Characterization of Maize Chlorotic Mottle Virus

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ABSTRACT

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Maize chlorotic mottle virus (MCMV) is an icosahedral plant virus 30 nm in diameter, composed of a single 25-kDa capsid protein subunit and a 4.4-kb single-stranded, positive-sense genomic RNA. The genomic RNA is capped at the 5′ terminus with m^7GpppA, and no genome-linked protein was detected. MCMV infection produces two discrete double-stranded RNA species in infected maize plants, corresponding to single-stranded RNAs of 4.4 and 1.1 kb. The smaller double-stranded RNA corresponds to a 1.1-kb subgenomic messenger RNA that is homologous to the 3′-terminal region of MCMV genomic RNA and encodes the viral capsid protein. Virion RNA directs the synthesis of 110-, 50-, 44-, 41-, 32-, and 25-kDa polypeptides in a rabbit reticulocyte lysate in vitro translation system. Only the 25-kDa polypeptide is immunoprecipitated by MCMV capsid protein antiserum. The similarities between MCMV and carnation mottle and turnip crinkle carmoviruses in their amino acid sequences, genome organization, and gene expression strategies suggest that MCMV is evolutionarily related to the carmoviruses. However, MCMV contains an additional open reading frame, does not produce a second subgenomic RNA, and has no capsid protein amino acid sequence identity with the carmoviruses. Given these distinctions, we propose that MCMV should be considered the type member of a new plant virus group.

The complete nucleotide sequence of the maize chlorotic mottle virus (MCMV) genome has been determined (28). The viral genome contains four open reading frames (ORFs), which are capable of encoding polypeptides of 111, 33, 32, and 25 kDa. The ORF encoding the 111-kDa polypeptide (p111) is punctuated by an in-frame amber terminator generating a pre-read-through ORF encoding a 50-kDa polypeptide (p50). Similarly, the internal ORF encoding the 33-kDa polypeptide (p33) is interrupted by an in-frame opal terminator to yield an ORF encoding a 9-kDa polypeptide (p9) (Fig. 1). Overall, the MCMV genome organization is similar to that of members of the carmovirus group. The 111-kDa polypeptide possesses considerable amino acid sequence similarity with putative RNA polymerases encoded by the carmoviruses carnation mottle virus (CarMV) and turnip crinkle virus (TCV), red clover necrotic mosaic dianthovirus, and several tombusviruses (16,37). In addition, amino acid sequence similarity exists at the carboxy terminus of the MCMV 9-kDa, TCV 8-kDa, and CarMV 7-kDa polypeptides (28). However, unlike the carmoviruses, MCMV contains an additional ORF encoding a 32-kDa polypeptide (p32), which overlaps the pre-read-through portion of the p111 ORF at the 5′ end of the genome (Fig. 1). Also, the MCMV capsid protein is smaller than those of the carmoviruses, dianthoviruses, and tombusviruses, and there is no obvious sequence similarity between MCMV and these other viruses (16,28,37).

Even though the complete genome of MCMV has been cloned and sequenced, the virions and viral gene products have yet to be characterized. In this report we characterize the MCMV virions and present the in vitro translation profile of the genomic RNA and its relationship to the ORFs deduced from the nucleotide sequence. MCMV was tentatively assigned to the sobemovirus group on the basis of physicochemical properties similar to those of members of that group and the ability of beetles to transmit these viruses (13,33). Our results concerning the RNA structure, protein sequence relationships, genome organization, and gene expression strategies show that MCMV is distinct from both the sobemoviruses and the carmoviruses, and we propose that it should be considered the type member of a new plant virus group.

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MATERIALS AND METHODS

Virus and RNA purification and analysis. The MCMV Kansas serotype 1 isolate (34) was propagated and maintained in *Zea mays* (N28Ht) under glasshouse conditions. The virus was purified as described by Lommel et al (22), except that after precipitation in polyethylene glycol 6,000, it was resuspended and maintained in 1 mM Tris HCl, pH 7.0. The RNA was extracted from purified virions with phenol (23). Virion RNA species were separated by formaldehyde-denaturing agarose gel electrophoresis (29). Virus-specific double-stranded RNA (dsRNA) was isolated from maize plants 7 days postinoculation and analyzed by polyacrylamide gel electrophoresis (PAGE) according to Morris and Dodds (26). Virion-associated proteins were analyzed by sodium dodecyl sulfate PAGE (SDS-PAGE) according to Laemmli (20).

