Properties of Single- and Double-stranded Ribonucleic Acid from Barley Plants Infected with Bromegrass Mosaic Virus

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Incorporation of \(^{32}P\) into nucleic acids in barley plants infected with bromegrass mosaic virus (BMV) was analyzed by chromatography on methylated albumin kieselguhr (MAK) columns. Treatment with actinomycin D reduced the synthesis of ribosomal ribonucleic acid (RNA) to low levels and allowed the detection of the three components of BMV-RNA in vivo. The kinetic study on \(^{32}P\) incorporation into these BMV-RNA components suggested that a single cleavage occurred in some of the intact RNA shortly after completion of its synthesis, giving rise to the small and medium components. Chromatographic analyses also revealed a double-stranded, ribonuclease-resistant RNA which has been purified by differential extraction, sucrose-density gradient centrifugation, and MAK column chromatography. This RNA sediments at approximately 14S, is alkali-labile, and has a sharp thermal transition with a \(T_m\) of 96°C in 0.1X standard saline citrate buffer, as determined by susceptibility to ribonuclease. The RNA is absent in uninfected barley plants.

Ribonucleic acid (RNA) isolated from bromegrass mosaic virus (BMV) consists of three distinct components of molecular weight: 0.3 \(\times\) 10\(^6\), 0.7 \(\times\) 10\(^6\), and 1.0 \(\times\) 10\(^6\) (3). To understand the replication of BMV-RNA, it is of interest to analyze RNA synthesis in infected plants and to seek the origin of the small- and medium-size components. The purpose of this paper is to describe some results of such studies and to report the occurrence and properties of a double-stranded viral RNA, like those discovered in a number of RNA virus-infected systems (8, 13, 14). The present studies were facilitated by the finding that host nucleic acid synthesis could be inhibited by treatment with actinomycin D while allowing viral RNA synthesis to proceed. Preliminary reports of the present work appeared elsewhere [C. Hikuri and P. Kaesberg, Phytopathology, p. 1061 (abstr.), 1965; C. Hikuri, Proc. Intern. Congr. Genet. Tokyo, p. 32, 1968].

MATERIALS AND METHODS

Virus growth. A standard strain of BMV of H. H. McKinney (ATCC 66) was used. Moore barley (Hordeum vulgare L.) was grown in vermiculite in a controlled environment at 21°C with 16-hr light periods at intensities of approximately 2,000 foot-candles and at 16°C with 8-hr dark periods and at relative humidities of 60%. Plants, at the three-leaf stage, were inoculated by rubbing carborundum-dusted leaves with cheesecloth moistened with virus inoculum in 0.01 m sodium acetate buffer (pH 5.5) containing 0.001 m MgCl\(_2\) and 0.001 m CaCl\(_2\).

The nutrient solution contained 0.002 m KNO\(_3\), KH\(_2\)PO\(_4\), and MgSO\(_4\).7H\(_2\)O, and 0.003 m Ca(NO\(_3\))\(_2\).4H\(_2\)O, all combined with the micronutrients of the formula suggested by Johnson et al. (6), except that an iron chelate, Versenol FL (Dow Chemical Co., Midland, Mich.), was substituted at the rate of 1 ml/liter for FeSO\(_4\) and silicon in the form of Na\(_2\)SiO\(_3\).9H\(_2\)O was added at the rate of 10 mg/liter (15). Watering with the nutrient solution was made daily.

\(^{32}P\) labelling and actinomycin D treatment. At various intervals after inoculation, 30 to 50 plants were removed from pots, cut under distilled water at the base of shoot, and placed in 25 to 50 ml of distilled water containing 2 to 10 mc of \(^{32}P\) (obtained as carrier-free \(^{32}P\)-orthophosphate from Oak Ridge National Laboratory or from Tracerlab, Waltham, Mass.). When actinomycin D was used, the severed plants were allowed to stand in the solution (40 \(\mu\)g/ml) for 24 hr in the dark and then \(^{32}P\) was added to the solution. Labeling was terminated by washing the lower part of shoots in distilled water and freezing at \(-15°C\).

