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Characterization of the Subunit Structure of the Ribonucleic Acid Genome of Influenza Virus

L. J. LEWANDOWSKI, J. CONTENT,[1] AND S. H. LEPPLA

Department of Molecular Biology and Virus Laboratory, University of California, Berkeley, California 94720

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Ribonucleic acid extracted from influenza virus was labeled at the 3′ termini with ^{3}H and analyzed by polyacrylamide gel electrophoresis. Influenza virus was found to contain a minimum of seven and possibly as many as 10 polynucleotide chains, most of which appear to terminate at the 3′ end in uridine.

Although the ribonucleic acid (RNA) genome of influenza virus appears, by analysis on polyacrylamide gels, to consist of several subunit species (7, 9, 16, 18), the exact relationship of the subunits to the whole genome has not been clearly defined. In particular, it is not known whether these subunits result from replication and assortment into the virion of a number of discrete polynucleotide chains or whether they result from the endonucleolytic cleavage of the genome at some time after it is packaged. It seemed likely that these possibilities could be distinguished by analysis of the termini of the subunits since, generally speaking, separately replicated, chemically unmodified polynucleotide chains would end in 5′ polyphosphate and 3′ hydroxyl groups, whereas subunits resulting from the cleavage of a single large RNA genome by most of the commonly known endonucleases would have 5′ hydroxyl and 3′ monophosphate termini. Young and Content (21), in applying this concept to the 5′ termini of influenza RNA, found an amount of pppAp which suggests that all of the fragments terminate in this nucleotide and, therefore, that they replicate as discrete units.

We chose to study the apparently multicomponent structure of the influenza virus genome by analysis of the 3′ termini of the RNA subunits. The 3′ termini were specifically labeled by reduction with ^{3}H-labeled sodium borohydride after oxidation with periodate, a technique first used successfully with RNA by Leppla et al. (12) and Rajbhandary et al. (17). These procedures have recently been used to show that the double-stranded RNA genomes of both cytoplasmic polyhedrosis virus (13) and reovirus (15) exist in 10 distinct polynucleotide segments present in the intact virus in equimolar amounts. In the present study, we have observed that influenza virus strain WSN contains a minimum of seven and possibly as many as 10 polynucleotide chains, most of which appear to terminate at the 3′ end in uridine.

MATERIALS AND METHODS

Preparation of viruses and viral RNA. Influenza virus strain WSN was grown in confluent sheets of bovine kidney cell line MDBK by the method of Choppin (3). Twenty hours after infection at a multiplicity of 1 plaque-forming unit (PFU) per cell, the medium was collected and the virus was purified by the procedure of Duesberg (8). For ^{32}P-labeled virus, monolayer cultures were infected under the above conditions in low-phosphate medium and immediately received 5 mCi of ^{32}P per 10 ml of media. RNA was extracted from gradient-purified virus by the phenol-sodium dodecyl sulfate (SDS) method, and after precipitation with ethanol the RNA was dissolved in 0.001 M tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 7.4), containing 0.001 M ethylenediaminetetraacetic acid (EDTA), and was purified in the following manner. A 50-μg amount of RNA was combined with a small amount of ^{32}P-labeled viral RNA in 200 μliters of buffer, heated for 2 min at 85 C, quenched in ice, and sedimented in a 10 to 25% (w/v) sucrose gradient in 0.1 M LiCl, 0.01 M Tris-hydrochloride (*p*H 7.4), 0.001 M EDTA, and 0.1% SDS for 9 hr at 193,000 $\times$ *g* in the SW41 Spinco rotor at 20 C. Portions of the fractions were counted to determine the profile of the ^{32}P-labeled marker RNA. The fractions containing the RNA sedimenting faster than 4*S* were pooled, and the RNA was precipitated with ethanol. The RNA pellet was redissolved in standard buffer and resedimented, as above, in sucrose. The fast sedimenting RNA was collected, precipitated, and stored at −20 C. R17 phage RNA was kindly provided by W. Parenchych, and 36*S* RNA of avian sarcoma virus B77 was a gift of P. H. Duesberg.

Oxidation and reduction. The 3′ terminal ribose of the RNA components was oxidized to yield the dialdehyde by a modification of the technique of Leppla et al. (12). Approximately 40 μg of purified RNA was resuspended in 0.05 M sodium cacodylate, 0.001 M EDTA (*p*H 7.3), 0.2% SDS at a concentration of 100 μg/ml. This solution was adjusted to *p*H 5.5 with

[1] Present address: Institut Pasteur du Brabant, Brussels, Belgium.