In vitro translation and immunoprecipitation analysis. The translation of viral RNAs and the analysis of the translation products by SDS-PAGE were performed essentially as described earlier (36).

m^7G cap and penultimate residue analysis. The 5′ penultimate residue of genomic RNA of MCMV was [^{3}H]-methylated using the (nucleoside-2′-)-methyltransferase activity associated with infectious vaccinia virus (27). After methylation, RNA was digested with nuclease P1, and the products were analyzed together with m^7GpppA^m and m^7GpppG^m standards by ascending paper chromatography. Labeled products were eluted from the paper, further digested with snake venom pyrophosphatase and bacterial alkaline phosphatase, and reanalyzed by ascending paper chromatography with A^m and G^m standards (27). For both cap and penultimate residue analysis, turnip yellow mosaic virus (TyMV) RNA was coprocessed as a (cap) positive control.

cDNA cloning of the MCMV genome. The synthesis and construction of the nearly full-length MCMV cDNA clone pMCM16 was described previously (28). The numerical designations for particular nucleotides used here were assigned according to Nutter et al (28).

RESULTS

Virion properties. Denaturation and SDS-PAGE of purified

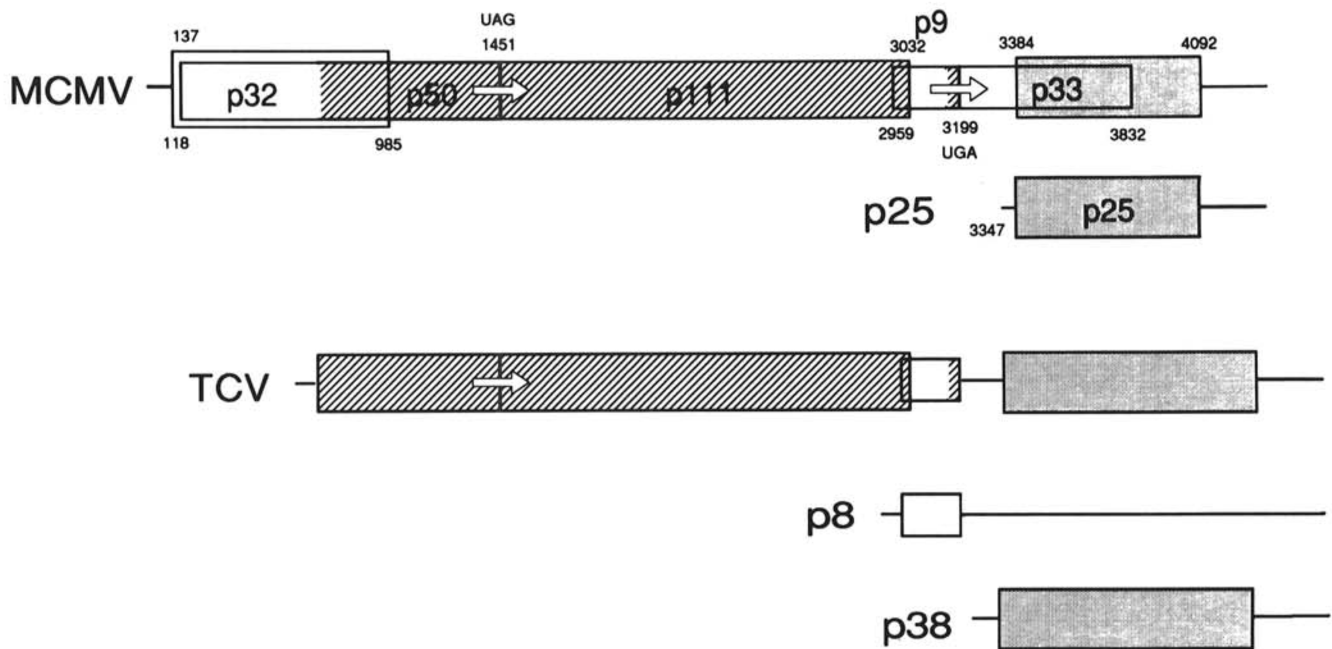

Fig. 1. Organization and expression of the maize chlorotic mottle virus (MCMV) and turnip crinkle carmovirus (TCV) genomes aligned over the amber termination codons, which interrupt the polymerase open reading frame (ORF). The boxes represent ORFs. The numbers above and below the boundaries of a box are the nucleotide numbers of the initiation and termination codons of the ORF. Boxes are labeled according to the size of the polypeptide that the ORF is capable of encoding (e.g., p32 for a 32-kDa polypeptide). The hatched areas represent amino acid sequences in which the two viruses have more than 36% amino acid identity (5,28). The shaded boxes denote capsid protein cistrons. The horizontal arrows identify termination codons that, when suppressed, generate an extended read-through ORF. Identified subgenomic RNAs and their 5′-proximal ORFs are drawn below the complete genome maps of both viruses (5,21).

