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NOTES

Structure and Molecular Length of the Large Subunits of RD-114 Viral RNA[1]

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By electron microscopy, the large subunits of RD-114 RNA have a molecular weight of 5.0×10^6; they all have a characteristic secondary structure feature close to the middle.

RD-114 is an endogenous feline type C virus that is immunologically and biochemically distinct from the conventional feline leukemia-sarcoma viruses (1, 4, 5, 7-9, 12, 13, 15, 16). We wish to report here on our preliminary electron microscope studies of the properties of the large molecular weight subunits of the 60 to 70*S* RNA complex of this virus.

Virus was grown as described previously (J. E. Flbert, R. M. McAllister, M. O. Nocolson, and R. V. Gilden, Proc. Soc. Exp. Biol. Med., in press). Heavily grown monolayers of RD-114 cells were labeled with [5-^{3}H]uridine (28 Ci/mmol, 40 μCi/ml), and the culture medium was harvested at 3-h intervals. Virions were isolated, and the 60 to 70*S* RNA complex was extracted and purified as described in the legend to Fig. 1.

When the 60 to 70*S* complex is mounted for electron microscopy under the more or less standard spreading conditions involving 50 to 60% formamide that are effective for extending single strands of DNA (2), the RNA is collapsed into a "bush-like" structure due to intramolecular base pairing, and it is impossible to study the topology of the molecules. If the formamide concentration is raised to 80%, molecules are more extended but still not traceable. We therefore treated the 60 to 70*S* complex with glyoxal under conditions previously described (M.-T. Hsu, H. J. Kung, and N. Davidson, Cold Spring Habor Symp., in press) (see also Fig. 1 legend). Glyoxal is a reagent that disrupts the secondary structure of a polynucleotide by selective reaction with the guanine residues in such a way as to block their hydrogen bonding functions. The resulting molecules were well spread and suitable for length measurement. An example is shown in Fig. 1a. A histogram of the length distribution is given in Fig. 2a. It may be seen that the 60 to 70*S* complex is dissociated by this treatment to yield a high molecular weight component (defined by the shaded area in Fig. 2) and a large number of smaller RNA molecules that are quite heterogeneous in size. The mean length of the large component, as indicated by an arrow in Fig. 2a, is 3.74 ± 0.32 μm. This corresponds to a molecular weight of 5.03×10^6 when using *Escherichia coli* 23*S* rRNA as a standard ($<L_n>$ = 0.80 ± 0.05 μm, M = 1.08×10^6 daltons [M.-T. Hsu, H. J. Kung, and N. Davidson, Cold Spring Habor Symp., in press]).

We believe that the 3.74-μm component is an intrinsic subunit present in the RD-114 genome. Most of the smaller molecules are probably breakdown products due to nuclease action. Qualitatively similar distributions are seen when the 70*S* complexes of other RNA tumor viruses are subjected to denaturing conditions (3, 11).

The RNA molecules were also examined after spreading from a denaturing urea-formamide spreading solution (14, 17). The resulting histogram of the length distribution is shown in Fig. 2b. The measured number average length of the large subunit is 3.72 ± 0.33 μm, which is in agreement with the results from the glyoxal spreadings.

Spreading from the denaturing urea-formamide solvent has proven to be particularly effective for identifying regions of stable secondary structure in an RNA (17). The large sub-