1 M acetic acid and oxidized with excess (0.0025 M) freshly prepared sodium metaperiodate for 60 min at 0 C in the dark. Excess periodate was destroyed by the addition of propylene glycol to a final concentration of 1%, and the RNA was precipitated by ethanol. The RNA was then dissolved in 0.5 ml of 0.2 M borate buffer (*p*H 8.5), containing 0.001 M EDTA and 0.2% SDS, cooled to 0 C, and reduced by the addition of 5 mCi of ^{3}H-sodium borohydride (15 Ci/mM). After 2 hr, excess borohydride was decomposed by the addition of glacial acetic acid to 0.1 M. The sample was precipitated with cold EtOH and the pellet was washed repeatedly with cold 70% EtOH to remove the bulk of nonbound ^{3}H. The RNA was again dissolved, and the 10 to 25% sucrose gradient purification step was repeated. That portion of the gradient containing the influenza RNA (as determined by the ^{32}P profile) was collected and precipitated with ethanol.

Electrophoretic fractionation. The sample was analyzed by electrophoresis on a 2.5% polyacrylamide diacrylate cross-linked gel (7 by 0.6 cm diameter) by the method of Duesberg (7). After electrophoresis, the gel was frozen and sliced into 1-mm fractions which were then dissolved in 50 μliters of 2 M piperidine. A 2-ml amount of a toluene-based scintillation fluid containing 20% NCS solubilizer (Amersham/Searle) was added, and the samples were monitored for their ^{3}H and ^{32}P content. For preparative gels, 4% bisacrylamide was used as the cross-linker and the diameter of the gel column was increased to 1.0 cm. After electrophoresis, these gels were sliced and each fraction was minced and incubated with shaking overnight at 37 C, in buffer containing 0.2 M NaCl, 0.01 M Tris-hydrochloride (*p*H 7.4), 0.001 M EDTA, 1% SDS, 1% mercaptoethanol, and 0.01% dithiothreitol. The buffer phases were collected and sampled for their radioactive content. Fractions from the three major zones of RNA were pooled, extracted with SDS-phenol, and precipitated with ethanol.

An alternative to the above procedures was first to chromatograph samples of the unlabeled RNA on a preparative gel, elute and concentrate the three major polynucleotide fractions, and perform the oxidation and reduction on these electrophoretically purified fractions.

Hydrolysis of RNA and chromatographic analysis of 3′ termini. The oxidized-reduced RNA fractions resulting from the above procedures were hydrolyzed for 16 hr at 37 C in 200 μliters of 0.5 M NaOH in a mixture of 20 μg of each of the four common trialcohol markers prepared by the method of Khym and Cohn (11). Such alkaline hydrolysis releases the 3′ terminal residue as a hydroxymethyl diethylene glycol derivative, conveniently called a trialcohol. The digested samples were neutralized by passage through 0.5-ml columns of BioRex-70, NH_4^+, followed by lyophilization. When, as sometimes happened, solubilized polyacrylamide was carried through this process, it was removed either by precipitation with ethanol or by adsorption to Sephadex A-25.

The ^{3}H-labeled terminal residues were identified by chromatographing the hydrolysate on 10-ml columns of Whatman phosphocellulose (P-11) and comparing the position of the tritium to that of the markers. Elution was with a linear gradient of ammonium formate (*p*H 3.85), 0.01 to 0.6 M (22).

RESULTS

In several preliminary oxidation reduction experiments, a large excess of ^{3}H counts was found associated with what appeared to be extraneous non-RNA substrate present in the RNA extracts. Carbohydrates, in the form of glycoproteins, are known to comprise a substantial amount of the envelope of influenza virus. Such molecules are also known to be excellent substrates for periodate and borohydride. When present in RNA extracts, they not only competitively consume the reagents but also yield labeled macromolecules not easily separated from the RNA. In the work reported here, therefore, samples were prepared for labeling by the following purification procedure which successfully eliminated most, though certainly not all (as will be seen in Fig. 2), of such extraneous label. A trace amount of ^{32}P-labeled influenza virus was mixed with unlabeled purified virus (equivalent to approximately 40 μg of viral nucleic acid), and the RNA was extracted with phenol-SDS. This RNA was first purified by successive cycles on gradients of sucrose. A typical profile demonstrating the three major polynucleotide classes ordinarily observed in such extracts is seen in Fig. 1. In each cycle of purification, the RNA was selectively pooled as noted to exclude the trailing portion of the smallest class of subunits. Such selective exclusion was made necessary by the presence in the excluded portion of the gradients of the already noted extraneous low-molecular-weight non-RNA substrates.

