Configurational Variants of Oncornavirus RNAs

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Received for publication 17 February 1976

Heating oncornavirus RNAs at temperatures insufficient for complete denaturation results in forms migrating between the native form (vRNA) and the completely denatured form (vRNA') after gel electrophoresis. Intermediate forms from Rous sarcoma virus or murine leukemia virus were isolated after heating of vRNA's at 58°C and sedimenting in sucrose gradients, and at least four intermediates could be identified in each case. Melting of feline virus (RD-114) RNA produced one major intermediate, which required a comparatively high temperature to denature, and a second intermediate occurring in conditions of low ionic strength. Although the subunit model for oncornavirus RNA is not excluded by these data, we propose that vRNA, vRNA', and intermediates may be configurational variants of the same molecule, and a monomer model for oncornavirus RNA is presented.

Native oncornavirus RNA (vRNA) can be denatured to a form (vRNA') that sediments more slowly in sucrose gradients and migrates more rapidly during electrophoresis in polyacrylamide-agarose mixed gels (1, 11, 16, 23). This denaturation of vRNA occurs upon exposure to agents that dissociate hydrogen bonds, such as heat, dimethyl sulfoxide, urea, and formaldehyde. Early estimates of molecular weights of vRNA ranged from 9×10^6 to 12×10^6 (1, 11, 16, 23, 26), whereas that of vRNA' was found to be 2.5×10^6 to 4×10^6 . Electron microscopic examinations (18, 20) of partially denatured vRNA revealed molecules twice the length of the fully denatured vRNA', indicating that vRNA is composed of two subunits, and sedimentation equilibrium experiments (20, 25) suggest that vRNA has a higher molecular weight than vRNA'. Oligonucleotide analyses (3, 12, 24) showed that oncornavirus RNAs have a complexity equivalent to about 3×10^6 daltons, suggesting that the subunits are identical. On the basis of these observations, it now seems generally accepted that vRNA is composed of at least two subunits of vRNA', held together by hydrogen bonds.

Forms with electrophoretic migration intermediate between vRNA and vRNA' occur upon incomplete denaturation of vRNA (1, 16, 29). Such intermediate forms also are seen in virions harvested within a few minutes after completion by cells in culture (7, 8). The occurrence of intermediate forms seems inconsistent with a vRNA structure composed of two subunits, although discrete intermediate forms might be expected upon the loss of subunits from a ternary or quaternary structure. An examination

of intermediate forms of viral RNA is presented here.

Viral RNAs from Rous sarcoma virus (RSV), murine leukemia virus (MuLV), and RD-114 virus (RD-114V), representative of avian, murine, and feline oncornaviruses, respectively, were examined. The Bryan "high-titer" strain of RSV produced by chicken embryo cells contains a nontransforming helper virus, RAV1, but the genomic RNAs are of identical size and cannot be distinguished upon denaturation and analysis by gel electrophoresis (26). The Rauscher strain of MuLV produced by mouse splenic cells apparently is a homogenous virus, as is the RD-114V produced by human cells. As presented here, all three viruses contained RNAs that could be partially denatured, and intermediate forms of viral RNA could be isolated in each case. The results suggest that vRNA, vRNA', and intermediate forms may be configurational variants of the same molecule. Although the subunit model for oncornavirus RNA has not been excluded, a model for a monomer structure of vRNA and its denaturation to vRNA' and intermediates is proposed.

MATERIALS AND METHODS

Cells and viruses. Chicken embryo cells in secondary culture were infected with the Bryan high-titer strain of RSV and RAV1. After several passages at 3-day intervals, virtually all cells were transformed, and cultures were producing virus at a rate greater than 1 focus-forming unit/cell per h. Mouse splenic cells (JLSV5) were a continuous line, infected several years earlier with the Rauscher strain of MuLV, and continued to produce that virus. Human rhabdomyosarcoma cells (MXC line) continuously produced RD-114V, the feline virus.

Vesicular stomatitis virus was propagated in chicken embryo cells after infection at low virus/cell multiplicity, and virions were collected 36 h after infection. Cells were grown in Eagle minimal essential medium containing added glucose (2 g/liter, final concentration) and supplemented with sodium pyruvate (5 mM), 5% fetal bovine serum, 10% tryptose phosphate broth (Difco), penicillin (50 U/ml), streptomycin (50 μ g/ml), tylocine (50 μ g/ml), and gentamycin (50 μ g/ml). When radioactive labeling was in progress, tryptose phosphate broth was eliminated from the medium.

