NOTES

Absence of Ribonucleic Acid Methylase in the Avian Myeloblastosis Virus Core

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N2-guanine-ribonucleic acid-methyltransferase, which is associated with avian myeloblastosis virus, is not a component of the viral core.

Our laboratory has recently shown that a specific ribonucleic acid (RNA) methylase activity (2), N2-guanine-RNA-methyltransferase, is associated with the RNA tumor virus, avian myeloblastosis virus (AMV). The enzyme activity has an absolute requirement for both surfactant and exogenous RNA. Information on the location of this enzyme within the virion may provide some insight into its biological role. For example, if it is located in the viral core, one might suspect that the viral RNA may be its natural substrate, whereas if it is not located in the core it seems more plausible to expect a host cell nucleic acid to be the natural substrate. This communication presents evidence that the methylase activity is unlikely to be a core component of the virion.

Figure 1 illustrates the sedimentation of methylase activity in two identical sucrose gradients. The intact virus control (Fig. 1A) was sedimented to equilibrium, and the virus peak had a buoyant density of 1.15 g/ml; the RNA methylase activity is found within the intact virus band. In Fig. 1B, an equal amount of the same intact virus preparation was treated with the surfactant, Sterox SL, and was sedimented simultaneously with the untreated preparation to give an absorbancy peak at 254 nm (A254) with a density of 1.27 g/ml (viral cores) and a second band, presumably lipoprotein, near the top of the sucrose gradient; the great bulk of methylase activity is apparent at the top of the gradient. Although 78% of the methylase activity was recovered, less than 4% is associated with the core fraction. These results clearly demonstrate that the specific methylase activity is not a core constituent under these conditions.

Figure 2 presents electron micrographs of core material, prepared simultaneously from treated and untreated virus preparations, from sucrose gradients identical to Fig. 1B. The homogeneity

Fig. 1. Isopycnic sucrose density gradient centrifugation of intact AMV (A) and surfactant-disrupted AMV (B). Virus from leukemic chick plasma which was purified by rate zonal centrifugation in a Ficoll gradient [5-25% w/v] in SET buffer consisting of 50 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, 1.0 mM ethylenediaminetetraacetic acid, 0.1 M NaCl, pH 7.4 at 0°C, was suspended in SET buffer (1.3 mg/ml) and divided into three 4-ml samples. Two
and morphological integrity of the viral cores are evident. The presence of both RNA-dependent deoxyribonucleic acid polymerase and the viral nucleic acid in these cores is shown in a more complete description of the isolation procedure (4) in which the polymerase specific activity is increased fivefold over that of the virion in the absence of added templates.

Samples were each incubated for 28 min at 0°C with 1 ml of Sterox SL (7.5%; v v; Monsanto Co.), while the third sample was incubated with 1 ml of SET buffer. The 3-ml samples were layered over 13-ml sucrose gradients prepared by layering 1 ml of 10% sucrose over a 12-ml gradient (25-65%, w w, in SET buffer containing 20 mM dithiothreitol). Samples were centrifuged for 16.5 hr at 0°C at 24,000 rpm in the SW25.3 rotor. After centrifugation, the gradients were displaced by saturated potassium tartrate to collect 1-ml samples with an ISCO model D density gradient fractionator equipped with a UA2 ultraviolet monitor. Sedimentation is from left to right. Incorporation of radioactivity into N2-methylguanamine was carried out as previously reported (2). Briefly, each mixture contained 50 mM Tris-hydrochloride (pH 8.3), 60 mM NaCl, 16 mM magnesium acetate, 20 mM dithiothreitol, 0.5% Sterox SL, 800 μg of transfer RNA (Escherichia coli B), 2.0 μCi of S-adenosymethionine-methyl-3H (New England Nuclear; specific activity, 4.0 Ci mmole), and 0.2 ml of the gradient fraction in a total volume of 0.4 ml. After incubation for 2 hr at 37°C, the RNA was precipitated with 50%, cold trichloroacetic acid, centrifuged, and the precipitate was suspended in 1 ml Tris-hydrochloride, pH 8.3; the precipitation-suspension cycle was repeated a total of four times. Finally, the RNA was hydrolyzed for 30 min in 3 ml of 1 N HCl at 95 to 99°C and was chromatographed on a Dowex 50 column (see reference 1 for details). \( {^{3}H} \) incorporation, ○; sucrose density, ●.

Because AMV replicates by budding from the plasma membrane, it is difficult to evaluate the significance of a given enzyme activity in the proliferation of this virus. Its presence could simply be the result of incorporation of cell membrane components or spurious entrapment of soluble cellular material (3). Alternatively, the methylase enzyme may be a loosely bound constituent of the core which is leached out during the process of core isolation. However, the morphological integrity of the core preparation argues against this possibility. The absence of a constituent from the viral core does not necessarily mean that it is not essential for viral proliferation. Consequently, in view of the evidence relating aberrant methylation of nucleic acids to malignant conversion of cells (1) and the fact that AMV is an oncogenic virus, it is important to establish whether there is a relationship between the specific RNA methylase activity and the virus life cycle.

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LITERATURE CITED