Virus purification. Frozen leaves, usually 10 to 50 g, were quickly cut into pieces 1 cm long and ho-
mogenized in 0.5 M KH$_2$PO$_4$, pH 4.5, in a Waring Blender at room temperature. The homogenate was filtered through cheesecloth and allowed to stand at room temperature for 1 to 2 hr while green coagulated material precipitated. The following steps were carried out at 0 to 4 C. The sap was centrifuged at 1,500 X g for 20 min in a refrigerated centrifuge (model HR-1, International Equipment Co., Boston, Mass.). The supernatant liquid was then centrifuged at 6,500 X g for 20 min to remove remaining insoluble material. The virus in that liquid was then sedimented into a pellet by centrifuging at 79,000 X g for 90 min (Spinco model L, no. 30 rotor), suspended in standard buffer, and subjected to two further cycles of differential centrifugation (high: 105,000 X g for 60 min, discard supernatant; low: 6,500 X g for 20 min, discard pellet, no. 40 rotor). The final virus pellets were colorless. The yield was 1 to 1.5 g per liter of sap.

Isolation of nucleic acids from leaves and from purified virus. Ten to 50 g of frozen leaves, cut into small pieces, was homogenized at room temperature in a Waring Blender in the presence of 2 ml of water-saturated phenol and 1 ml of an extracting solution [2% sodium dodecylsulfate, 0.05 M ethylenediaminetetraacetic acid (EDTA), 1% bentonite, 0.3% NaCl, 0.03 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 7.8] per 1 g of leaves. The resulting homogenate was filtered through cheesecloth to remove debris. The debris was reextracted with a small amount of the phenol and extracting solution. The filtered slurry was kept at 0 C and centrifuged at 6,500 X g for 20 min. The aqueous phase was carefully removed and shaken two times with the phenol solution. Phenol was then removed by shaking with ether three times. The nucleic acids were twice precipitated with two volumes of cold 95% ethyl alcohol and dialyzed against standard buffer at 4 C for 24 hr. The final material was suspended in a buffer containing 0.005 M Tris-hydrochloride and 0.02 M EDTA, pH 7.0.

RNA from purified BMV was prepared by a similar procedure except that 0.05 M EDTA was excluded from the extracting solution.

Purification of double-stranded RNA. Two methods were used to isolate the RNA of peak III and these yielded products whose properties were very similar and were characteristic of double-stranded RNA.

(i) The nucleic acids from 8-day-infected, actinomycin D-treated, and 32P-labeled leaves were subjected to sucrose density gradient centrifugation, and the fractions from the 12 to 16S region were pooled. This material was dialyzed against 0.1 M NaCl in 0.05 M phosphate buffer for 18 hr at 4 C; the salt concentration was then adjusted to 0.4 M. This was chromatographed on a methylated albumin kieselguhr (MAK) column. The material from the peak III region was applied to a Sephadex G25 column (2 by 22 cm) saturated with 0.1 M NaCl. The effluents were digested with 20 µg of crystalline pancreatic deoxyribonuclease per ml in 0.001 M MgCl$_2$ at 37 C for 10 min. After the chromatography on a MAK column, the peak III RNA was concentrated by ethyl alcohol precipitation. The amount of the ribonuclease-resistant RNA isolated was 3.8% of the starting total nucleic acids as judged by radioactivity.

(ii) An improved method has been reported for isolating a replicative form of RNA from M12 phage-infected cells (1). Nucleic acids were extracted according to this procedure from 8-day-infected, actinomycin D-treated, and 32P-labeled barley leaves. RNA was digested, 15 mg at a time, for 1 hr at 25 C with 10 µg of deoxyribonuclease per ml in 0.3 M NaCl, 0.001 M MgCl$_2$, pH 7.0. After adding EDTA to 0.002 M, the deoxyribonuclease digest was diluted with three volumes of distilled water, and an equal volume of ethyl alcohol was added. This was incubated at 76 C for 10 min and quickly cooled in an ice bath. After adding two more volumes of ethyl alcohol, the suspension was centrifuged at 6,500 X g for 20 min at -10 C. The precipitate was dissolved in 0.1 M NaCl; the single-stranded plant ribosomal and BMV-RNA molecules were precipitated by adding NaCl to 10% and were removed by centrifugation at 6,500 X g for 10 min at -5 C. The supernatant fraction from the second centrifugation was adjusted to 0.4 M NaCl and was rechromatographed on a MAK column. The amount of the ribonuclease-resistant RNA isolated was 4.5% of the starting total nucleic acids.