Once sufficiently purified, the RNA was oxidized with metaperiodate and reduced with ^{3}H-sodium borohydride. The sample was then examined by polyacrylamide gel electrophoresis with the results shown in Fig. 2. Several pieces of information can be gleaned from the analysis. The molecular weights of the RNA subunits were estimated by assuming that the distance migrated is an inverse linear function of molecular weight (2) and by using as standards the migration in a parallel gel of ribosomal RNA markers of known molecular weight (19). These values are plotted in Fig. 3. To determine whether the individual peaks represented more than one component, the observed distribution of ^{32}P was compared with the theoretical ^{32}P content of the genome as estimated from the molecular weight determinations of Fig. 3 and the number of peaks in Fig. 2. The delineation of the boundaries of each ^{32}P-labeled peak was facilitated by the

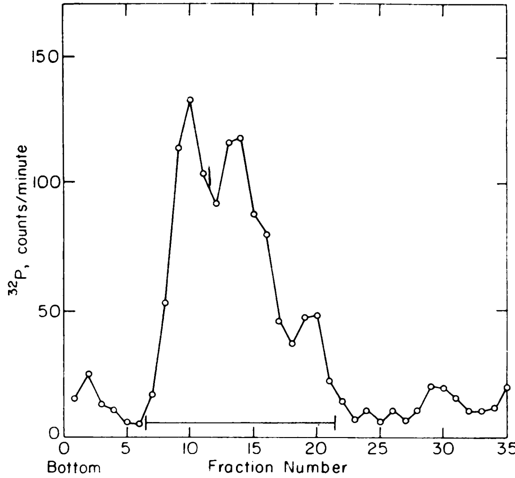

FIG. 1. *Sucrose gradient centrifugation of influenza virus RNA. A 50-μg amount of RNA was combined with a small amount of ^{32}P-labeled viral RNA, heated for 2 min at 85 C, and quenched in ice. Sedimentation was performed on a 10 to 25% (w/v) sucrose gradient containing 0.01 M Tris hydrochloride (pH 7.4), 0.001 M EDTA, 0.1 M LiCl, and 0.1% SDS, in the SW41 rotor at 193,000 × g for 9 hr at 20 C. Fractions marked by the bar were pooled and repurified on a second gradient.*

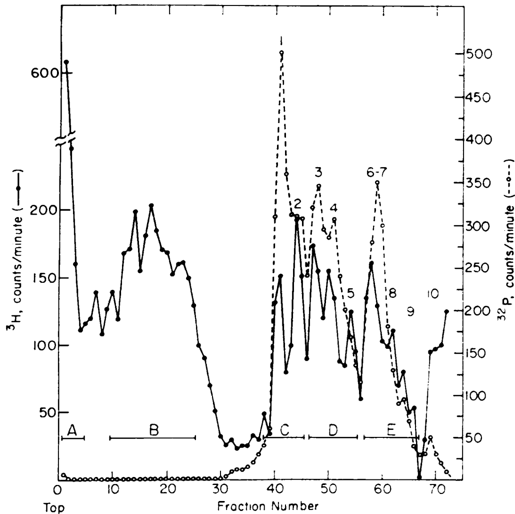

FIG. 2. *Electrophoretic separation of terminally ^{3}H-labeled influenza virus RNA subunits. Migration is from left to right in a 2.5% polyacrylamide diacrylate cross-linked gel (7 by 0.6 cm diameter) for 3 hr at 56 v. After electrophoresis, the gel was frozen and sliced into 1-mm fractions which were analyzed as detailed in the text. The lettered bars delineate those pools which were analyzed for their trialcohol content. ^{3}H-labeled material (solid line, closed circles); ^{32}P marker RNA (dashed line, open circles).*