Radioactive labeling of viral RNA. Cells were exposed to [3 H]uridine (150 μ Ci in 6 ml per 10-cm petri dish) for 6 to 8 h. Radioactive medium was removed, and the cultures were rinsed with warm growth medium before addition of non-radioactive growth medium. At 2-h intervals, culture medium was collected and replaced. Collected fluids were kept cold until processed. Several collections were pooled and centrifuged at $600 \times g$ for 10 min, and then at $12,000 \times g$ for 30 min, to remove cells and debris. Virion-containing fluids were made 0.5 M in NaCl, polyethylene glycol (7%, final concentration) was added, and the mixture was stirred for 2 h on ice. After centrifugation ($12,000 \times g$, 30 min), the pellet was resuspended in 0.05 M Tris (pH 7.0) plus 0.001 M EDTA and added to the top of 20% sucrose in Tris-EDTA (0.1 M NaCl, 0.01 M Tris, pH 7.4, 0.001 M EDTA) layered over 55% sucrose. The virions were sedimented ($190,000 \times g$, 1 h) to a band at the interface between the two sucrose solutions. The band was collected and diluted with Tris-EDTA, and the virions were centrifuged to a pellet. Virions were resuspended in Tris-EDTA and then disrupted with sodium dodecyl sulfate (1%, final concentration). Viral RNA was examined directly or after three serial extractions with phenol. When virions were labeled with $^{32}\text{PO}_4^{3-}$, phosphate was omitted from the growth medium, and culture fluids were collected after 18 h of continuous exposure to the radioactive medium. RNA from these virions was used as the vRNA marker. Vesicular stomatitis virus RNA was labeled in a similar manner. No longer than 36 h elapsed between the isolation of radioactive virions and eventual electrophoretic analysis of viral RNA.

Electrophoresis in polyacrylamide gels. Resolution of RNA by electrophoresis in polyacrylamide (2%)-agarose (0.5%) mixed gels was described earlier (1). In many instances the duration of electrophoresis was extended from 1.5 to 3 h to allow a reasonable distance between native and denatured forms. For any given experiment, samples to be compared were run in the same slab gel. Chicken rRNA's (molecular weight, 1.6×10^6 and 0.7×10^6) (19), *E. coli* rRNA's (molecular weight, 1.1×10^6 and 0.56×10^6) (19), and vesicular stomatitis virus RNA (molecular weight, 4.0×10^6) usually were run in the same or adjacent tracks as reference standards.

Sucrose gradient sedimentations. Viral RNA was layered in 0.1 ml over a 5 to 20% linear sucrose gradient containing Tris-EDTA and 0.2% sodium dodecyl sulfate. The RNA was sedimented at $200,000 \times g$ for 50 min. Sodium acetate (2%, final concentration) and phenol-extracted yeast RNA (10 μ g) were

added to each fraction, the detergent was removed with phenol, and the RNA was precipitated by the addition of 2 volumes of cold ethanol. After sedimentation, the RNA pellet was dissolved in low-ionic-strength "electrophoresis buffer" (1) for gel analysis.

RESULTS

Denaturation of viral RNAs. In the gel electrophoresis system used here, the apparent molecular weight of vRNA is proportional to the molecular weight of its denatured form (vRNA') (Fig. 1). Extrapolated vRNA molecular weights of RSV (5.2×10^6), MuLV (6.0×10^6), and RD-114V (6.0×10^6) were double those found after denaturation by heat, i.e., RSV, 2.6×10^6 ; MuLV, 3.0×10^6 ; and RD-114V, 3.0×10^6 .

Earlier studies with avian myeloblastosis virus (16) and with MuLV (1) suggested that heating of native viral RNA at temperatures less than that required for complete denaturation to vRNA' resulted in forms intermediate between vRNA and vRNA'. Similar experiments were performed on MuLV vRNA, RSV vRNA, or RD-114V vRNA, using undenatured or fully denatured viral RNAs as internal markers. Samples of extracted vRNA (in 0.1 M NaCl, pH 7.4) were heated at various temperatures between 50° and 75°C. A typical result of heating radioactive RNA extracted from MuLV is shown in Fig. 2. Heating at 55°C produced a

FIG. 1. Electrophoretic mobilities of native and denatured viral RNAs. Virions of MuLV, RSV, RD-114V, or vesicular stomatitis virus, labeled with [3 H]uridine, were exposed to 1% sodium dodecyl sulfate. Samples from each virus preparation were heated at 100°C for 3 min. Chicken and bacterial rRNA's were added to each sample before electrophoresis in 2% acrylamide-0.5% agarose mixed gels. Molecular weights of radioactive viral RNAs are projected from molecular weights of stained rRNA markers (●).

