# Deoxycholate Releases RNA from Rauscher Murine Leukemia Virus[1] (34939)

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(Introduced by A. Granoff)

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Relatively little is known about the internal structure of avian and mammalian oncogenic RNA viruses. These viruses mature by budding from cell membranes, acquiring a lipid-containing envelope in the process. Ultrastructural examinations of the material enclosed by the viral envelope have been inconclusive, though some workers have observed what they considered to be helically symmetrical viral nucleocapsids (1).

On the other hand, among the nononcogenic enveloped RNA viruses, efforts to determine nucleocapsid structure by electron microscopy have generally, but not invariably, met with more success. The nucleocapsids of paramyxoviruses and of rhabdoviruses are clearly helical (2, 3). However, definitive evidence is lacking that the same holds true for the influenza viruses (4), and nucleocapsid anatomy of the enveloped arboviruses has not yet been completely resolved (5, 6).

Supplementary information about substructure in nononcogenic RNA viruses has come from physical–chemical studies. Thus, the strongly ionic detergent, sodium dodecyl sulfate (SDS), releases RNA from these viruses (7, 8, 10) while the nonionic and weakly ionic detergents, including the commonly employed sodium deoxycholate (DOC), release nucleocapsids (3, 4, 8–12). The nucleocapsids of all of these viruses sediment three to four times faster than the free viral RNA's (Table I), reflecting in each case the added mass of capsid proteins.

In view of these results, it was pertinent to disrupt a representative oncogenic RNA virus, Rauscher murine leukemia virus (RLV) (13), with DOC and look for a subviral entity, or nucleocapsid, composed of RNA and protein, which sedimented faster than the viral RNA.

*Materials and Methods.* The established mouse cell line, JLS-V5, which continuously produces RLV *in vitro* (14) was used as the source of virus. Cells were grown in Eagle's minimum essential medium (15) with 10% fetal calf serum at 37° in 5% $CO_2$ in air. To label viral RNA, 50 $\mu$Ci/ml of $^3$H-5-uridine (Schwarz BioResearch, sp act 20 Ci/mmole) were added to the medium. To label virus proteins, cells were incubated in the same medium with 5 $\mu$Ci/ml of $^{14}$C amino acids (Schwarz mixture). After 24 hr incubation with labeled precursors culture fluids were removed and centrifuged at 1000*g* for 20 min to remove floating cells and debris. Virus was concentrated from culture fluids using methods similar to those of Duesberg and Robinson (16). Five milliliters of sucrose, 65% (w/w), having a density of 1.32 g/ml made up in 0.005 *M* Tris–HCl, 0.1 *M* NaCl and 0.001 *M* EDTA, pH 7.4 (TEN buffer) were placed at the bottom of a 1 × 3-in. centrifuge tube and overlaid with 5 ml of a 15% (w/w) sucrose solution in the same buffer; 20 ml of tissue culture fluid were added, and the preparation was centrifuged in a Spinco SW 25.1 swinging-bucket rotor at 20,000 rpm for 2 hr at 4°. A faintly turbid band appearing at the interface between the

[1] Supported by Childhood Cancer Research Center Grant 2 PO2 CA 08480 from the National Cancer Institute and Public Health Research Grant 5 RO1 AI 05343 from the National Institute of Allergy and Infectious Diseases, and by ALSAC.

[2] Recipient of Research Career Development Award 5 K3 HD 14491 from the National Institute of Child Health and Human Development.

TABLE I. Sedimentation Coefficients of Nucleocapsids and RNA's from Enveloped RNA Viruses.

| Virus | Sedimentation coefficient ($S_{20,w}$) | | Reference |
|---|---|---|---|
| | Nucleocapsid | RNA | |
| Arbovirus (western equine encephalomyelitis virus) | 140 | 40 | 10 |
| Influenza virus (Type A, WSN strain) | 64[a] | 18 | 8 |
| | 56[a] | 15 | 8 |
| Paramyxovirus (Newcastle disease virus) | 200 | 50 | 12 |
| Rhabdovirus (rabies virus) | 200 | 45 | 3 |

[a] Several species of RNA and nucleocapsid occur in this virus (8,9).