Fig. 2. Electrophoretic analysis of the maize chlorotic mottle virus (MCMV) capsid protein, RNA from virions, and double-stranded RNA extracted from MCMV-infected maize. **A,** 12% SDS-PAGE gel stained with Coomassie Brilliant Blue R, containing capsid proteins of brome mosaic virus (BMV), 20.3 kDa (1); tobacco mosaic virus (TMV), 17.6 kDa (12); MCMV, estimated to be 25 kDa; and southern bean mosaic virus (SBMV), 28.2 kDa (35). **B,** 1% agarose denaturing gel stained with ethidium bromide, containing viral RNA of SBMV, 4,194 nucleotides (35); MCMV, 4,439 nucleotides; TMV, 6,395 nucleotides (12); and BMV, 3,234, 2,865, 2,114, and 876 nucleotides (1). **C,** 6% polyacrylamide gel stained with ethidium bromide, containing double-stranded RNAs isolated from plants infected with SBMV, MCMV, BMV, and TMV.

MCMV virions yielded a single capsid protein species of 25 kDa (Fig. 2A). Under denaturing conditions, the single MCMV genomic RNA was calculated to be 4.4 kb in length (Fig. 2B). No distinct RNAs smaller than genome size appeared to be present in virion preparations, either when 50 ng was loaded (as illustrated in Fig. 2B) or when the gel was was overloaded with 500 ng (data not shown). Two distinct dsRNA species were isolated from MCMV-infected maize: a large dsRNA (approximately 4.4 kb), presumed to be the genomic RNA replicative form, and a smaller dsRNA (1.1 kb) (Fig. 2C).

The 5′ penultimate purine residue of MCMV RNA was [^{3}H]-methylated and digested with nuclease P1, and the digestion products were separated by ascending paper chromatography. The labeled product migrated with the m^{7}GpppA^m standard (Fig. 3A). Further digestion with snake venom pyrophosphatase and bacterial alkaline phosphatase followed by chromatographic analysis of the labeled cap dinucleotide indicated that the methylated penultimate purine was an A^m (Fig. 3B). The mobility of the cap standard and the MCMV cap correlated well with the mobility of the m^{7}GpppG cap of TyMV genomic RNA and the m^{7}GpppA cap of TyMV capsid protein subgenomic RNA (4,14). These data indicate that the 5′ terminus of MCMV genomic RNA is capped with m^{7}GpppA. Attempts to detect an MCMV genome-linked protein (VPg) by Na[^{125}I]-Iodo-Gen labeling (10) and SDS-PAGE of the labeled samples together with cowpea mosaic virus (32) as a positive control and tobacco mosaic virus (12) as a negative control were unsuccessful. Therefore, MCMV RNA does not have a 5′-terminal-linked VPg.