MAK column chromatography. Up to 3 mg of nucleic acid in 0.4 M NaCl, 0.05 M phosphate buffer (pH 6.7) was applied to MAK columns prepared as previously described (7), or by a modified method. Gradient elution was carried out at room temperature, with 0.4 to 1.6 M NaCl used in the same phosphate buffer. Fractions (4 ml) were collected and examined for absorbance at 260 nm (A$_{260}$), infectivity, and 32P radioactivity.

Sucrose density gradients. The procedures for sucrose density gradient centrifugation and analyses were similar to those described previously (3).

Spectrophotometry. Ultraviolet absorption was measured with a Cary model 11 recording spectrophotometer equipped with quartz absorption cells of 1-cm light path.

Radioactivity measurements. Samples were placed on filter paper discs (2.3 mm in diameter, Whatman 3 mm) in glass scintillation vials. They were dried in an oven at 50 C for 30 min, and 5 ml of scintillation fluid [4 mg of 2,5-diphenyloxazole and 0.3 mg of 1,4-bis-[2-(5-phenyloxazolyl)]-benzene in 1 ml of toluene] was added. Assays were made in a Packard series 3000 liquid scintillation counter.

Infectivity assays. Infectivity assays were made by the half-leaf method using Chenopodium hybridum L. (11).

Base analyses. Desalted material was hydrolyzed with 0.3 N NaOH for 18 hr at 37 C. The hydrolysate was neutralized with Dowex 50 (hydrogen) resin and subjected to electrophoresis on Whatman 3 MM paper (38 by 620 mm) in 0.05 M sodium formate buffer, pH 3.5, at 24 v/cm for 6.5 hr in a water-cooled flat-plate apparatus. Each electrophoresis strip was cut perpendicular to the direction of migration into 1-cm segments; the radioactivity of each segment was
measured in a liquid scintillation spectrometer. In some cases nonlabeled mononucleotides were added as carriers and revealed with an ultraviolet lamp.

**Ribonuclease treatment.** RNA fractions (1 ml) were treated with 100 μg of ribonuclease (pancreatic ribonuclease A, phosphate-free, Worthington Biochemical Corp.) for 20 min at 37°C, except as stated otherwise. Samples were chilled, 0.2 ml of 60% trichloroacetic acid and 400 μg of bovine serum albumin were added per tube, and the tubes were kept at 4°C for 20 min. The precipitates were collected on membrane filters (Millipore Filter Corp., Bedford, Mass.; type HA, pore size 0.45 μm), washed with cold 10% trichloroacetic acid and cold ethyl alcohol, and assayed for radioactivity.

**RESULTS**

Incorporation of 32P into nucleic acids of infected barley plants. At 14 days after inoculation, barley cuttings were cultured for 6, 12, and 24 hr in distilled water containing 32P. The nucleic acids were extracted, and the extent of 32P-incorporation into the several types of nucleic acids was determined by measurement of the radioactivity of fractions separated by MAK column chromatography. There are at least seven distinguishable regions of radioactivity (Fig. 1). The nucleic acid in some of the regions is identifiable by comparison with chromatographic analyses of purified nucleic acids. Regions I and II contain soluble RNA and possibly also degradation products of larger host and viral nucleic acids. Peak III contains double-stranded RNA of viral origin and also host deoxyribonucleic acid. Peak IV is the BMV-RNA small component. The fifth and sixth regions contain ribosomal RNA (rRNA) and the medium component of BMV-RNA. Region VII contains infectious BMV-RNA.

It is apparent (Fig. 1) that 32P incorporation into RNA components increases with prolonged labeling time, and that synthesis of rRNA and viral RNA proceeds at approximately the same rate.

**Synthesis of BMV-RNA in barley treated with actinomycin D.** Detection of BMV-RNA in infected plants is facilitated if host rRNA synthesis is suppressed with actinomycin D. Figure 2 shows the rRNA portions of MAK chromatograms from healthy plants, untreated and treated with actinomycin D, respectively. Obviously RNA synthesis is much reduced in the latter. However, in actinomycin D-treated, infected plants, BMV-RNA synthesis was not suppressed (Fig. 3). The peak of the medium component becomes apparent in the rRNA region, and the peak of the small component is much more evident.