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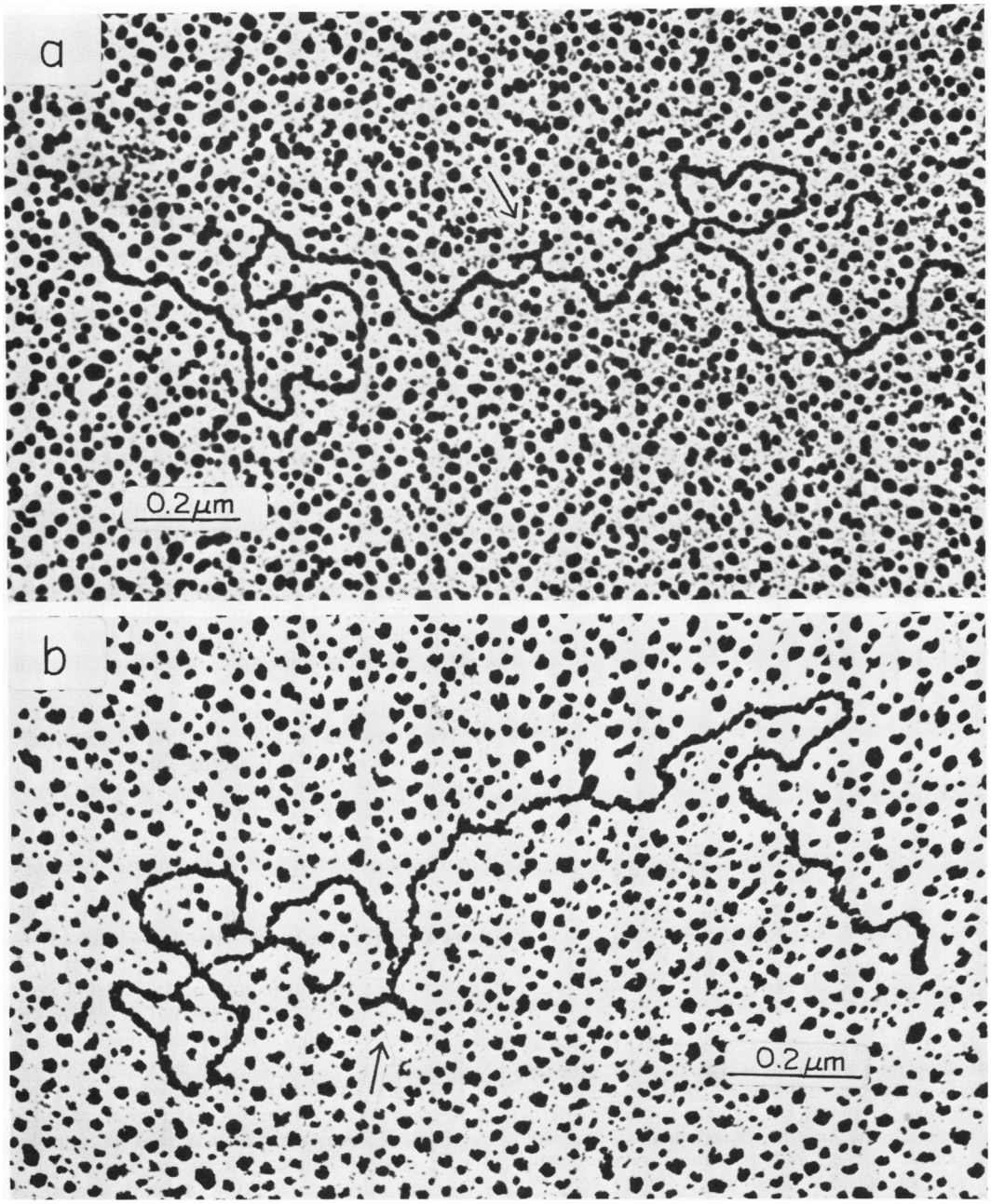

FIG. 1. *Electron micrographs of the large molecular weight subunits of RD-114-70S RNA. Arrows indicate the secondary structure feature.* ^{3}*H-labeled viruses in culture medium were loaded on SW27 tubes underlayered with 5 ml of 20% TNE (0.1 M NaCl, 0.01 M Tris, 1 mM EDTA, pH 7.2) and 0.5 ml of 65% sucrose in TNE. After centrifugation at 2 × 10^4 rpm for 2 h, the upper layer was carefully removed, and the bottom 1 to 1.5 ml, corresponding to the virus band, was gently dispersed with a syringe. The virus concentrate was then diluted with TNE to 20% surcrose or less and pelleted by centrifugation at 27 × 10^3 rpm for 90 min. RNA was extracted by adding 0.2 to 0.4 ml of self-digested Pronase (2 h, 37 C, 0.02 M Tris, pH 7) (500 μg/ml) containing 0.5% sodium dodecyl sulfate to the virus pellet and incubating at 37 C for 30 min. The RNA extract was layered directly onto 5 ml of a 10 to 30% sucrose (in TNE) gradient in an SW50.1 tube and centrifuged at 45 × 10^3 rpm, 5 C for 2 h. Fractions from the 60 to 70S RNA region were used for electron microscopy. (1a) Glyoxal-formamide spreading. The RNA (~10 μg/ml) was dialyzed against 0.5 M glyoxal, 0.01 M PO_4, pH 7 at 37 C for 1 h, and then diluted ca. 20-fold into 40% formamide, 0.1 M EDTA, pH 8.5, 50 μg of cytochrome c per ml and spread onto a 10% formamide hypophase with one-tenth the electrolyte concentration. (1b) Urea-formamide spreading. 5 λ of the RNA sample (~10 μg/ml) was mixed with 40 λ of pure formamide to which urea is added to 8 M, 5 λ 1 M Tris-0.1 M EDTA, pH 8.5, 2.5 λ cytochrome c (1 mg/ml) and spread onto a hypophase containing 0.0083 M Tris and 0.00083 M EDTA, pH 8.5. Films were picked up within 10 s after spreading.*