In vitro translation analysis. Unfractionated MCMV virion RNA directs the synthesis of six distinct polypeptides, with estimated sizes of 110, 50, 44, 41, 32, and 25 kDa, in a rabbit reticulocyte lysate in vitro translation system (Fig. 4). The 110-kDa polypeptide was synthesized in extremely low amounts from virion RNA and was observed only after long (more than 4-day) exposures of the fluorographs. The 44-, 41-, and 25-kDa proteins were the most abundant products; the 32- and 50-kDa polypeptides were produced in lesser amounts. The relative intensities of the products were consistently reproduced in independent translation experiments. The MCMV capsid protein antiserum immunoprecipitated only the 25-kDa polypeptide from total MCMV translation products, establishing it as the capsid protein (Fig. 4).

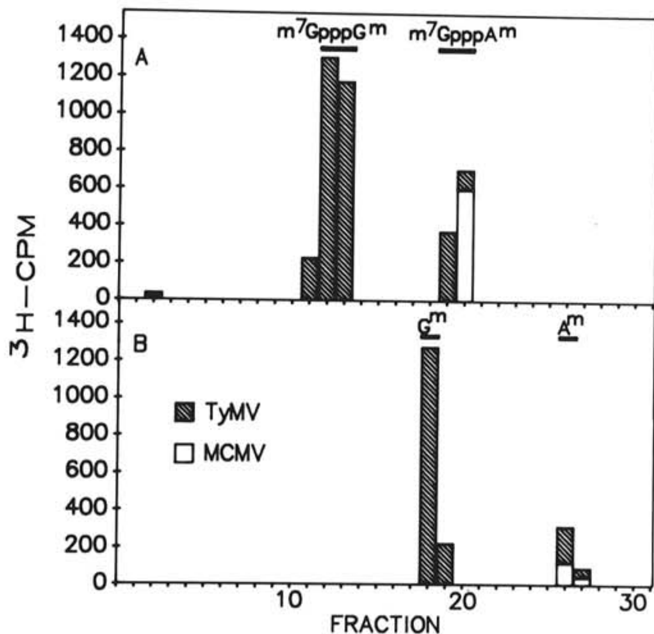

Fig. 3. Identification of the 5′-terminal cap structure and penultimate purine residue of genomic RNA of maize chlorotic mottle virus (MCMV) by ascending paper chromatography of [^{3}H]-methyl-labeled RNA digestion products (diagrammatic representations of the 5′-terminal dinucleotide and penultimate residue chromatographs). **A,** Tritiated RNA from MCMV and turnip yellow mosaic virus (TyMV) was digested with nuclease P1 and subjected to ascending paper chromatography in a mixture of isobutyric acid and 0.5 M NH_4OH (10:6, v/v) on Whatman No. 1 paper. The locations of the cap standards are marked by solid horizontal lines. **B,** The tritiated dinucleotide was eluted from the paper and further digested with snake venom pyrophosphatase and bacterial alkaline phosphatase and chromatographed in a mixture of isopropanol, H_2O, and concentrated NH_4OH (7:2:1). The locations of the methylated purine residue standards are marked by solid horizontal lines.