Base compositions were determined of three RNA fractions obtained by MAK chromatography from infected plants and of two fractions of rRNA from uninfected plants (Table 1). The base compositions of RNA fractions from infected, actinomycin D-treated plants were close to those of the three components of BMV-RNA and were somewhat different from those of rRNA from uninfected plants. It may be concluded that, in these actinomycin D-treated barley leaves, BMV-RNA, but not much rRNA, is made. Regions I and II exhibit some radioactivity which possibly suggests some host soluble RNA is made in the presence of actinomycin D.

When the nucleic acid from infected, actinomycin D-treated plants (e.g., the material of Fig. 3) is sedimented through sucrose density gradients, four peaks of radioactivity occur, corresponding to sedimentation coefficients of 27S, 20S, 14S, and 4S. From their sedimenta-

![Fig. 1. MAK chromatographic detection of the relative rate of incorporation into nucleic acid fractions in BMV-infected barley. Labeled at (A) 6 hr, (B) 12 hr, and (C) 24 hr. Symbols: (●—●) absorbance at 260 nm; (○—○) counts/min of 32P; (●—○—○) relative infectivity.](http://jvi.asm.org/)

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**Table 1.** Base compositions of RNA fractions from BMV-infected barley treated with actinomycin D, and from uninfected barley. The base compositions are given as mol%.

<table>
<thead>
<tr>
<th>Region</th>
<th>RNA Fractions</th>
<th>Base Compositions (mol%)</th>
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<tbody>
<tr>
<td>I</td>
<td>Small</td>
<td>A: 23.0 B: 34.0 C: 43.0</td>
</tr>
<tr>
<td>II</td>
<td>Medium</td>
<td>A: 27.0 B: 30.0 C: 43.0</td>
</tr>
<tr>
<td>III</td>
<td>Large</td>
<td>A: 32.0 B: 28.0 C: 40.0</td>
</tr>
<tr>
<td>IV</td>
<td>Viral</td>
<td>A: 30.0 B: 30.0 C: 40.0</td>
</tr>
<tr>
<td>V</td>
<td>Infectious</td>
<td>A: 30.0 B: 30.0 C: 40.0</td>
</tr>
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</table>

**Fig. 1.** MAK chromatographic detection of the relative rate of incorporation into nucleic acid fractions in BMV-infected barley. Labeled at (A) 6 hr, (B) 12 hr, and (C) 24 hr. Symbols: (●—●) absorbance at 260 nm; (○—○) counts/min of 32P; (●—○—○) relative infectivity.
Fig. 2. Comparison of $^{32}$P incorporation into rRNA of actinomycin D-treated and untreated healthy barley leaves. (A) RNA extracted from untreated healthy barley; (B) RNA from actinomycin D-treated healthy barley. Symbols: (●) absorbance at 260 nm; (○) counts per minute of $^{32}$P.

Fig. 3. Chromatographic separation of BMV-RNA components from total nucleic acids extracted from BMV-infected barley leaves. (A) Eight-day-infected with BMV, without actinomycin D; (B) 12-day-infected with BMV, without actinomycin D; (C) 8-day-infected with BMV and treated with actinomycin D; (D) 12-day-infected with BMV and treated with actinomycin D. Symbols: (●) absorbance at 260 nm; (○) counts per minute of $^{32}$P.
showed that in regions I and II. About 40% of the 4S peak eluted in regions I and II. The former is refractive to ribonuclease but becomes sensitive upon heating to 100 C and quickly cooling. Chromatographic analysis of the 14S peak showed that it consisted of two major components: (i) ribonuclease-resistant RNA which was eluted in region III, and (ii) ribonuclease-sensitive RNA which was eluted in region IV. The former is thus the highly labeled RNA of Fig. 1 and the latter is the small component of BMV-RNA.

Specific 32P activity of three pieces of RNA from purified BMV. The above experiments suggest that the two smaller BMV-RNA components are produced in the plant. It is conceivable, though, that they are artifacts of the RNA isolation. If it could be shown that the components were not synthesized in parallel, it would follow that they are not artifacts. To test whether the three components are synthesized at the same time, their specific radioactivity (counts per min per A260) was measured after short-time labeling, as above. Figure 4 shows the data of 32P incorporation into the three components of BMV-RNA extracted from purified virus particles and fractionated by sucrose gradient centrifugation. The ratios of radioactivity to absorbance (at 260 nm) of the large and small components of BMV-RNA are nearly the same and are higher than that of the medium component in all cases. Since absorbance is a measure of the steady-state concentration of the components whereas radioactivity is a measure of the concentration of components synthesized and persisting for the fixed periods before harvest, it is evident that the small and medium components are real. The data are consistent

![Graph](Figure 4. Incorporation of 32P into the large (L), medium (M), and small (S) components of BMV-RNA.)
with the hypothesis that, when the large component is synthesized, some of it is subsequently broken down in the plant to yield the small and medium components.

