Characterization of the Peanut Mottle Virus Genome by in Vitro Translation

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ABSTRACT

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Peanut mottle virus (PMoV) RNA was translated in the rabbit reticulocyte lysate (RRL) and wheat germ systems. The translation products were analyzed by immunoprecipitation with antisera to several potyviral proteins. Comparisons were made between the RRL products translated in the absence and presence of dithiothreitol (DTT) and between the translation products formed in the absence of DTT subjected to subsequent incubation in the absence and presence of DTT. Proteolytic processing of the in vitro translation products was apparent. In the absence of DTT, PMoV RNA translation in RRL produced M_r 84,000 (84K) and 210–250K polyproteins, which were subsequently cleaved into smaller proteins during incubation with DTT. Based on the analysis of the in vitro translation products by antisera to potyviral proteins and by the precursor-product relationships observed during manipulation of the proteolytic processing, a PMoV genomic map was proposed from 5′ to 3′ termini as follows: 34K unknown protein, 50K helper component protein, 42K unknown protein, 68K cylindrical inclusion protein, 50K nuclear inclusion protein, 53K nuclear inclusion protein, and 34K coat protein.

The translational strategy and the genome structure of a number of potyviruses have been studied extensively by the in vitro translation of potyviral RNA in cell-free systems. These studies have made it possible to construct a potyviral genome model (8,10–14,22,25,29). One report involving the in vitro translational analysis of the papaya ringspot virus genome (29) presented evidence that a polyprotein, translated from the viral RNA, was subsequently cleaved. Sequence data for the genomes of tobacco etch virus (TEV) and tobacco vein mottle virus (TVM) provide evidence for the synthesis and processing of a polyprotein in potyviral genomic expression (2,3,9).

Peanut mottle virus (PMoV) was studied by in vitro translations for comparative purposes with other potyviruses and to better understand its genomic organization and the translational strategy. PMoV is a typical member of the potyvirus group. It is a flexuous, filamentous virus approximately 740–750 nm long. PMoV consists of a single-stranded, positive-sense RNA of 3.1×10^6 daltons (21). The estimated molecular weight (M_r) of the coat protein (CP) is 34,000 (34K) (21). Infection induces cylindrical inclusions (CI) in the cytoplasm (6,15,23) and nuclear inclusions (NI) (28) in the nuclei of the infected cells. The CI consist of a 68K protein (26–28). Although the molecular weights of the PMoV NI proteins have not been determined, they are serologically related to tobacco etch virus (TEV) NI proteins (26). In this report, further evidence of proteolytic processing involved in the expression of the potyviral genome in the cell-free system is presented. Polyproteins, viral-specific proteins, and intermediate products in the proteolytic processing were analyzed by various potyviral protein antisera. A genetic map for the PMoV genome is proposed.

MATERIALS AND METHODS

Virus source and purification. The PMoV isolate used in this study was obtained from J. W. Demski of the Georgia Agricultural

Experimental Station, Experiment, GA. The virus was maintained in *Pisum sativum* L. 'Little Marvel' in a greenhouse.