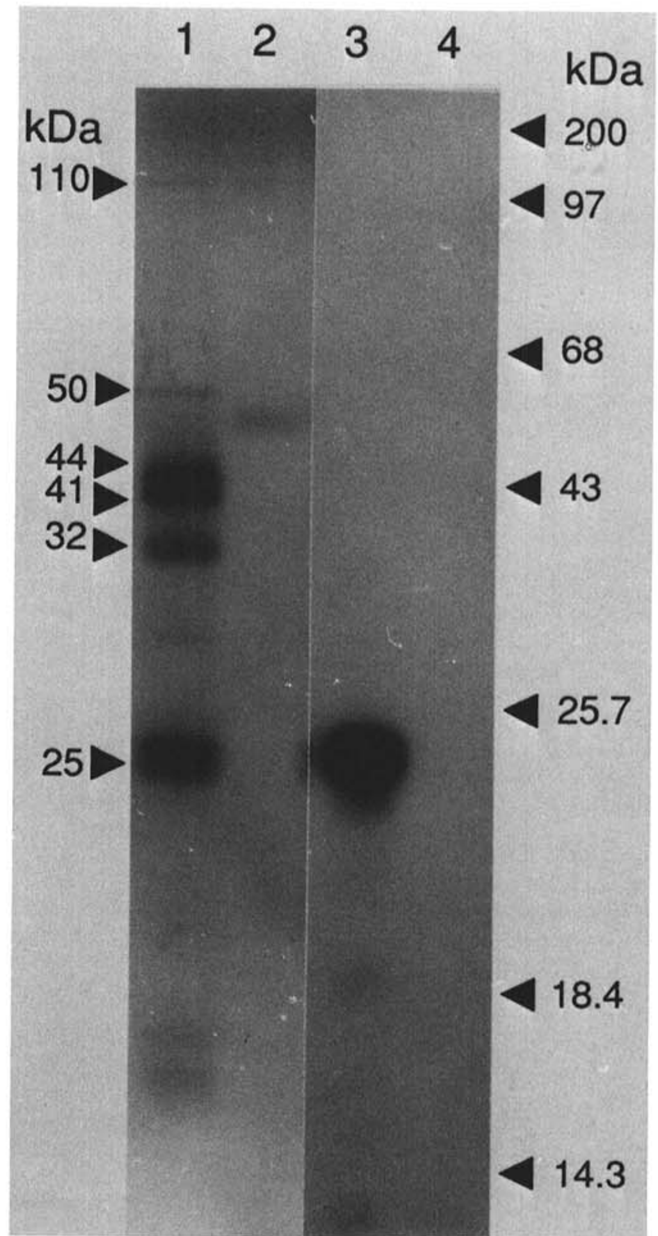

Fig. 4. Immunoprecipitation and 12.5% SDS-PAGE analysis of RNA-directed in vitro translation products of maize chlorotic mottle virus (MCMV). Fluorograph of [^{35}S]-methionine-labeled translation products separated by 12.5% SDS-PAGE, directed by MCMV RNA (lane 1) or by no exogenous RNA (lane 2); MCMV translation products immunoprecipitated with MCMV capsid protein antisera (lane 3); and MCMV translation products immunoprecipitated with preimmune antisera (lane 4). The sizes (in kilodaltons) of the major MCMV translation products are marked on the left. The mobility of the [^{14}C]-labeled protein molecular weight markers used to estimate the size of the MCMV-directed products are marked on the right: myosin, H-chain (200 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), α-chymotrypsinogen (25.7 kDa), β-lactoglobulin (18.4 kDa), and lysozyme (14.3 kDa).

DISCUSSION

MCMV is physicochemically similar to a number of icosahedral plant viruses 25–32 nm in diameter with a single-stranded RNA genome (9). The genomic RNA, and presumably small quantities of subgenomic RNA (21), are packaged into 30-nm icosahedral virions together with multiple copies of a 25-kDa polypeptide. The genome is composed of a 4.4-kb positive-polarity, single-stranded RNA that has a 5′-terminal m^7GpppA cap, instead of the more typical m^7G cap linked to a penultimate guanine residue (9).

Morris (25) suggested that the detection of virus-specific dsRNA smaller than genome length indicates the in vivo synthesis of subgenomic messenger RNA for the expression of internal cistrons. Consequently, we predicted that the dsRNA smaller than genome length detected in MCMV-infected maize plants represents a subgenomic RNA synthesized in vivo. A single 1.1-kb virus-specific RNA was detected by northern hybridization using MCMV cDNA probes (21). The subgenomic RNA was mapped to the 3′-proximal ORF, and high translational activity for capsid protein was observed from the subgenomic RNA species (21). Together, these data suggest that capsid protein is synthesized in vivo from a subgenomic RNA (Fig. 1).