15% and 65% sucrose solutions was isolated, diluted with TEN buffer to reduce its density, and centrifuged in a 28-ml linear 15–65% sucrose gradient in TEN buffer at 20,000 rpm for 7 hr at 4° in the swinging-bucket rotor. Fractionation and analysis of such gradients revealed coincidence of turbidity at 254 mμ and acid-insoluble radioactivity, either $^{3}H$ or $^{14}C$, at the density of RLV, 1.16 g/cm$^3$ (16), halfway down the gradient.

*Results.* The material which banded at 1.16 g/cm$^3$ was virus, since SDS released RNA from it which sedimented much faster than chick embryo ribosomal RNA's centrifuged under the same conditions (Fig. 1A, 20–28 ml). The distribution of RNA in this rapidly sedimenting peak was broad, indicating heterogeneity of size or configuration, as has been reported for RLV and for other tumor virus RNA's (16–18). When the SDS-treated virus was heated to disrupt hydrogen bonds, the rapidly sedimenting RNA was converted to a number of slower-sedimenting components, as Blair and Duesberg (17) have reported (Fig. 1B).

Contrary to results with nononcogenic enveloped RNA viruses, DOC released RNA which sedimented no more rapidly than the RNA released by SDS (Fig. 1C). In addition, the broadness of the peak was similar to the SDS-released material, indicating the same sedimentation heterogeneity. Of the total radioactivity applied to the gradient, 94% was recovered in the gradient after DOC treatment. To test whether viral protein cosedimented with viral RNA, $^{14}C$ amino acid-labeled virus was treated with DOC and centrifuged in the same way. Little of the $^{14}C$ label was recovered in the region of the gradient corresponding to the position of viral RNA (Fig. 1C), indicating that little, if any, viral protein was associated with viral RNA released by DOC. The viral RNA released by DOC was completely sensitive to pancreatic ribonuclease (Table II), contrasting with the resistant behavior of the RNA in paramyxovirus and rhabdovirus nucleocapsids (3, 12). The RNA of intact virions was slightly sensitive to ribonuclease (Table II), possibly reflecting slight contamination with cell RNA.

*Discussion.* We found that when the murine leukemia virus, RLV, was treated with DOC, a weakly ionic detergent, RNA was released. This RNA did not behave as if it were in a nucleocapsid: it sedimented at the same rate as RNA extracted with the strongly ionic detergent, SDS, or RNA obtained by phenol extraction (16, 17) and little, if any, viral protein sedimented with it. The sensitivity of the viral RNA to ribonuclease indicates that it was exposed by DOC to the environment, but this does not, by itself, rule out association of protein with the RNA. With influenza virus and arboviruses, nucleocapsid structure is such that viral RNA is accessible to ribonuclease (8–11). More extensive studies by P. H. Duesberg (22) show that DOC and nonionic detergents release RNA free of viral protein from the avian oncogen, Rous sarcoma virus. Thus, a nucleocapsid, defined as an ordered structure containing viral nucleic acid and protein, may not be present in enveloped, oncogenic RNA viruses.

