Influence of Defective Virion Core Proteins on RNA Maturation with an Avian Sarcoma Virus

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The RNA of the avian sarcoma virus B77 temperature-sensitive mutant LA334 was investigated using electrophoretic analysis. The RNA from mutant virus grown at the nonpermissive temperature (42°C) showed a heterogeneous peak between 80 and 125S, and another at about 35S. The RNA of the mutant virus grown at the permissive temperature (35°C) behaved like wild-type B77 virus RNA, exhibiting a major peak at 70S. The homology between the various RNA fractions and virus-specific DNA probe was determined, indicating that mutant virus grown at the nonpermissive temperature contains relatively large amounts of nonviral-specific RNA.

LA334 is a temperature-sensitive mutant of avian sarcoma virus B77, which exhibits two separate genetic defects, one affecting the transformed phenotype of the infected cells, and one affecting the replication of progeny in the infected cells (7). The latter genetic defect has been identified as being caused by defective processing of a precursor protein, pr76 (10), which in LA334 at the nonpermissive temperature is incorrectly cleaved (6, 8a). This incorrect processing leads to the production of proteins defective in formation of a functional viral core. Electron microscopy has shown that at the nonpermissive temperature, LA334-infected cells exhibit a significant suppression of physical particle release, and an abnormal accumulation of particles in the budding stage (4a). Furthermore, noninfectious particles that are formed at the nonpermissive temperature are structurally labile (8a) and morphologically atypical (6, 8a). Rohrschneider et al. (8a) have recently shown that all of these defects in the core probably derive from the assembly of an atypical protein, p23, into the structurally and morphologically atypical and nonfunctional particles at the nonpermissive temperature. This protein is derived from an erroneous cleavage in the normal pathway of synthesis of the viral core proteins p19 and p27.

The purpose of this communication is to describe the results of some studies on the RNA components of LA334 matured at the permissive (35°C) and nonpermissive (42°C) temperatures. Chicken embryo cells were prepared from chick helper factor-negative (5) C/BDE phenotype embryos obtained from Heisdorf and Nelson Farms, Redmond, Wash. LA334, subgroup C, and its parental wild-type virus, avian sarcoma virus B77, subgroup C, were maintained by cloning as single foci at each stage of stock preparation. The growth medium for virus radioactive labeling was either Dulbecco-modified Eagle medium supplemented with [5-3H]uridine (25 Ci/mmol, Amersham) to a concentration of 200 μCi/ml, or [2-14C]uridine (>50 mCi/mmol, Amersham) to 25 μCi/ml, or Eagle minimal essential medium lacking all phosphate, but supplemented with 2 μCi of [32P]orthophosphate per ml (Amersham). For both media, 5% heat-inactivated calf serum and 1% dimethyl sulfoxide were added. Chicken embryo cells confluently infected with mutant or wild-type virus were maintained for 10 days, and transferred two to three times at 35°C before beginning the virus labeling procedure. If labeling was to be performed at 42°C, then cells were transferred to, and maintained at, 42°C for at least 2 days before being exposed to the medium containing isotope. After 12 h of labeling, radioisotope-free medium was added to the cells, and three separate harvests with cold medium were taken at 3, 6, and 9 h. Cells could be used for several such labeling cycles. Virus was purified from these 3-h harvests as rapidly as possible using equilibrium sucrose density gradients. After sodium dodecyl sulfate (0.5%)/Pronase (0.5 mg/ml) digestion and phenol extraction of purified virus, RNA was precipitated from the aqueous phase by ethanol and collected for electrophoretic analysis by centrifugation.

Figure 1 shows the coelectrophoresis of RNA extracted from LA334, grown either at 42°C (Fig. 1A) or 35°C (Fig. 1B), with B77 wild-type
virus grown at 42°C. It is immediately apparent that the mutant virus preparation from the nonpermissive temperature exhibits an abnormal RNA pattern; few counts are present in the region of the typical oncornavirus native RNA at 70S (8). Instead, a heterogeneous spread of radioactivity between 80 and 125S was observed, with a significant peak also apparent at about 35S, equivalent to the characteristic size of the oncornavirus RNA subunit (3). Figure 1B shows that mutant virus RNA grown at 35°C is indistinguishable from that of wild-type B77 virus.

Figures 2A and B illustrate the components of viral RNAs prepared as in Fig. 1, after denaturation at 100°C for 45 s. Figure 2A shows that the mutant virus prepared at the nonpermissive temperature contains some 35S RNA that is presumably virus specific, but that the majority of the RNA species present in the virion exhibit a broad heterogeneous smear below 35S. A quantitatively more significant fraction of the wild-type virus RNA, or mutant RNA prepared at the permissive temperature, was found in the peak at 35S.