In vitro translation of MCMV genomic RNA resulted in the synthesis of a complex profile of proteins relative to those of the sobemoviruses (31) and carmoviruses (6). Several of the products correlate in size with the putative products of ORFs deduced from the nucleic acid sequence (28), but others do not. The 50- and 110-kDa products are most likely encoded by the p50 and p111 read-through ORF. The reduced level of 110-kDa protein synthesis relative to the accumulation of the 50-kDa polypeptide is also consistent with amber termination codon read-through, as has been described for tobacco mosaic virus (2,3). The 41- and 44-kDa products observed in vitro do not correlate with any of the predicted ORFs. If each polypeptide were unrelated, the coding capacity of the MCMV genome would be insufficient to encode all of the observed products. Correlating the observed products with the deduced ORFs allows us to conclude that some of the smaller noncapsid protein products are fragments of the larger products. These polypeptides may result in vitro by premature termination or internal initiation within the p50 or the p111 ORF. The observed 32-kDa product is translated either from the 5′-most p32 ORF or from the potential internal p33 ORF, which arises by suppression of the p9 ORF termination codon (Fig. 1). However, protein synthesis that results from opal termination codon read-through would be expected to occur only relatively infrequently, if at all (2). No 9-kDa protein was detected in the MCMV RNA translation profile. In vitro synthesis of 9-kDa polypeptide could not be detected in high-percentage SDS-PAGE gels designed to resolve low-molecular-weight proteins (data not shown).

By correlating the MCMV genome map (Fig. 1) with the in vitro translation data (Fig. 4), we can make several predictions about the various gene expression mechanisms utilized by MCMV. The 5′-proximal p32 ORF is most likely translated directly from genome-length RNA. According to the ribosome scanning model (19), it would be the first ORF encountered. As suggested by Nutter et al (28), the p32 ORF initiation sequence is not in an optimal context for translation initiation. Consequently, a small percentage of the attached ribosomes should continue to scan past the p32 ORF initiator and initiate translation at the second AUG codon (p50-p111 ORF initiator), which is in a more favorable sequence context. Therefore, the 50-kDa product would also be expected to be translated directly from full-length viral RNA. The 110-kDa protein would arise by read-through of the in-frame amber termination codon at position 1451 (Fig. 1). Amber codon suppression has also been implicated in the expression of the RNA polymerase genes in the carmoviruses (5,15) and tombusviruses (16,30).

How the internal p9 ORF and the potential opal terminator read-through p33 ORF are expressed in vivo is not clear. The MCMV p9 ORF contains carboxy-terminal amino acid sequence identity with similarly located small ORFs from the carmoviruses TCV (Fig. 1) and CarMV. For the carmoviruses these small ORFs are expressed from a second subgenomic RNA (7,8). We could not detect a second subgenomic RNA with the potential to express the p9 ORF by northern blot analysis (23), and no dsRNA species indicative of a second subgenomic RNA was detected (Fig. 2C).

Other than the additional 5′-terminal p32 ORF and the lack of capsid protein amino acid sequence similarity, the genome organization of MCMV is very similar to that of the carmoviruses. MCMV appears to be an example of a plant virus that has been compiled by "modular evolution" (38) from various modules shared by the carmoviruses in one or more recombination events. In Figure 1, the MCMV and TCV genomes are aligned at the amber terminator read-through site and the highly conserved domain of the RNA polymerase, which contains the GDD sequence motif (15). As illustrated in Figure 1, the 5′-terminal 1 kb of MCMV, which contains the p32 ORF (module), with no counterpart in the genome of TCV, is fused to the conserved polymerase cistron. Presumably the 32-kDa gene product serves a unique function.

The number and sizes of the MCMV capsid protein, encapsidated RNAs, and dsRNAs are within the ranges typical of members of the sobemovirus group (13,17). Like southern bean mosaic virus (SBMV) (11,35), MCMV produces a 3′-coterminal subgenomic RNA that encodes the capsid protein. These properties, as well as the fact that MCMV can be transmitted by beetles, have led to consideration of MCMV as a member of the sobemovirus group (13). However, it remains uncertain if beetles are the natural vector (18). In greater contrast, MCMV possesses other properties quite unlike those of the sobemoviruses. For example, MCMV RNA does not have a 5′-terminal, covalently linked VPg, but rather is capped with an m^7GpppA (Fig. 3) (24). The size and number of the MCMV-directed translation products are distinct from those of SBMV. MCMV has a different genome organization and utilizes a different gene expression strategy for the nonstructural proteins, and no amino acid sequence similarity is apparent between the two viruses (35). Given these rather profound differences, we propose that MCMV should not be considered a sobemovirus or a carmovirus but rather the type member of a new plant virus group.