Judging by results obtained with the

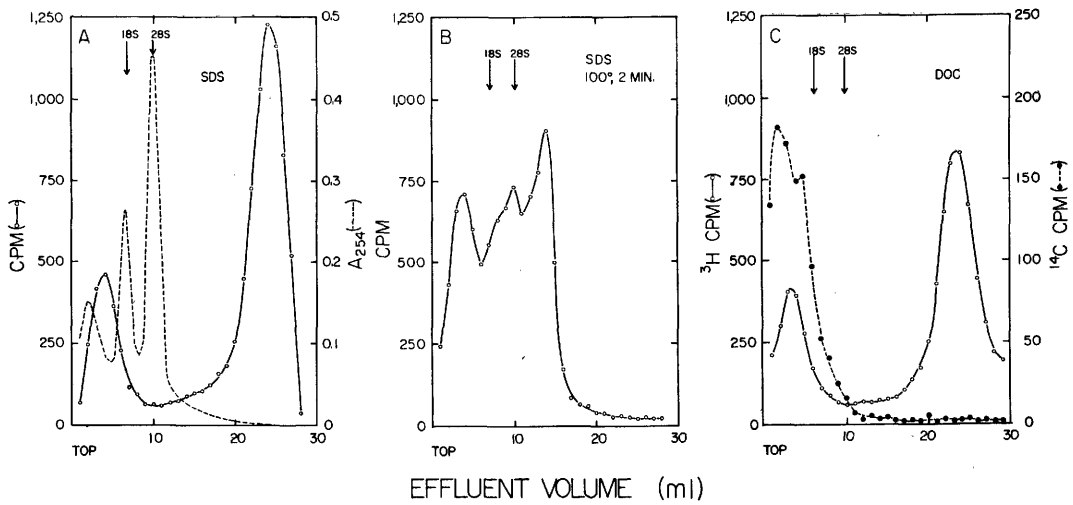


FIG. 1. Sucrose gradient rate zonal centrifugation of RLV RNA. Purified virus labeled biosynthetically with $^3$H-uridine or $^{14}$C-amino acids, in TEN buffer, was mixed with either SDS or DOC, and centrifuged in a 28-ml linear 15% (w/w) to 30% (w/w) sucrose gradient containing 0.5% of the respective detergent in TEN buffer. The gradients were centrifuged at 18,000 rpm, 20°, for 16 hr in a Spinco SW 25.1 swinging-bucket rotor. Gradients were fractionated automatically into 1-ml fractions, and acid-insoluble radioactivity was determined. A. SDS, 0.5%; the uv absorbance tracing was obtained from chick embryo cell ribosomal RNA centrifuged separately under the same conditions. B. SDS, 0.5%; heated at 100° for 2 min. C. DOC, 0.5%; $^3$H- and $^{14}$C-labeled virions were centrifuged separately, but under the same conditions.

nononcogenic viruses, DOC and similar surface-active agents disrupt enveloped viruses by a lipid solvent action, and not by affecting any other intermolecular bonds. However, RLV RNA might, in virions, be complexed with protein by some type of bond that is labile to DOC. Alternatively, lipids might hold the viral RNA and internal viral proteins together. "Nucleoids" released from RLV by ether have little discernible substructure but they contain viral RNA and group-specific internal protein antigens (19, 20). The relatively low density (1.22 g/m$^3$) of the nucleoids (20) may reflect the presence of lipids. Ether may be relatively inefficient in disrupting lipid-containing viral structures compared to detergents like DOC, and it is possible that these nucleoids represent an intermediate stage in virus disruption. Indeed, structures much smaller than nucleoids (2.7S), containing the internal group-specific protein antigens, have been obtained by some workers instead of nucleoids upon treatment of a murine leukemia virus with ether (21).

*Summary.* When purified Rauscher murine leukemia virus (RLV) was treated with sodium deoxycholate (DOC), an RNA-con-

TABLE II. Ribonuclease Sensitivity of RNA Released from RLV by DOC.

| Sample | Acid-insoluble | | |
|---|---|---|---|
| | No ribonuclease (cpm) | After ribonuclease[a] (cpm) | % Ribonuclease-resistant |
| Virus in TEN buffer | 2060 | 1675 | 81 |
| Virus in TEN buffer + 0.5% DOC | 2020 | 51 | 2.5 |

[a] Pancreatic ribonuclease A (10 μg/ml) was added, and samples were incubated 30 min at 37° before the reaction was stopped with 5% trichloroacetic acid.

taining structure was released. This structure sedimented at the same rate as free viral RNA, displayed the same sedimentation heterogeneity, had little, if any, protein associated with it, and was solubilized by ribonuclease. It is, therefore, improbable that RLV RNA resides in a subviral structure which resembles nucleocapsids isolated from noncogenic enveloped RNA viruses.

Miss Peggy Cook provided skilled technical assistance.

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Received Mar. 23, 1970. P.S.E.B.M., 1970, Vol. 134.