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Isolation of a Ribonucleoprotein Structure from Oncornaviruses

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Detergent treatment releases from both avian and mammalian oncornaviruses a structure containing the viral RNA and a major arginine-rich protein, as well as reverse transcriptase activity.

Morphologically the oncornaviruses have at least two features in common with several other enveloped RNA viruses (for reviews, D. P. Bolognesi, 1973, Advan. Virus Res., in press; R. C. Nowinski et al., 1973, Methods Cancer Res., in press). These are: (i) the presence of a bilayer lipid membrane with exterior projections (10, 13) and (ii) the presence within the viral core of stranded material, presumably ribonucleoprotein (RNP), which in some negatively stained preparations appears to have helical symmetry (9, 15). Between the RNP and the viral envelope is a core shell, apparently composed of capsomeres (10, 19). With respect to its situation in the virion the core shell is thus comparable to the structure formed by the membrane or matrix proteins of influenza and rhabdoviruses (16, 21). Protein analyses of intact cores (including both the core shell and the RNP) from avian and mammalian oncornaviruses are now available (Stromberg et al., submitted for publication; Bolognesi et al., in press). A knowledge of the protein composition of viral RNP's therefore would provide information not only on the structure of the RNP itself but also (indirectly) on the nature of the core shell proteins. We report here the isolation of viral RNP's consisting of the viral RNA complexed with a major arginine-rich structural protein and retaining a significant portion of the reverse transcriptase activity originally present in the virion.

In these studies we have utilized two strains of avian leukosis virus (AvLV), MC29 and avian myeloblastosis virus (AMV), and the Rauscher strain of murine leukemia virus (MuLV). The avian viruses were grown in chicken embryo fibroblasts and the Rauscher virus in a continuously producing rat cell line (RRTC, Microbiological Associates) as previously described (5, 11). Radioactive labeling and purification of virus and procedures for gel filtration of viral proteins in the presence of 6M guanidine hydrochloride (GuHCl) have been reported (5, 11). Reverse transcriptase activities were assayed by the procedures of Kelloff et al. (8) or Stephenson et al. (18), utilizing ^{3}H-deoxythymidine triphosphate as substrate and poly rA·oligo dT as template.

To isolate viral RNP's we adopted a modification of the procedure used by Coffin and Temin (2) and by Davis and Rueckert (4), centrifuging virus through a sucrose layer containing the detergent, Nonidet P-40 (NP-40), and dithiothreitol (DTT). As shown in Fig. 1, a characteristic subviral particle, 10-fold enriched in RNA/protein ratio, was released by this treatment, its yield depending on the NP-40 concentration used. More complex core structures, with lower RNA/protein ratios, could not be detected by this procedure. The recovery of the RNP band in fractions 3 and 4 (density 1.27 g/cm^3) can be ascribed to the steep gradient in viscosity at this sucrose concentration, rather than to the density of the RNP per se. Thus when RNP's from such a band were fixed in 7.7% glutaraldehyde and centrifuged to equilibrium in a CsCl gradient, most of the radioactivity was recovered at a density of 1.38 g/cm^3. The latter density is consistent with a ribonucleoprotein composed of 20 to 25% RNA.

An RNP preparation from MC29 AvLV containing radioactive protein (cf. Fig. 1A) was subjected to gel filtration in 6 M GuHCl, with the results shown in Fig. 2B. Only one major protein species was detectable, corresponding to the p12(N) component of virus (Fig. 2A). (Protein designations used in this report are those adopted by the Oncornavirus Discussion Group at a meeting in New York, June 1973. See legend to Table 1. A proposal outlining this new nomenclature will be published separately.) By

