV shares extensive or complete sequence homology with the DNA of avian cells, and that populations of individual sequences are present in both types of DNA in about the same amount. Virion DNA of RSV is therefore probably derived in a random fashion from DNA of the avian host cell.

Purified preparations of a number of DNA viruses contain virions that enclose host cell DNA rather than viral genome (*13*). These "pseudovirions" can generally be distinguished and purified by virtue of their unique buoyant densities. We have centrifuged RSV—doubly labeled with [^{3}H]thymidine and [^{14}C]uridine—to equilibrium in shallow density gradients of CsCl. There is no appreciable discrepancy between the densities of the particles containing DNA and RNA. However, the significance of this negative result is uncertain, because nucleic acids comprise only 1 to 2 percent of the total mass of RSV (*1*) and therefore contribute very little to the density of the virus particle.

At present, there is no evidence to suggest that virion DNA might be essential to the biological activity of RNA tumor viruses. We have examined this issue further by performing the following experiment. RSV-infected cells were exposed to bromodeoxyuridine (BUdR: 5 or 50 μg/ml) for 36 hours. Virus was harvested from these cells during the final 12 hours, purified, and tested for photosensitivity. Irradiation with visible light had no effect on its biological activity, which was measured by focus assay (*14*). The amount of BUdR and the dose of irradiation were similar to those used by Boettiger and Temin to photoinactivate infectious centers induced by RSV (*15*). The extent to which BUdR has sub-

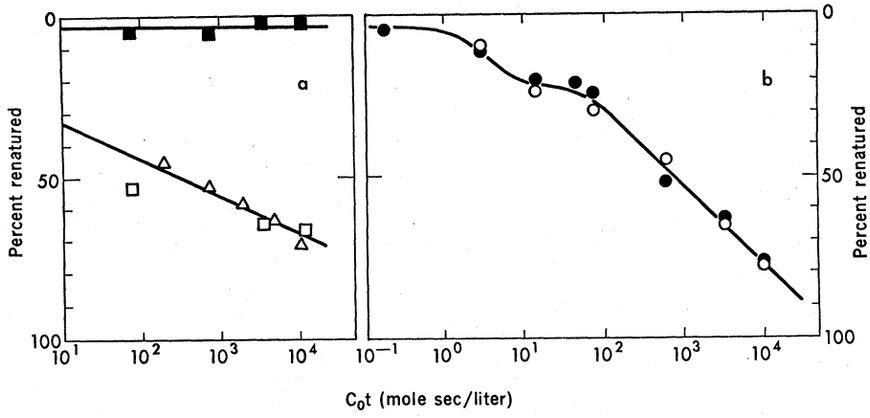

Fig. 2. Homology between RSV virion DNA and avian cell DNA. DNA was extracted from purified RSV (labeled with [^{3}H]thymidine) and from unlabeled chick embryos (*8*). Salmon sperm DNA (Sigma) was deproteinized by two extractions with a mixture of chloroform and isoamyl alcohol. The DNA's were sheared in a pressure cell at 50,000 pounds per square inch (American Instrument), then denatured by boiling in 0.003*M* EDTA for 5 minutes. Sodium phosphate (*p*H 6.8) was added to a concentration of 0.40*M*, and the solutions were incubated at 68°C. Samples were withdrawn periodically, and the extent of reassociation of DNA was determined by analysis on hydroxyapatite. The amount of salmon sperm or avian DNA in the hydroxyapatite eluates was determined by optical measurements (absorbance at 260 nm). Virion DNA (^{3}H-labeled) in the eluates was measured by acid precipitation. The extent of reassociation is plotted as a function of the product of the concentration of DNA and time of incubation (C_0t) (*12*). (a) Virion DNA in the presence of salmon sperm DNA. The true concentration of virion DNA was indeterminate. Consequently, the extent of reassociation is plotted here and in (b) against the C_0t values for cell DNA. The reassociation of salmon sperm DNA occurred as expected on the basis of previous results (*12*). ■—■, Virion DNA (radioactivity); □—□, salmon sperm DNA, 2.7 mg/ml (optical density); and △—△, salmon sperm DNA, 3.7 mg/ml (optical density). (b) Virion DNA in the presence of chick embryo DNA. ●—●, Virion DNA (radioactivity); ○—○, chick embryo DNA, 2.7 mg/ml (optical density).