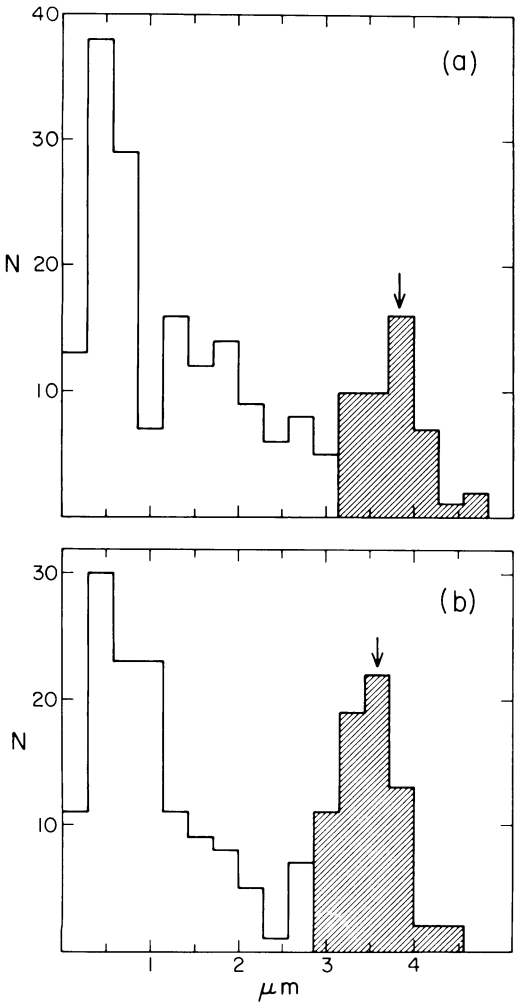

FIG. 2. *Histograms of the length distributions of the RNA from the RD-114 70S complex. Average lengths and standard deviations reported in the text for the large subunits were calculated from the lengths of all molecules in the shaded areas in the two histograms. Arrows indicate the number average lengths. (a) Length distribution obtained by the glyoxal-formamide method; (b) length distribution obtained by spreading from urea-formamide.*

units of RD-114 RNA show such a secondary structure feature (Fig. 1b). In appearance, it resembles a "rabbit-ear" television antenna. We refer to it as the SSF. The SSF was present in over 90% of the molecules of length greater than 3.4 μm in urea-formamide spreadings. It was also present in many of the glyoxal-treated molecules (including the one shown in Fig. 1a) but at a lower frequency. In the class of full-length molecules, defined as those with a length greater than 3.4 μm in the urea-formamide spreadings, the SSF was always close to the center of the molecule with an average fractional distance from the closer end of 0.443 $\pm$ 0.025 (relative to the length of the given molecule). We estimate the contour length of the SSF as about 900 $\pm$ 150 nucleotides. Almost all of the molecules of length greater than 2.0 μm have the SSF, but the shorter the molecule the more this feature is off center. The obvious interpretation of this result is that the intermediate length molecules are breakdown products of a full-length 3.7-μm subunit.

It is conceivable that the "subunit" of length 3.7 μm (molecular weight 5.03×10^6) is not a continuous covalent chain, but consists of two smaller molecules of length 1.6 and 2.1 μm (molecular weights 2.2 and 2.8×10^6) joined by base pairing within the SSF. We believe this is unlikely because over 50% of the full-length molecules in the glyoxal spreads were quite smooth or showed only small residual bumps in the middle. Therefore, we believe the 5.0×10^6 strand is, most probably, a single covalent chain.

In collaboration with Peter Vogt, we are studying the molecular lengths of the large subunits of PR-RSV-C RNA. Our preliminary result is that this length corresponds to a molecular weight of 3.2×10^6, which is in approximate agreement with values deduced from sedimentation and electrophoresis studies (3). The molecular weights of the large subunits of Kirsten murine sarcoma and leukemia viruses and of a number of feline leukemia viruses lie in the range 2.0 to 2.5×10^6 (10, 18). It thus appears that the large subunit of RD-114 viral RNA has a molecular length that is considerably larger than that of other common type C viruses.

The stable secondary structure feature is due to a region which has a high guanine plus cyctosine content and/or a rather perfect inverted repeat sequence. It occurs in all of the large subunits of the RD-114 60 to 70*S* RNA complex. This result is consistent with the hypothesis that the subunits are identical in sequence. If they are not identical, they all have the same kind of strongly base-paired sequence in the middle.

It should be noted that Granboulan et al. (6) observed that long strands with estimated molecular weights of 10^7 were seen when purified RNA from avian myleoblastosis virus was spread for electron microscopy under less denaturing conditions than used here.

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