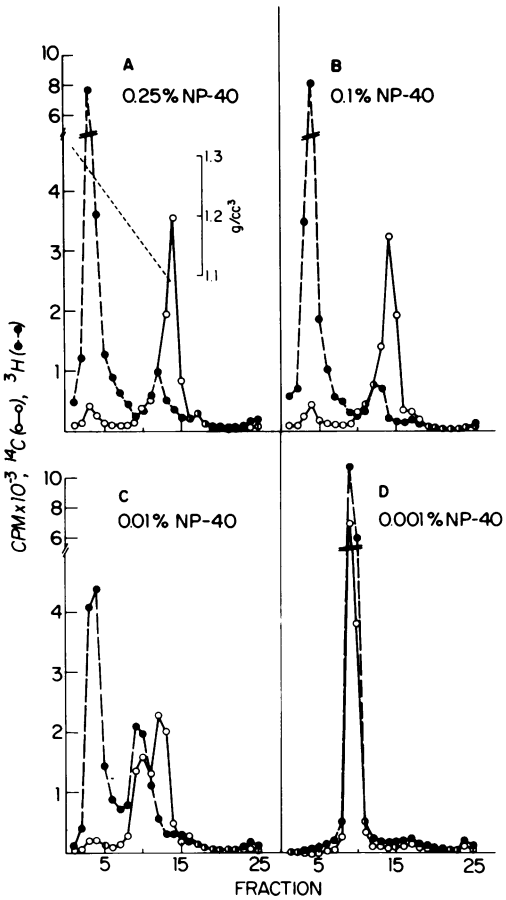

FIG. 1. *Effect of different concentrations of detergent (NP-40) on the release of RNP's from MC29 virus. A 6-ml gradient of 30 to 70% sucrose was constructed as described by Coffin and Temin (2) (the 70% sucrose stock being made up in D_2O). Above this was layered 1.5 ml of 26% sucrose containing 0.01 M Tris-hydrochloride, pH 8.0, 1 mM EDTA, 1% DTT, and a concentration of NP-40 as indicated in each panel of the figure. Above the 26% sucrose layer was placed 1 ml of 22% sucrose in the same Tris-EDTA buffer, and above this was layered 4 ml of a 1:2 dilution of virus banded in a sucrose gradient. (Virus had been labeled in vivo with ^{14}C-amino acid mixture [1 μCi/ml] and ^{3}H-uridine [10 μCi/ml].) After centrifugation at 36,000 rpm for 16 h in the Spinco SW41 rotor at 4 C, 0.5-ml fractions were collected from the bottom of the tube. The detergent layer appears in fractions 13 to 15, intact virus in fractions 9 and 10, and viral RNP's in fractions 3 and 4. Filled circles, ^{3}H-RNA; open circles, ^{14}C-protein. The density gradient is shown for fractions 1 to 15 (panel A).*

gel filtration analysis this component represents about 10% of the protein in the virion (5), which agrees with the recovery of 10% of the total virion protein in the RNP (Fig. 1). An enrichment of the p12(N) protein in RNP structures from AvLV has been reported by other workers (4, 12); in addition, these workers found significant amounts of other proteins in their preparations (though in lower yields than the p12(N) component). When processing large quantities of virus we have also observed other protein components in RNP preparations, e.g., minor species in the 60,000 to 90,000 molecular weight range which may represent reverse transcriptase subunits (2, 7, Stromberg and Wilson, in press; see below).

When the procedure of Fig. 1A was applied to radiolabeled Rauscher MuLV, an RNP band could again be obtained, containing on the average about 70% of the viral RNA and 9% of the viral protein. Gel filtration of the material from such a band in 6M GuHCl resulted in the protein pattern shown in Fig. 3B. Apart from protein eluting at the void volume of the column, only one major protein species was again detectable, at the position of the smallest viral structural protein (p10 (N)). The protein material eluting at the void volume was judged not to

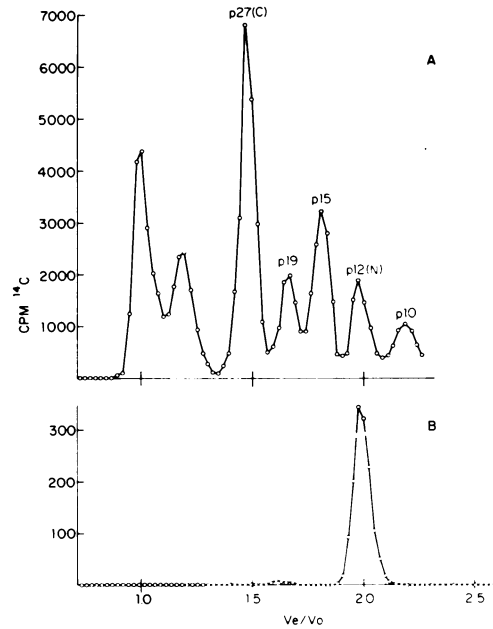

FIG. 2. *Comparison of proteins from MC29 virus with protein from viral RNP's by gel filtration in 6 M GuHCl. A mixture of ^{14}C-labeled amino acids was used to label virus in vivo. RNP's were isolated as in Fig. 1A, dialyzed rapidly to get rid of sucrose, lyophilized, and subjected to gel filtration in 6 M GuHCl and 0.01 M DTT. The protein pattern for whole virus is shown in panel A, and the pattern for RNP's in panel B. For protein designations see legend to Table 1.*