FIG. 2. Thermal denaturation of MuLV RNA. Virions of MuLV labeled with [^3H]uridine were extracted, and the RNA was suspended in Tris buffer (0.1 M NaCl, 0.001 M EDTA, 0.01 M Tris, pH 7.4) containing 1% sodium dodecyl sulfate. Samples were heated for 3 min at 55, 60, 65, or 70°C and then cooled rapidly to room temperature. The samples were analyzed by electrophoresis in polyacrylamide (2%)-agarose (0.5%) mixed gels. Molecular weights extrapolated from RNA standards are 6.0×10^6 for vRNA and 3.0×10^6 for vRNA'.

small amount of vRNA' and other RNA migrating faster than vRNA but slower than vRNA' (Fig. 2A and B). The major peak at the vRNA region was skewed toward the leading edge, and possibly the vRNA peak itself migrated farther than in unheated vRNA.

More pronounced denaturation was found after heating vRNA at 60 or 65°C. The slowest migrating RNA after 60°C treatment ran faster than vRNA, and a form(s) intermediate between vRNA and vRNA' was found. Native vRNA disappeared with 65°C treatment, and RNA migrating more slowly than vRNA' was found as well as the completely denatured vRNA'. This latter intermediate form migrated more rapidly than intermediate forms observed in 60°C samples. Exposure to 70°C usually resulted in the complete denaturation of vRNA to vRNA', although small amounts of RNA migrating more slowly than vRNA' occasionally were seen.

Resolution of a true intermediate form of viral RNA demanded vRNA of high molecular integrity, since losses of scissioned segments at intermediate temperatures could give the results presented here. The sharpness of the leading edge of vRNA', and the sharp resolution of the vRNA' peak, can be taken as measures of nondegraded vRNA. Other experiments demonstrated that virtually all the radioactivity in

a preparation containing intermediate forms could be recovered in vRNA' after heating at 100°C for 3 min.

Similar results were found with RSV (Fig. 3). As in the case of MuLV vRNA, partial melting of the vRNA form was found at 55°C, and melting increased with increasing temperature until complete denaturation occurred after heating at 70°C.

In the case of RD-114V, virtually complete loss of the vRNA peak occurred with heating at 55°C (Fig. 4), resulting in a single intermediate (extrapolated molecular weight, ca. 4.5×10^6). No further change was found after heating at 60°, 65°, or 70°C, although heating at 100°C denatured the RD-114V RNA completely to the vRNA' form (Fig. 4), as shown by East et al. (15).

Extraction and heating (50°C) of RD-114V RNA in 1% sodium dodecyl sulfate (approximately 0.03 M Na^+) without added NaCl resulted in two peaks intermediate between vRNA and vRNA' (Fig. 5), both of which were converted to vRNA' by heating at 100°C. These intermediates were less prevalent after heating at 60°C, and denaturation to vRNA' was complete in samples heated at 70°C.

Isolation of intermediates. The regular observation of RNAs of intermediate mobility convinced us of the existence of intermediate

FIG. 3. Thermal denaturation of RSV vRNA. Viral RNA labeled with [^3H]uridine was suspended in Tris buffer and heated at 55, 60, 65, or 70°C before electrophoresis in polyacrylamide (2%)-agarose (0.5%) mixed gels.

FIG. 4. Thermal denaturation of RD-114V RNA. Virions of RD-114 were labeled with [^3H]uridine and exposed to 1% sodium dodecyl sulfate in Tris buffer. Samples were heated (3 min) at 5-degree increments between 55 and 70°C, and one portion was heated at 100°C. After heating, all samples were mixed with ^{32}P -labeled MuLV RNA (○) before gel electrophoresis. Patterns of samples heated at 60, 65, and 70°C were essentially identical to that heated at 55°C.

forms of viral RNA, although some smearing or overlap of vRNA and vRNA' peaks with RSV or MuLV RNAs could be considered. As a further demonstration of intermediate forms, ^3H -labeled vRNA was heated at temperatures insufficient for complete denaturation and then sedimented in sucrose gradients (5 to 20%) containing 0.2% sodium dodecyl sulfate. A gradient profile from heated (58°C) RSV RNA is shown

in Fig. 6. Forms sedimenting between the anticipated vRNA region and completely denatured vRNA' were found.