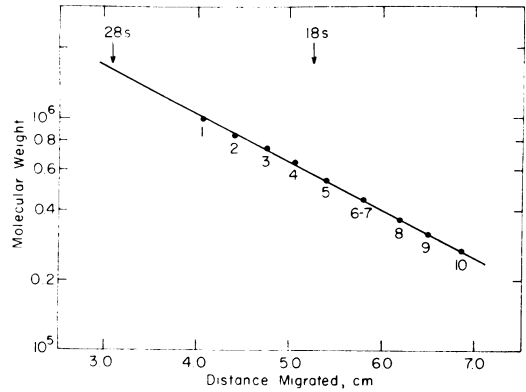

FIG. 3. *Semilog plot of molecular weight versus distance migrated of influenza virus RNA subunits in the polyacrylamide gel shown in Fig. 2. The arrows mark the positions of marker 28 and 18S mammalian ribosomal RNA of known molecular weight (19) electrophoresed in a parallel gel.*

presence of ^{3}H in the 3′ terminal residue of the individual RNA subunits. The results are recorded in Table 1. It can be seen that the first five peaks each appear to represent a single species, present in equimolar amounts. The next peak, on the other hand, appears to consist of two species (components 6 and 7), whereas the last three peaks each seem to contain less than one species equivalent. The decreasing relative amounts of ^{32}P radioactivity in the three fastest migrating components may have resulted from the previously mentioned selective exclusion of a portion of the smaller RNA components during the extensive gradient purification. Alternatively, the material in these peaks may represent breakdown products of the larger components. Based on this analysis of ^{32}P content, influenza virus could therefore contain a minimum of seven and possibly as many as 10 polynucleotide species. If we assume equimolar amounts of each species to be present in each virion, the maximum molecular weight of the total RNA content would be approximately 5×10^6 daltons per virion.

The second major piece of information in Fig. 2 derives from the pattern of the ^{3}H label. The presence of ^{3}H in each component suggests that all of the RNA species possess nonphosphorylated 3′ termini which are susceptible to labeling by the present technique. In addition to ^{3}H radioactivity which migrates with the ^{32}P marker RNA, Fig. 2 also shows a slower migrating broad peak of ^{3}H free of the marker. To investigate the nature of the ^{3}H label in these components, label was eluted from the gel slices and pooled in five fractions, A through E (Fig. 2). Recoveries were approximately 85%. These pools were concen-

TABLE 1. *Analysis of radioactivity in the influenza virus RNA subunits*

Peaks[a]	Mol wt[b] (daltons × 10^{-6})	^{32}P counts/min		^{32}P ratio (observed/estimated)	Estimated no. of subunits per virus	^{3}H counts/min observed
		Observed	Estimated[c]			
1	1.00	1,250	1,250	1.00	(1)	395
2	0.85	1,035	1,060	0.98	(1)	469
3	0.75	943	938	1.01	(1)	435
4	0.65	952	840	1.13	(1)	440
5	0.55	570	687	0.85	(1)	335
6 (7)	0.45	1,185	562	2.10	(2)	560
8	0.36	350	450	0.78	(<1)	245
9	0.32	275	400	0.69	(<1)	215
10	0.27	150	338	0.44	(<1)	700

[a] Numerical classification of the components refers to Fig. 2.
[b] Estimated from Fig. 3.
[c] Based on the distribution of ^{32}P in the five larger RNA species of Fig. 2 and the molecular weight distributions obtained from Fig. 3, we have assigned the value of 1,250 counts per min per 10^6 daltons of RNA, i.e., total counts/min in components 1 to 5 (4,750 counts/min) divided by total estimated molecular weight of components 1 to 5 (3.80 × 10^6 daltons).

trated, hydrolyzed, and chromatographed (Fig. 4). The marker absorbancy profiles are presented at the top of each panel. The designation NTM indicates the elution position of nonterminal materials including nucleotides, and U, G, A, and C represent the elution positions of the four common trialcohols. As a control to the efficiency of the above labeling and analytical procedures, R17 phage RNA was handled in parallel. The results (Fig. 4) agree with published values for the 3′ termini of the RNA phages (5, 6, 10, 20).

It can be seen that the label from pools A and B, which migrated free of any ^{32}P marker RNA, elutes in the position of NTM; these two pools would therefore be composed of labeled extraneous non-RNA substrates or non-specifically labeled nucleotides (or both). With pools C and E containing the hydrolysates of the largest and the smallest viral RNA species, most of the label migrates with the marker trialcohol derivative of of uridine although approximately 6% of total label in each pool elutes as NTM. With the hydrolysate of the medium-sized subunits in pool D, however, approximately 67% of the trialcohol-specific label elutes with the marker uridine derivative, 23% with the adenosine derivative, and the remainder with the guanosine and cytidine derivatives. These values are recorded in Table 2.