LITERATURE CITED

1. Ahlquist, P., Luckow, V., and Kaesberg, P. 1981. Complete nucleotide sequence of brome mosaic virus RNA3. J. Mol. Biol. 153:23-28.
2. Beier, H., Barciszewska, M., Krupp, G., Mitnacht, R., and Gross, H. J. 1984. UAG readthrough during TMV RNA translation: Isolation and sequence of two $tRNAs^{Tyr}$ with suppressor activity from tobacco plants. EMBO J. 3:351-356.
3. Beier, H., Barciszewska, M., and Sickinger, H.-D. 1984. The molecular basis for the differential translation of TMV RNA in tobacco protoplasts and wheat germ extracts. EMBO J. 3:1091-1096.
4. Briand, J.-P., Keith, G., and Guilley, H. 1978. Nucleotide sequence at the 5′ extremity of turnip yellow mosaic virus genome RNA. Proc. Natl. Acad. Sci. USA 75:3168-3172.
5. Carrington, J. C., Heaton, L. A., Zuidema, D., Hillman, B. I., and Morris, T. J. 1989. The genome structure of turnip crinkle virus. Virology 170:219-226.
6. Carrington, J. C., and Morris, T. J. 1985. Characterization of the cell-free translation products of carnation mottle virus genomic and subgenomic RNAs. Virology 144:1-10.
7. Carrington, J. C., and Morris, T. J. 1986. High resolution mapping of carnation mottle virus-associated RNAs. Virology 150:196-206.
8. Carrington, J. C., Morris, T. J., Stockley, P. G., and Harrison, S. C. 1987. Structure and assembly of turnip crinkle virus. IV. Analysis of the coat protein gene and implications of the subunit primary structure. J. Mol. Biol. 194:265-276.
9. Davies, J. W., and Hull, R. 1982. Genome expression of plant positive-stranded RNA viruses. J. Gen. Virol. 61:1-14.
10. Fraker, P. J., and Speck, J. C. 1978. Protein and cell membrane iodinations with sparingly soluble chloramide, 1,3,4,6-tetrachloro-2,6-diphenylglycouril. Biochem. Biophys. Res. Commun. 80:849-857.
11. Ghosh, A., Rutgers, T., Mang, K.-Q., and Kaesberg, P. 1981. Characterization of the coat protein mRNA of southern bean mosaic virus and its relationship to the genomic RNA. J. Virol. 39:87-92.