Individual fractions from the gradients were mixed with ^{32}P -labeled untreated RSV vRNA, and the mixture was subjected to gel electrophoresis. Fractions from RSV vRNA heated at 58°C produced a spectrum of forms intermediate between vRNA and vRNA' (Fig. 7). At least

FIG. 5. Intermediates of RD-114V RNA. Virions of RD-114V labeled with [^3H]uridine were exposed to 1% sodium dodecyl sulfate in low-salt buffer (0.001 M EDTA, 0.01 M Tris, pH 7.4). The sample was heated at 50°C, and one-half was heated at 100°C to completely denature the RNA before addition of a mixture of ^{32}P -labeled MuLV RNA and ^{32}P -labeled MuLV RNA' (○). (A) RD-114V RNA heated at 50°C. (B) RD-114V RNA heated at 50°C and then at 100°C.

FIG. 6. Sedimentation of partially denatured vRNA. RSV virions labeled with [^3H]uridine were exposed to 1% sodium dodecyl sulfate in Tris buffer and heated at 58°C for 3 min. The sample was layered over a 5 to 20% sucrose gradient containing 0.2% sodium dodecyl sulfate and centrifuged at $200,000 \times g$ for 50 min. A sample from each fraction was taken for counting before processing for electrophoresis. Arrows indicate peak regions anticipated of 60S vRNA and 35S vRNA'.

four, and probably more, intermediate forms were detected in this and other preparations of this avian virus RNA.

The possibility that isolated intermediate forms were degradation products of vRNA was examined. Samples from gradient fractions containing intermediates were heated at 70°C before electrophoresis with unheated ^{32}P -labeled vRNA. The ^3H activity originally found in intermediate forms was recoverable as a sharp peak in the vRNA' region (Fig. 7e). Recovery from several fractions is shown in Table 1. The small losses of radioactivity after heating at 70°C are insufficient to account for the differences in mobility between vRNA' and intermediate forms. Also, no discrete peaks of radioactivity migrating ahead of the vRNA' region were discernible; small amounts of radioactivity were found at random locations between vRNA' and the electrophoretic front. It should be noted that the denaturing conditions used here may be insufficient to displace the reverse transcriptase primer from viral RNA (6). These observations demonstrate that viral RNA intermediates can be converted to vRNA' and that RNA molecules smaller than vRNA' constitute an insubstantial part of intermediate forms.

FIG. 7. Isolation of intermediate forms of RSV RNA. Fractions obtained from the sucrose gradient of Fig. 6 were mixed with ^{32}P -labeled vRNA, extracted with phenol, and precipitated with ethanol. Samples from fractions c, d, and e were heated at 70°C to produce vRNA' before mixing with ^{32}P -labeled vRNA. Resuspended RNAs were analyzed by electrophoresis in a polyacrylamide-agarose slab gel. The peak gel fractions of ^{32}P are represented by the dashed line, denoted fraction 0. The peak of vRNA' placed at fraction 20 was determined from the distance between vRNA and the completely denatured samples. Typical ^{32}P -labeled vRNA ($\circ$) patterns are presented for fractions b and e. The completely denatured vRNA' (Δ) in fraction e was produced from the same fraction showing intermediate; these samples were run in adjacent gel tracks.

In experiments similar to that described above, intermediate forms of MuLV RNA were isolated after heating at 58°C and separating by sedimentation in sucrose gradients. As with RSV, the RNAs from MuLV (Fig. 8) were found in a variety of forms, with electrophoretic mobilities different from either native vRNA or denatured vRNA'. In contrast to RSV, the most rapidly sedimenting fractions contained a single predominant intermediate form (Fig. 8a, b, and c). Although similar in mobility to the RD-114V intermediate described above, this form could be denatured at a temperature (65°C in 0.13 M Na^+) inadequate to denature the RD-114V intermediate. Heating MuLV intermediate forms at 75°C produced sharp radioactive bands in the vRNA' region (Fig. 8e), and most of the radioactivity in intermediates could be recovered in the vRNA' region (Table 1).