In contrast to the above sequence of operations, a portion of influenza RNA was first purified by standard electrophoresis in polyacrylamide gels prior to the oxidation-reduction procedures. The analyses from this sample are recorded in the parentheses of Table 2 and parallel the results obtained by the initial sequence of procedures. Whether the viral genome is subfractionated prior to oxidation-reduction or first oxidized-reduced and then subfractionated, the bulk of what appears to be terminal-specific ^{3}H label migrates with the trialcohol marker derivative of uridine. Thus, most of the RNA components extracted from purified influenza virions appear to terminate at their 3′ ends in uridine, whereas a minor portion, particularly from the medium-sized RNA pool, terminates for the most part in adenosine.

For comparative purposes, a second mammalian viral RNA, the 36*S* polynucleotide species from the 60 to 70*S* genome of avian sarcoma virus B77, was included for parallel analysis. Table 2 shows that the distribution of ^{3}H after oxidation and reduction of avian sarcoma virus RNA is quite similar to that found with the genome of influenza virus. Although a minor portion of ^{3}H label elutes as the terminal adenosine derivative, the bulk of the terminal specific ^{3}H label cochromatographs with the trialcohol marker derivative of uridine. When the reduction with ^{3}H-borohydride was performed on unoxidized viral RNA either from influenza virus or from avian sarcoma virus, no incorporation of label into a trialcohol derivative was observed. Under these conditions, however, label was incorporated into material which eluted with NTM fraction.

The next piece of information presented in the analysis of Fig. 2 relates to the absolute amount of ^{3}H counts present in each distinct peak (last column, Table 1). If the RNA extract is composed of polynucleotide species with one ^{3}H-labeled terminal residue per molecule, and if each species is present in equimolar amounts, we

would expect to find the same number of ^{3}H counts in each peak regardless of the molecular length of the particular species. This is approximately what is observed, at least for the larger components. The progressively decreasing amounts of ^{3}H in the faster migrating components, up to peak 10, may reflect the initial selective exclusion of the smaller RNA species during gradient purification. Although the last peak, peak 10, probably contains a distinct polynucleotide species, the excess of ^{3}H label in the absence of excess ^{32}P suggests the presence, as well, of extraneous non-RNA material accumulating at the membrane at the base of the gel column.

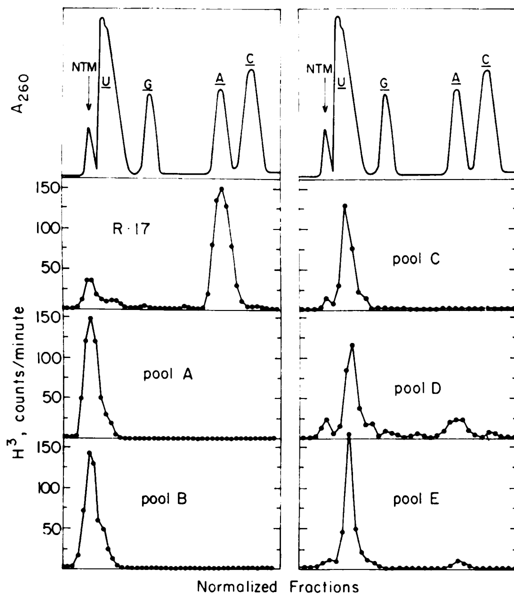

FIG. 4. *Identification of the ^{3}H-labeled termini of the separated RNA components. Terminally labeled RNA was recovered from the five regions of a gel (as in Fig. 2) and hydrolyzed in the presence of nonradioactive trialcohol markers. The neutralized hydrolysates were applied to 10-ml columns of phosphocellulose (Whatman P-11) and eluted by linear gradients of ammonium formate (pH 3.85), 0.01 to 0.60 M, in total volumes of 60 ml. The eluates were passed through an ultraviolet monitoring apparatus to record A_{260} (top panels) and collected directly in scintillation vials to determine ^{3}H (lower panels).*

DISCUSSION

Exclusive of its use in the chemical identification of the terminal residue proper, terminal labeling of RNA has a wide variety of potential applications. It can give information on the number and size, as well as the chemical integrity of distinct RNA classes of a particular genome, their molar amounts per class (and by inference, per virion), and whether the different RNA species in multicomponent genomes have the same 3′ terminus. Furthermore, it provides the basis for an analysis of the terminal nucleotide sequences of the individual components of the viral genome.