12. Goelet, P., Lomonossoff, G. P., Butler, P. J. G., Akam, M. E., Gait, M. J., and Karn, J. 1982. Nucleotide sequence of tobacco mosaic virus RNA. Proc. Natl. Acad. Sci. USA 79:5818-5822.
13. Gordon, D. T., Bradfute, O. E., Gingery, R. E., Nault, L. R., and Uyemoto, I. K. 1984. Maize chlorotic mottle virus. Descriptions of Plant Viruses, No. 284. Commonwealth Mycological Institute and Association of Applied Biologists, Kew, Surrey, England.
14. Guilley, H., and Briand, J. P. 1978. Nucleotide sequence of turnip yellow mosaic virus coat protein mRNA. Cell 15:113-122.
15. Guilley, H., Carrington, J. C., Balazs, E., Jonard, G., Richards, K., and Morris, T. J. 1985. Nucleotide sequence and genome organization of carnation mottle virus. Nucleic Acids Res. 13:6663-6677.
16. Hearne, P. Q., Knorr, D. A., Hillman, B. I., and Morris, T. J. 1990. The complete genome structure and synthesis of infectious RNA from clones of tomato bushy stunt virus. Virology 177:141-151.
17. Hull, R. 1988. The sobemovirus group. Pages 113-146 in: The Plant Viruses, Vol. 3. R. Koenig, ed. Plenum, New York.
18. Jensen, S. G. 1985. Laboratory transmission of maize chlorotic mottle virus by three species of corn rootworms. Plant Dis. 69:864-868.
19. Kozak, M. 1986. Regulation of protein synthesis in virus-infected animal cells. Adv. Virus Res. 31:229-292.
20. Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227:680-685.
21. Lommel, S. A., Kendall, T. L., Xiong, Z., and Nutter, R. C. 1991. Identification of the maize chlorotic mottle virus capsid protein cistron and characterization of its subgenomic messenger RNA. Virology 181:382-385.
22. Lommel, S. A., McCain, A. H., and Morris, T. J. 1982. Evaluation of indirect enzyme-linked immunosorbent assay for the detection of plant viruses. Phytopathology 72:1018-1022.
23. Lommel, S. A., Morris, T. J., and Pinnock, D. E. 1985. Characterization of nucleic acids associated with Arkansas bee virus. Intervirology 23:199-207.
24. Mang, K., Ghosh, A., and Kaesberg, P. 1982. A comparative study of the cowpea and bean strains of southern bean mosaic virus. Virology 116:264-274.
25. Morris, T. J. 1983. Double stranded RNA: Functional role in virus infection. Pages 80-83 in: Plant Infectious Agents. H. E. Robertson et al, eds. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
26. Morris, T. J., and Dodds, J. A. 1979. Isolation and analysis of double-stranded RNA from virus-infected plant and fungal tissue. Phytopathology 69:854-858.
27. Moss, B. 1977. Utilization of guanyltransferase and methyltransferases of vaccinia virus to modify and identify the 5′-terminals of heterologous RNA species. Biochem. Biophys. Res. Commun. 74:374-383.
28. Nutter, R. C., Sheets, K., Panganiban, L. C., and Lommel, S. A. 1989. The complete nucleotide sequence of the maize chlorotic mottle virus genome. Nucleic Acids Res. 17:3163-3177.
29. Rave, N., Crkvenjakou, R., and Boedtker, H. 1979. Identification of procollagen mRNAs transferred to diazobenzyloxymethyl paper from formaldehyde agarose gels. Nucleic Acids Res. 6:3559-3567.
30. Rochon, D. A., and Tremaine, J. H. 1989. Complete nucleotide sequence of the cucumber necrosis virus genome. Virology 169:251-259.
31. Salerno-Rife, T., Rutgers, T., and Kaesberg, P. 1980. Translation of southern bean mosaic virus RNA in wheat embryo and rabbit reticulocyte extracts. J. Virol. 34:51-58.
32. Stanley, J., Rottier, P., Davies, J. W., Zabel, P., and Van Kammen, A. 1978. A protein linked to the 5′ termini of both components of the cowpea mosaic virus genome. Nucleic Acids Res. 5:4505-4522.
33. Tremaine, J. H., and Hamilton, R. I. 1983. Southern bean mosaic virus. Descriptions of Plant Viruses, No. 274. Commonwealth Mycological Institute and Association of Applied Biologists, Kew, Surrey, England.
34. Uyemoto, J. K., Bockelman, D. L., and Claflin, L. E. 1980. Severe outbreak of corn lethal necrosis disease in Kansas. Plant Dis. 64:99-100.
35. Wu, S., Rinehart, C. A., and Kaesberg, P. 1987. Sequence and organization of southern bean mosaic virus genomic RNA. Virology 161:73-80.
36. Xiong, Z., Hiebert, E., and Purcifull, D. E. 1988. Characterization of the peanut mottle virus genome by in vitro translation. Phytopathology 78:1128-1134.
37. Xiong, Z., and Lommel, S. A. 1989. The complete nucleotide sequence and genome organization of red clover necrotic mosaic virus RNA-1. Virology 171:543-554.
38. Zimmern, D. 1988. Evolution of RNA viruses. Pages 212-240 in: RNA Genetics, Vol. 2. E. Domingo, J. J. Holland, and P. Ahlquist, eds. CRC Press, Boca Raton, FL.