DISCUSSION

Forms of oncornavirus RNA different from native vRNA or denatured vRNA' were isolated from vRNA heated at temperatures insufficient for complete denaturation. Resolution

by gel electrophoresis revealed a relatively broad distribution of intermediate forms for RSV and MuLV, suggesting the occurrence of transitional forms rather than distinct species. In contrast, two distinct intermediates were identified after heating RD-114V RNA.

Other published reports dealing with oncornavirus RNA have failed to establish a cogent model for the structure of vRNA. All agree that viral RNA as extracted from virions changes its properties after treatment with heat or other hydrogen bond-dissociating agents. Increases in electrophoretic mobility of viral RNA in gels, and decreases in sedimentation rate (1, 11, 16, 23), suggested that denaturation resulted in a decrease in molecular size, and these observations are the basis of the subunit hypothesis (11). Denatured forms of viral RNA with molecular weights smaller than 2×10^6 (9, 21, 30), excepting the low-molecular-weight primer used for DNA synthesis (6), probably can be attributed to partial degradation of vRNA. Viral RNA of high molecular integrity produces a single sharp band of vRNA' after denaturation and electrophoresis. Thus, if vRNA is

TABLE 1. Recovery of vRNA and intermediates as vRNA' after heating^a

Expt no.	Viral RNA	Treatment (°C)	Fraction	% Recovery
1	RSV	60	e	87
		65	b	79
2	RSV ^b	58	c	68
			d	92
			e	96
3	MuLV ^c	58	b	93
			c	88
			d	84
			e	88

^a Extracted viral RNA was heated at the indicated temperature and sedimented in a sucrose gradient, and fractions were collected. Samples from some fractions were heated at 70°C (75°C in experiment 3) before electrophoresis. Recovery is presented as percentage of radioactivity originally migrating in the regions between and including vRNA and vRNA' found in the vRNA' peak after heating at 70°C.

^b Same experiment as Fig. 7.

^c Same experiment as Fig. 8.

composed of subunits, the subunits are identical in size.

Some avian sarcoma viruses contain vRNA' of a size (class a) larger than the vRNA' (class b) of their nontransforming counterparts (13). When the cloned transforming virus mutates to the nontransforming form, only the class b forms are found (14). One would expect a deletion of a functional site in one subunit to leave other subunits intact, and mixed-size subunits should be found if subunit functions are unique. These observations, therefore, suggested that vRNA is composed of identical subunits.

Experiments involving reassociation kinetics of nucleic acids produced a genomic complexity of 9×10^6 molecular weight for both avian (28) and murine (17) oncornavirus RNA, suggesting a unique function for each subunit. However, oligonucleotide patterns of nucleoside digests gave a figure of less than 3×10^6 daltons for the genomic complexity of an avian sarcoma virus (3, 12, 24). The difficulties in providing adequate quantitative controls for reassociation studies, and the homogeneity of denatured forms of transformation-defective sarcoma viruses mentioned above, indicate that the lower-molecular-weight (3×10^6) estimate for genomic complexity is correct. Dissociation of vRNA into identical sized subunits of the genetic complexity of a single subunit brought the conclusion that oncornaviruses contained polyploid genomes (12).

It is difficult to reconcile the notion of viral RNA subunits with the occurrence of interme-

FIG. 8. Isolation of intermediate forms of MuLV RNA. RNA from MuLV virions was heated at 58°C and sedimented in a sucrose gradient, and fractions were isolated as in Fig. 6. Unheated MuLV RNA labeled with ³²P (○) was mixed with each fraction before electrophoresis. Typical ³²P patterns are depicted in b and e. Portions from several samples were heated at 75°C to complete denaturation before mixing with ³²P-labeled vRNA. The completely denatured product of intermediate forms is shown in e (Δ).

mediate forms as presented here. Two subunits per vRNA molecule should give no intermediates. No single peak suggestive of a three-subunit model was found with RSV and MuLV RNAs, and even four- and five-subunit models for vRNA would produce discrete intermediates detectable by this gel electrophoresis system.

The possibility of more than four subunits, and probably even four subunits, is untenable on other grounds. In this gel system, the apparent molecular weight of vRNA is approximately double that of the vRNA' form. Few other determinations have ventured a molecular weight of vRNA more than three times that of vRNA'. Estimates of vRNA molecular weight higher than three times that of vRNA' size would require conformational attributes inconsistent with a number of observed properties of vRNA (1, 2, 4, 16, 25).