Possible contamination of the viral RNA with highly labeled RNA originating from host constituents could affect the above conclusions. To eliminate this uncertainty, uninfected barley plants (34 g) were labeled with 5 mc of 3P for 24 hr and mixed with unlabeled 14-day-infected barley (45 g). The sap of the mixed sample was subjected to the usual preparative procedures to obtain purified virus; radioactivity was checked at each stage in purification. Starting sap material had a radioactivity 7 x 10 counts per min per ml, whereas the final virus preparation and the RNA prepared from it had no measurable radioactivity. It is concluded that the present procedure yields BMV-RNA uncontaminated with host constituents.

Some properties of double-stranded RNA. The RNA, as prepared by either procedure, exhibited a single, symmetrical peak of radioactivity and absorbancy upon MAK chromatography. It sedimented as a single band of 14S. Neither fact is very surprising since these procedures were used for purification. Nevertheless, as was reported above, these characteristics were already evident in the unpurified samples.

Base composition. The ratio guanine-cytosine-adenine-uridine is 24.2:24.4:25.7:25.7; thus, the molar ratio of guanine is almost equal to that of cytosine, and that of adenine is equal to that of uracil, as is to be expected for double-stranded RNA.

Infectivity. Infectivity was examined on Chenopodium hybridum by the half-leaf method after treating in the various ways listed below. In none of the cases was infectivity detected. (i) A 2.4-mg amount of total RNA, from infected leaves in 0.005 M Tris-hydrochloride, 0.02 M EDTA (pH 7.0), was treated with 0.5 µg of ribonuclease for 20 min at 20 C and fractionated by sucrose density gradient centrifugation (5 to 23% sucrose in 0.02 M EDTA, 0.005 M Tris-hydrochloride, pH 7.0) and 12 to 16S fractions were tested. (ii) A 1.4-mg amount of total leaf RNA was treated with 0.1 µg of ribonuclease in 0.002 M phosphate buffer, containing 0.03% EDTA and 0.8 M NaCl (pH 7.2), for 20 min at 20 C, fractionated by sucrose density gradient centrifugation (5 to 23% sucrose in 0.02 M EDTA, 0.005 M Tris-hydrochloride, pH 4.8), and tested. (iii) The fractions from (ii) were heated for 1 min in a water bath at 94 C in 0.3 M NaCl, 0.002 M Tris-hydrochloride, 0.01 M EDTA, quickly cooled in an ice bath, and then tested. (iv) The region III fraction obtained by MAK chromatography without ribonuclease treatment was tested.

Nuclease resistance. The ribonuclease susceptibility of the double-stranded RNA strongly depends upon salt concentration (Table 2). In 5 x SSC (0.15 M NaCl plus 0.015 M sodium citrate) buffer, 70% of the RNA was resistant to ribonuclease, whereas, in 1 x SSC, only 46% was resistant, and in 0.1 x SSC 31% remained resistant. The nucleic acid was completely resistant to deoxyribonuclease even at low salt concentration and together with ribonuclease (Table 3).

Melting point. A sample was dialyzed against 0.1 x SSC, and susceptibility to ribonuclease was determined after heating to different temperatures for 10 min and then cooling. Susceptibility

| Table 2. Effect of salt concentration on ribonuclease susceptibility |
|--------------------------|---------------------|-----|
| Assay condition          | Acid precipitates  | %  |
|                          | (counts/min)        |    |
| 1 x SSC, no ribonuclease | 4,426               | 100|
| 5 x SSC                  | 3,138               | 71  |
| 2.5 x SSC                | 2,538               | 57  |
| 1 x SSC                  | 2,022               | 46  |
| 2/3 x SSC                | 1,865               | 42  |
| 1/2 x SSC                | 1,768               | 40  |

* The samples (1 ml each) were incubated at 37 C for 20 min with 10 µg of ribonuclease per ml.