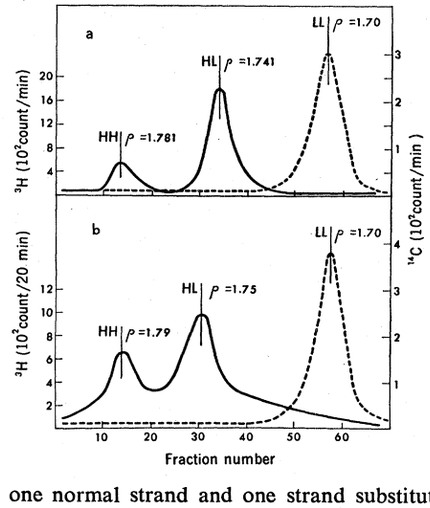

Fig. 3. Equilibrium centrifugation of BUdR-substituted DNA. RSV-infected cells were exposed to BUdR (5 μg/ml) for 24 hours, followed by a 12-hour exposure to both BUdR (5 μg/ml) and [^{3}H]thymidine (50 μc/ml). At the conclusion of this 12-hour period, virus was harvested, and DNA was extracted from the cells (*22*). DNA was subsequently extracted from the purified virus in the same manner. Virion and cellular DNA were analyzed separately by equilibrium centrifugation in CsCl [40 rotor, 33,000 rev/min, 25°C, 60 hours; see (*21*)]. Normal avian DNA, labeled with [^{14}C]thymidine, was included in both cases as a density marker ($\rho = 1.70$ g/cm^3). The gradients were fractionated and samples were taken for determination of density and acid-precipitable radioactivity. *LL*, DNA containing two strands of normal density; *HL*, DNA containing one normal strand and one strand substituted with BUdR; *HH*, DNA containing both strands substituted with BUdR. (a) Cellular DNA. ——, ^{3}H radioactivity (BUdR-substituted cellular DNA); – – –, ^{14}C radioactivity (normal cellular DNA). (b) Virion DNA. ——, ^{3}H radioactivity (BUdR-substituted virion DNA); – – –, ^{14}C radioactivity (normal cellular DNA).

stituted for thymidine in virion DNA was determined by equilibrium centrifugation in CsCl (Fig. 3). The distributions of BUdR-substituted virion and cellular DNA's in these gradients are virtually identical. This observation supports our previous suggestion that virion DNA is derived from the normal pool of cellular DNA without special selection. The densities of the once-replicated (HL, $\rho = 1.74$–1.75) and twice-replicated (HH, $\rho = 1.78$–1.79) DNA's are indicative of maximum substitution by BUdR (*16*).

We conclude that the DNA associated with purified virions of RSV consists of a random sample of cellular DNA in a low-molecular-weight form. The possibility that the DNA is enclosed in "pseudovirions", rather than in biologically active virions, is still undetermined. However, the presence of small amounts of ribosomal RNA in RSV (*1*, *6*) and other RNA tumor viruses (*1*) does point to the inclusion of normal cellular elements in virus preparations, either as adventitious contaminants or as virion constituents. A report that DNA is associated with the plasma membrane of human diploid cells (*17*) raises the possibility that the DNA found in oncornaviruses is an envelope constituent, acquired when virions are released from the host cell. Examination of purified viral nucleoids (*18*) for the presence of DNA should provide a test of this suggestion. Whatever its source, the virion DNA of RNA tumor viruses must be taken into account in any study of the RNA-directed DNA polymerase present in these viruses. We have evidence that as much as 5 to 10 percent of the total double-stranded DNA synthesized in vitro by enzyme-active virions consists of transcripts of the avian DNA associated with RSV virions. Other investigators have suggested that virion DNA might serve as the primer for initiation of RNA-directed synthesis within the virion (*19*), but more recent observations indicate that this function is served by a ribopolynucleotide (*20*). For the present, we consider the most reasonable conclusion to be that the DNA present in preparations of RSV is probably devoid of any special function in the life cycle of the virus.

WARREN E. LEVINSON
HAROLD E. VARMUS
AXEL-CLAUDE GARAPIN
J. MICHAEL BISHOP
Department of Microbiology,
University of California,
San Francisco 94122

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Systemic Activity of a Juvenile Hormone Analog

Abstract. *The peptidic analog of insect juvenile hormone, ethyl pivaloyl-L-alanyl-p-aminobenzoate with enormous biological activity on the red cotton bug,* Dysdercus cingulatus, *has pronounced systemic effect in sunflower plants. The compound is absorbed by the plant tissues and appears to be translocated throughout the plant system in active form. There is some evidence that juvenile hormone analogs of other types also have similar systemic effects. The discovery of systemic action should aid in the possible utilization of juvenile hormone analogs in insect control programs.*

Juvenile hormone (JH) inhibits cell differentiation in insect metamorphosis and stimulates growth of the ovaries in adult life. Many natural and synthetic compounds exhibit JH activity (*1*) and are effective in contact applications as well as in the diet. Because of their high biological activity and selective action, the juvenile hormone analogs (JHA's) are considered to be potential pesticides (*2*).

In order to explore all possible methods of application of the JHA, we have studied their systemic activity. By analogy with conventional insecticides we realized that such a systemic JHA must be absorbed by the plant system, should be relatively stable, enormously active on insects in peroral application, and at least partly water soluble. With this in mind we selected a few compounds that seemed to have at least some of the above-mentioned qualities. Special attention was paid to the recently discovered (*3*, *4*) group of peptidic JHA which are relatively stable, are highly active on *Dysdercus cingulatus*, and are active when administered in solution in drinking water. We selected the ethyl ester of pivaloyl-L-alanyl-*p*-aminobenzoic acid since as little as 0.00004 μg topically applied to freshly molted last instar larvae will cause formation of half-larval adultoids which are unable to reproduce. When administered in drinking water, adultoids appear already at the concentration of 0.0001 part per million in water.

Since the terrestrial seed feeding Hemiptera like *Dysdercus* invariably require water for their development, they suck the sap of any available plants, in the absence of other water. We used this phenomenon as an indicator of the presence or absence of the compound in the untreated parts of the plant system. We used sunflower plants that were approximately 20 cm long, cultured under greenhouse conditions. Freshly molted last instar larvae of *Dysdercus cingulatus* reared up to this stage on dry cotton seeds and