In the present study, we have consistently observed that the RNA released from gradient-purified influenza virus consists of from 7 to 10 polynucleotide species. Such RNA would have a maximum total molecular weight of approximately 5×10^{6} daltons if it is assumed that each virion contains one molecule of each species and that each species is part of the viral genome. Furthermore, molecules in each of the three major size classes seem to possess nonphosphorylated 3′ termini, the bulk of which appears to be uridine. The major exception to this is

TABLE 2. *Analysis of the distribution of ^{3}H label after oxidation and reduction of RNA with ^{3}H-sodium borohydride*

RNA source	Per cent NTM[a]	Per cent of total nucleoside trialcohols[b]				
		U	G	A	C	Other
R17 bacteriophage	15	5	—	95	—	—
Influenza virus RNA						
Pool A	100	—	—	—	—	—
Pool B	100	—	—	—	—	—
Pool C	6	100 (92)	— (–)	— (4)	— (4)	— (–)
Pool D	10	67 (75)	4 (2)	23 (21)	4 (2)	2 (–)
Pool E	6	94 (92)	— (–)	6 (6)	— (2)	— (–)
Avg totals		87%	1%	10%	2%	.3%
Avian sarcoma virus B77	30	92	—	8	—	—

[a] NTM, nonterminal materials.

[b] U, G, A, and C represent the trialcohol derivatives of uridine, guanosine, adenosine, and cytidine, respectively.

[c] Values in parentheses refer to influenza virus RNA samples purified by electrophoresis in polyacrylamide gels before the oxidation-reduction procedures, whereas the other values were derived from viral RNA samples first oxidized and reduced with ^{3}H-$NaBH_4$ and then subfractionated.

seen in the hydrolysate of the pooled medium-sized components where approximately 22% of specific trialcohol label migrates with the marker trialcohol derivative of adenosine. One explanation for the presence of this terminal adenosine, which represents 10% of the terminal label in the total RNA extract, may lie in the fact that host ribosomal RNA apparently can be incorporated into the virus superstructure during virion maturation (*unpublished data*). When virus is grown on cells which have been pulse-labeled with ^{32}P prior to infection, the RNA extracted from purified virus invariably contains both 28 and 18*S*-labeled ribosomal RNA species, the absolute amounts of which seem to vary with the individual virus preparation. Similar observations have been published in the case of the RNA tumor viruses (1). Thus the adenosine trialcohol obtained from hydrolysis of oxidized-reduced RNA from purified influenza virus may reflect the content of contaminating ribosomal RNA indiscriminately packaged as free RNA or more probably in ribosomes along with viral RNA during viral morphogenesis. Alternatively, of course, the presence of these adenosine termini (as well as trace amounts of cytidine and guanosine) may reflect minor amounts of virus-specific RNA other than those species which appear to terminate in uridine. The present chemical determinations cannot distinguish between these possibilities. The marked similarity of the 3′ termini of both influenza and avian sarcoma virus RNA species does suggest, however, that the predominant termination of the viral polynucleotides in uridine, together with a minor amount of terminal adenosine, may be a common feature of the multipartite genomes of animal viruses containing single-stranded RNA.

In any case, our results suggest that the multicomponent structure typically observed for the genome of influenza virus is not an artifact of covalent bond breakage. Not only is the subunit pattern of the RNA extract highly reproducible, but each of the three major size-classes appears to contain molecules with nonphosphorylated 3′ termini. We interpret these findings to mean that the subunit structure probably results from replication of discrete polynucleotide chains. The question arises, however, of how it is possible for a genome consisting of many, apparently separate, polynucleotides to replicate and assort so as to yield virions containing one molecule of each size class. Li and Seto (14) have recently reported that by using the Kleinschmidt extraction procedure they can observe in the electron microscope molecules whose lengths are great enough to correspond to approximately 4.5×10^6 daltons of RNA. These authors suggest that the inability to isolate intact molecules of this size physically may be due to the presence of extremely labile linkers which hold the pieces together inside the virion. Our present studies certainly do not rule out any such physical linkage but do indicate that the genome of influenza virus does exist in, or is readily converted to, subgenomic segments. Indeed, the lack of infectivity of isolated influenza virus RNA could be due to the physical separation of the RNA subunits upon phenol extraction or to the loss of some protein which might be required for infectivity, for example, the transcriptase activity recently shown to be part of the virion (4). Precedence for a situation of this type in multicomponent single-stranded viral RNA systems can be found in the studies (13) on the multicomponent double-stranded RNA genome of cytoplasmic polyhedrosis virus of the silkworm, *Bombyx mori*.