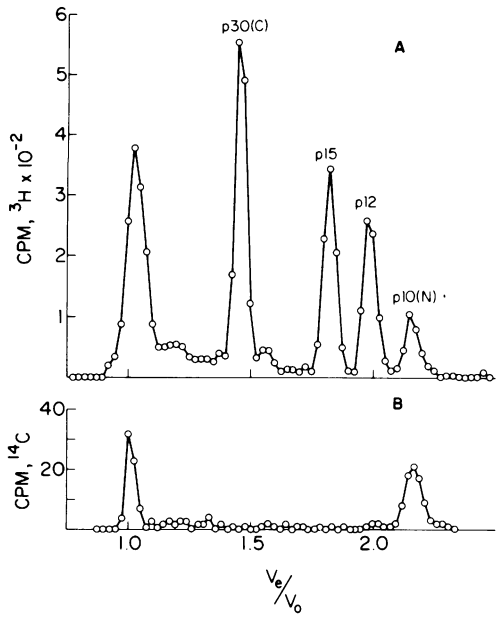

FIG. 3. *Comparison of proteins from Rauscher MuLV with proteins present in an RNP preparation from the same virus. The method for RNP preparation was that shown in Fig. 1A; subsequent steps were as in Fig. 2. The protein pattern for whole virus (^{3}H-amino acid label) is shown in panel A, and the pattern for the RNP preparation (^{14}C-amino acid label) in panel B. For protein designations see legend to Table 1.*

be part of the RNP structure from MuLV because (i) it represented aggregates which could be separated from the RNP's by differential centrifugation (after appropriate dilution) of sucrose banded RNP's, and (ii) in polyacrylamide gel electrophoresis this material consisted largely of viral glycoprotein plus a species of molecular weight about 40,000. Analysis of proteins from whole MuLV preparations has shown the latter species to be present in variable amounts, depending on the age and condition of the particular cell culture from which virus was collected. Given its molecular weight and its capacity to aggregate both in sucrose gradients (17) and in 6 M GuHCl (6), it is possible that this component represents an actin-like protein derived from microfilaments of the plasma membrane, and indicative of some vesicular material in virus preparations.

Since the p12(N) protein component of AvLV was known to be arginine-rich (5), the relative arginine content of the RNP-associated protein from MuLV, p10(N), was determined (Table 1). It can be seen that for both types of virus the RNP-associated protein is the most arginine-rich of the virion internal proteins.

For AMV and MC29, 30 to 50% of the reverse transcriptase originally present in virions could be recovered in RNP preparations (assaying the enzyme activity with exogenous template). In RNP preparations from MuLV about 10% of the original enzyme activity was recovered, the remainder of the activity being found in the detergent layer of the sucrose gradient. Thus the association of the enzyme with the RNP structure appears to be less stable to detergent for MuLV than it is for AvLV.

It has been reported that the RNP structures of influenza, parainfluenza, and rhabdoviruses contain significant amounts of the corresponding virion transcriptase activities (1, 3, 14, 20). As in the case of these other groups of enveloped RNA viruses, the oncornaviruses appear to contain an RNP with a single major structural protein, as well as an associated transcriptase; however, in agreement with Davis and Rueckert (4), we find that oncornavirus RNP's contain a much higher percentage of RNA (20 to 25%) than do the RNP's of these other viruses.

TABLE 1. *Relative arginine contents of avian and murine viral proteins*

AvLV (MC29)		MuLV (Rauscher)	
Protein species[a]	Counts per min of arginine/ counts per min of mixed amino acids[b]	Protein species[a]	Counts per min of arginine/ counts per min of mixed amino acids[b]
p27(C)	1.0	p30(C)	1.0
p19	0.3		
p15	0.7	p15	0.3
p12(N)	1.8	p12	0.7
p10	1.0	p10(N)	1.6

[a] Protein species are designated by their molecular weights (in thousands of daltons) as determined by gel filtration in 6 M GuHCl (5, 11). The suffix (C) denotes the major viral core protein (Stromberg et al., submitted for publication; Bolognesi et al., in press); (N) denotes the structural protein associated with the viral nucleoprotein.

[b] Radioactivity (counts per minute) incorporated in vivo into individual viral proteins with labeled arginine as precursor was divided by the counts per minute incorporated using a labeled amino acid mixture as precursor. The resultant ratios were normalized to a value of 1.0 for the major viral core protein (p27(C) or p30(C)). Proteins were analyzed for radioactivity after separation by gel filtration in 6 M GuHCl.

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