<table>
<thead>
<tr>
<th>Table 3. Deoxyribonuclease susceptibility</th>
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<tr>
<td>Treatment (37 C)</td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>Nilb</td>
</tr>
<tr>
<td>DNasec</td>
</tr>
<tr>
<td>DNase-NaCl-RNasee</td>
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<tr>
<td>NaCl-RNased</td>
</tr>
<tr>
<td>DNase-RNasec</td>
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</tbody>
</table>

* At time-zero, 1 ml of sample in 0.001 M Tris-hydrochloride (pH 7.0), 0.01 M NaCl, 0.001 M MgCl2 was taken as a control.

b Deoxyribonuclease (DNase) digestion (10 µg/ml) was carried out for 20 min.

c Deoxyribonuclease (DNase) digestion (10 µg/ml) was carried out for 20 min.

d DNase was omitted. Incubated with RNase in 0.8 M NaCl for 20 min.

e After digesting with DNase for 10 min, the NaCl concentration was adjusted to 0.8 M and the digestion was continued for another 10 min. Ribonuclease (RNase) digestion (10 µg/ml) was carried out for 10 min.

f After digesting with DNase for 10 min, RNase was added and the digestion was continued for another 10 min.
to ribonuclease begins to change at approximately 90°C and rises to a maximum at 100°C (Fig. 5). The 50% digestion point is 96°C and is consistent with the expected melting temperature of double-stranded RNA of this composition under these ionic conditions.

DISCUSSION

There are at least three possible ways in which the three components of BMV-RNA may be produced. (i) The medium and small components are the degradation products produced in vitro; (ii) the medium and small components are naturally occurring RNA and are independent of the synthesis of the large component; and (iii) the medium and small components are the products of the large component as the result of a single cleavage which occurs at a specific point in some of the large components in vivo.

The in vitro degradation does not appear to be an intrinsic reason explaining the production of three BMV-RNA components, although certain experimental conditions might enhance the specific cleavage of viral RNA in vitro (2). The medium and small components which are similar to those isolated from purified BMV can be chromatographically detected in the total RNA extracted directly from infected leaves (Fig. 3 and Table 1). Since the direct extraction of RNA is considered to minimize the chance of RNA degradation which might occur during virus purification, this indicates that most of the three components occur in vivo under our conditions. Furthermore, in vitro degradation experiments of BMV-RNA using barley nuclease failed to demonstrate this specificity in the cleavage of BMV-RNA (3).

The second possibility appears to be unlikely. To produce the medium and small components independently, additional templates are required. If this were the case, two species of double-stranded RNA from the medium and small components should have been detected.

The specific radioactivity is nearly the same for the large and small components and is higher in these components than in the medium component (Fig. 4). This result would be best explained by assuming the following. (i) only the large component is initially synthesized; (ii) the synthesis of the large component starts from only one end and the RNA grows linearly toward the other end (4); and (iii) the single cleavage occurs at a specific point near the terminal of the RNA strand. A possibility of further cleavage of the medium component which gives rise to the small component (2), however, cannot be entirely ruled out. The cleavage must not result in physical separation of the products, because they are evidently enclosed in the same protein shell (5).

The above interpretation is consistent with the existence of a single species of double-stranded viral RNA rather than three. By analogy with the double-stranded forms of other viruses, a single-stranded RNA of 27S exhibits a sedimentation coefficient of about 14S when base-paired with its negative-strand counterpart. It has been recently demonstrated that the labeled RNA product of uridine triphosphate incorporation by the extracts from BMV-infected leaves is ribonuclease-resistant and sediments around 13 to 14S, suggesting the synthesis of double-stranded RNA in the system (12). The 14S double-stranded RNA described in this paper thus seems to be the counterpart of the BMV-RNA large component—and the medium- and small-component RNA molecules seem to have no double-stranded counterparts.

Thus it is believed that the BMV-RNA large component is synthesized by a mechanism involving a negative strand complementary to it, that a portion associates with coat protein to produce infectious virus, and a portion is cleaved
and results in virus-like particles containing the small- and medium-component RNA molecules.
These studies also show that host rRNA and viral RNA are synthesized concurrently and, under our conditions, at about the same rate. The rapid degradation of rRNA after BMV infection was not detected, although it has been reported to occur in tobacco leaves after infection with TMV (9, 10).

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LITERATURE CITED