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LITERATURE CITED

1. Bauer, H., and D. P. Bolognesi. 1970. Polypeptides of avian RNA tumor viruses. II. Serological characterization. Virology **42**:1113–1126.
2. Bishop, D. H. L., J. R. Claybrook, and S. Spiegelman. 1967. Electrophoretic separation of viral nucleic acids on polyacrylamide gels. J. Mol. Biol. **26**:373–387.
3. Choppin, P. W. 1969. Replication of influenza virus in a continuous cell line: high yield of infective virus from cells inoculated at high multiplicity. Virology **39**:130–134.
4. Chow, N.-L., and R. W. Simpson. 1971. RNA-dependent RNA polymerase activity associated with virions and subviral particles of myxoviruses. Proc. Nat. Acad. Sci. U.S.A. **68**:752–756.
5. Dahlberg, J. E. 1968. Terminal sequences of bacteriophage RNAs. Nature (London) **220**:548–552.
6. De Wachter, R., and W. Fiers. 1967. Studies on the bacteriophage MS2. IV. The 3′-OH terminal undecanucleotide sequence of the viral RNA chain. J. Mol. Biol. **30**:507–527.
7. Duesberg, P. H. 1968. The RNA's of influenza virus. Proc. Nat. Acad. Sci. U.S.A. **59**:930–937.
8. Duesberg, P. H. 1969. Distinct subunits of the ribonucleoprotein of influenza virus. J. Mol. Biol. **42**:485–499.
9. Etchison, J., M. Doyle, E. Penhoet, and J. Holland. 1971. Synthesis and cleavage of influenza virus proteins. J. Virol. **7**:155–167.
10. Glitz, D. G., A. Bradley, and H. Fraenkel-Conrat. 1968. Nucleotide sequences at the 5′-linked ends of viral ribonucleic acids. Biochim. Biophys. Acta **161**:1–12.
11. Khym, J. X., and W. E. Cohn. 1960. Characterizations and some chemical reactions of periodate-oxidized nucleosides. J. Amer. Chem. Soc. **82**:6380–6386.
12. Leppla, S. H., B. Bjoraker, and R. M. Bock. 1968. Borohydride reduction of periodate-oxidized chain ends, p.

236–240. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 12B. Academic Press Inc., New York.

13. Lewandowski, L. J., and S. Millward. 1971. Characterization of the genome of cytoplasmic polyhedrosis virus. J. Virol. 7:434–437.
14. Li, K.-K., and J. T. Seto. 1971. Electron microscope study of ribonucleic acid of myxoviruses. J. Virol. 7:524–530.
15. Millward, S., and A. F. Graham. 1970. Structural studies on reovirus: discontinuities in the genome. Proc. Nat. Acad. Sci. U.S.A. 65:422–429.
16. Pons, M. W., and G. K. Hirst. 1968. Polyacrylamide gel electrophoresis of influenza virus RNA. Virology **34**: 385–388.
17. Rajbhandary, U. L., A. Stuart, R. D. Faulkner, S. H. Chang, and H. G. Khorana. 1966. Nucleotide sequence studies on yeast phenylalanine sRNA. Cold Spring Harbor Symp. Quant. Biol. 31:425–434.
18. Skehel, J. J. 1971. Estimations of the molecular weight of the influenza virus genome. J. Gen. Virol. 11:103–109.
19. Weinberg, R. A., U. Loening, M. Willems, and S. Penman. 1967. Acrylamide gel electrophoresis of HeLa cell nucleolar RNA. Proc. Nat. Acad. Sci. U.S.A. 58:1088–1095.
20. Weith, H. L., G. T. Asteriadis, and P. T. Gilham. 1968. Comparison of RNA terminal sequences of phages f2 and Qβ: chemical and sedimentation equilibrium studies. Science 160:1459–1460.
21. Young, R. J., and J. Content. 1971. 5′-Terminus of influenza virus RNA. Nature New Biol. (London) 230:140–142.
22. Zapisek, W. F., A. G. Saponara, and M. D. Enger. 1969. Low molecular weight methylated ribonucleic acid species from Chinese hamster ovary cells. I. Isolation and characterization. Biochemistry 8:1170–1181.