To ascertain the degree to which RNA incorporated into the virion of LA334 is virus specific, nucleic acid hybridization experiments were performed using a complementary DNA probe made from the Prague strain of Rous sarcoma virus, subgroup C (Pr-C), which exhibits high base sequence homology to the B77 virus (9). Cuts were taken from the region of the gels indicated in Fig. 1A and B. [32P]RNA was reisolated by electrophoresis, and the degree of virus-specific sequences was determined by hybridization to Pr-C complementary DNA. Table 1 shows the results of such hybridization experiments, which indicate that the ultimate homology obtainable between Pr-C cDNA probe and B77 virus RNA under these conditions is
% With LA 334 grown at the nonpermissive temperature, the native viral RNA species that show most significant viral homology are those present in the 80–125S complex and in the 35S peak, which, even so, apparently contain significant amounts of RNA not derived from the virus. The mutant virus RNA prepared at the permissive temperature migrated almost exclusively as 70S material and exhibited a degree of homology approximately the same as that observed with the wild-type B77 virus.

The oncornavirus particle undergoes significant maturation of structural proteins and RNA after leaving the host cell (1, 2). The major RNA components observed immediately after particle release are the 35S subunits and certain smaller 4S to 12S species; within 1 h after release, particles exhibit the characteristic 70S RNA complex. It was a goal of this investigation to determine to what extent the RNA maturation processes would be disturbed in a virus that exhibits a documented failure in viral core protein synthesis and core assembly (6, 8a). Our results have shown two probably related deficiencies in mutant viral RNA assembled at the nonpermissive temperature. (i) The 70S RNA complex is not formed; rather, a fraction of the native viral RNA migrates as 35S, and a fraction assembles into a very heterogeneous atypical complex ranging from 80 to 125S. (ii) The virion assembled much more nonviral, presumably host-specific, RNA in each size category. The altered pattern of the RNA observed may result from RNase, which one could imagine to be preferentially incorporated into the mutant virion, or to have freer access to the RNA genome as a result of the defective core structure. The increased incorporation of host-specific RNA into the mutant virion suggests that the initial intracellular assembly of the viral core proteins occurring under the plasma membrane may play a role in selecting appropriate viral RNA species for ultimate inclusion. Since there is no evidence that the viral ribonucleoprotein, p12, carries a defect in LA 334 (8a), it seems possible that other internal proteins that are defective, notably p27 and p19, influence the selection of RNA species to be incorporated.

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<th>Table 1. Nucleic acid homology between various RNA fractions and virus-specific DNA probe</th>
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<td>Sample</td>
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*Chicken embryo cells infected with mutant or wild-type viruses were grown at either 42°C (nonpermissive temperature) or 35°C (permissive temperature) for 48 h before beginning the isolation of virus. Virus harvests were made every 3 h.

The solutions were prepared from polyacrylamide gels in the regions indicated in Fig. 1A and B.

DNA probe was prepared from cloned Rous sarcoma virus Pr-C in an incubation mixture (15 ml) containing 100 mM Tris-hydrochloride (pH 8.2); 10 mM MgCl₂, 10 mM dithiothreitol, 0.02% Nonidet P-40, 100 μg of actinomycin D per ml, 0.1 mM each of unlabeled dATP, dCTP, dGTP, and dTTP, and 20 mg of viral protein. After 4 h at 37°C, protein was digested by Pronase (500 μg/ml) in the presence of 0.5% sodium dodecyl sulfate, and nucleic acids were precipitated from the phenol-extracted aqueous phase by the addition of ethanol. Nucleic acids were pelleted by centrifugation, RNA was destroyed by treatment with alkali, and DNA was repurified with ethanol in the presence of Escherichia coli tRNA. In protection experiments with [32P]-labeled Pr-C 70S RNA, the DNA probe thus obtained was able to render the viral RNA 100% resistant against single-strand-specific S1 nuclease. Each of the reisolated [32P]PRNAs (750 cpm) were mixed with a 450-fold excess of Pr-C complementary DNA in a final volume of 20 μl containing 0.6 M NaCl, 0.04 M Tris-hydrochloride, pH 7.2, and 0.002 M EDTA, pH 7.0. The reactions were sealed in sterile micropipettes and kept at 68°C for 40 h. The percentage of double-strand hybrid formed was determined after digestion with S1 nuclease.

LITERATURE CITED