We suggest that vRNA, vRNA', and all intermediate forms may be configurational variants of the same molecule. One possible model for vRNA is shown in Fig. 9. The viral RNA

FIG. 9. Model for vRNA and denatured forms. The vRNA molecule, containing a protruding polyadenylic acid at the 3' end, is intermittently base paired. Exposure to temperatures insufficient for complete denaturation separates some base-paired sequences, leaving others intact. Reducing the temperature results in molecules of mixed configuration. Looping could be more complex than that portrayed here.

within the virion exists in a hairpin-twisted form, base paired intermittently throughout the long axis. The RNA remains somewhat in this form after extraction, unable to assume a "collapsed-coil" configuration. This configuration may encumber migration through gel pores during electrophoresis and, by virtue of compactness or orientation, sediments rapidly during centrifugation. Heating at temperatures insufficient for complete denaturation could untwine the ends to different extents, which upon cooling would become a collapsed coil. Molecules of mixed configuration might migrate intermediate to vRNA and vRNA' during electrophoresis and sediment as intermediates during centrifugation. Complete dissociation of all twists would result in a total collapsed coil that responds as typical RNA and whose molecular weight can be estimated accurately by a variety of techniques. This model is analogous to that proposed by Worcel and Burgi (31) for the isolated *Escherichia coli* chromosome. The chromosome initially has a

high sedimentation rate, which decreases after treatments that affect the configuration, but not the molecular weight, of the chromosome.

Regions of association probably are base paired, since hydrogen bond-dissociating agents are the consistent denaturants of vRNA, and the effects of salt concentration on denaturation are typical of base-paired molecules (1). If these base-paired regions are intermittent and separated by a reasonable distance, or the looping is more complex than that depicted, the likelihood of reassociation might be small, even under ideal conditions. This could explain the inability to renature vRNA from vRNA' and intermediate forms under a variety of conditions (6; unpublished observations).

The number of observed intermediate peaks of RSV and MuLV suggests that more than five base-paired sequences maintain the vRNA configuration. The accumulation of an intermediate of MuLV RNA migrating slightly faster than vRNA, when vRNA is heated at 58°C (see Fig. 8), and a more rapidly migrating interme-

diate when heated at 65°C (see Fig. 2) suggests that the intramolecular base-paired sequences are heterologous. With RD-114V, the prevalence of one intermediate species, and the comparatively high temperature required to completely denature the RNA, can be explained by a single sequence of high guanine plus cytosine (G+C) content. A second intermediate migrating near vRNA' also may contain a high proportion of G+C pairs, but other sequences of lower G+C content would melt out at lower temperatures and other intermediates would be undetectable.

Unraveling from the ends is not an obligate feature of this model. Selective denaturation of internal base pairs also could fail to reassociate, resulting in collapsed coils and molecules of mixed configuration.

The intermediate forms found in "rapid harvests" of avian oncornaviruses (7, 8), including RSV, can be explained by this model. As virions bud from the cell surface, the RNA may be only partially twisted. Conditions of incubation could favor the intravirion twisting and intramolecular base pairing of viral RNA, with partially twisted molecules eventually becoming converted to fully twisted forms.

Early estimates of 9×10^6 for the molecular weight of avian oncornavirus RNA based on RNA-to-particle ratios (5, 10) have recently been disputed by Bellamy et al. (4). Using sedimentation analysis and intensity fluctuation spectroscopy, they determined the molecular weights of RSV and avian myeloblastosis virus vRNA's to be 3.8×10^6 to 4.8×10^6 . This range is less than double the size of the vRNA' for this virus and is consistent with either a two-subunit model or a monomer model. Our results argue against, but do not exclude, the possibility of subunits, and we suggest that each oncornavirus virion studied here contains one large molecule of RNA, 2.6×10^6 daltons for RSV, 3×10^6 daltons for MuLV, and 3×10^6 daltons for RD-114V.

Kung et al. (18), using electron microscopy, have calculated a molecular weight of 5.7×10^6 for partially denatured RD-114V RNA and have shown that this molecule can be denatured further to about one-half the original size, 2.8×10^6 daltons. Their data indicate that RD-114V vRNA is composed of two subunits, and, if correct, our data would require an interpretation other than that presented here.

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