Virus purification. Pea tissue was harvested 14–20 days after inoculation. Leaf tissue (100 g) was homogenized in 200 ml of 0.5 M potassium phosphate, pH 8.0, containing 20 mM EDTA and 20 mM sodium sulfite, with a Waring blender for 1 min. The sap was emulsified with 50 ml of $CHCl_3$ and 50 ml of CCl_4, followed by centrifugation at 1,000 *g* for 5 min. The supernatant was filtered through two layers of cheesecloth and centrifuged at 12,000 *g* for 15 min. The pellet was saved for the purification of PMoV CI (26). Polyethylene glycol (PEG, Mr 8,000), NaCl, and Triton X-100 were added to the supernatant to final concentrations of 4% (w/v), 0.1 M, and 1% (v/v), respectively. After stirring for 1 hr at 4 C, the virus was collected by centrifugation at 10,000 *g* for 10 min. The pellet was resuspended in 1/10 of the original volume of 50 mM potassium phosphate, pH 8.0, 20 mM EDTA, and 20 mM sodium sulfite. The resuspended pellet was stirred at 4 C for 20 min and clarified at 10,000 *g* for 10 min. NaCl and PEG were then added to the supernatant to final concentrations of 0.1 M and 6% (w/v). The mixture was stirred at 4 C for 1 hr before being subjected to centrifugation at 10,000 *g* for 10 min. The final pellet was resuspended in a small volume of buffer (50 mM potassium phosphate, pH 8.0, containing 20 mM EDTA and 20 mM sodium sulfite). The partially purified viral preparation was further purified by density gradient centrifugation in cesium sulfate (10 g of cesium sulfate dissolved in 27 ml of the resuspension buffer) at 32,000 rpm in a Beckman SW 41 rotor or 36,000 rpm in a SW 50.1 rotor for 16 hr. The virus band was collected and the virus was precipitated by 8% PEG. The virus pellet was resuspended in 10 mM Tris buffer, pH 8.2.

RNA purification. Freshly purified virus preparations were dissociated by incubation in RNA dissociation buffer (0.2 M Tris, 2 mM EDTA, 2% sodium dodecyl sulfate [SDS], pH 9.0) for 2 min at 60 C. The preparation was quick cooled in an ice-water bath, and the RNA was isolated by linear-log, sucrose density gradient centrifugation as described elsewhere (11).

The integrity of the purified PMoV RNA was tested by formamide-denaturation before a second sucrose density gradient centrifugation. The RNA preparation was denatured at 85 C for 3 min with 50% resin bead-deionized formamide in 10 mM sodium phosphate buffer, pH 7.0. The denatured RNA preparation was diluted with 7 volumes of 0.1 M phosphate buffer, pH 7.0, and quickly cooled in an ice-water bath. The RNA preparation was then layered onto a linear-log sucrose gradient (4,5) and centrifuged in a SW 41 rotor at 32,000 rpm for 10 hr.

In vitro translation. Translation of PMoV RNA in rabbit reticulocyte lysate was performed as described by Dougherty and Hiebert (10,11) unless described otherwise. The rabbit reticulocyte lysate (RRL) was obtained from Green Hectares, Oregon, WI. The wheat germ system (WG) (a gift from W. G. Dougherty, North Carolina State University, Raleigh) was prepared as described by Marco and Dudock (18). The reaction for the WG system was carried out as described by Salerno-Rife et al (22). Up to 7 μg and 20 μg of RNA were used in 100 μl of RRL and WG mixtures, respectively. The reaction mixtures were incubated at 30 C for 90 min, and the reactions were terminated by adding 3 volumes of Laemmli dissociation buffer (62.5 mM Tris, pH 6.8, 2% SDS [w/v], 4% [v/v] 2-mercapthoethanol, and 10% [v/v] glycerol).

Analysis of the translation products. The immunoprecipitations by different potyviral protein antisera and the analysis by 7.5–15% sodium dodecyl sulfate-polyacrylamide gradient gel electrophoresis (SDS-PAGE) were carried out as described by Hiebert and Purcifull (16). Fifteen microliters of dissociated lysate was incubated with 9 μl of antiserum and 30 μl of NET buffer (150 mM NaCl, 5 mM EDTA, 50 mM Tris, pH 7.4) containing 0.05% Nonidet P-40 (NP-40; Sigma N6507), 1 mg/ml of ovalbumin, and 2 mM methionine. The mixture was incubated for 1 hr at room temperature. Fifteen microliters of *Staphylococcus aureus* strain Cowan solution (10%, v/v) was added to the mixture and further incubated for 30 min at room temperature. The immunoprecipitated proteins were collected by centrifugation and washed three times with NET buffer containing 0.05% NP-40. The final pellets were resuspended in the Laemmli dissociation buffer, heated for 2 min, and loaded onto a 7.5–15% SDS polyacrylamide gradient gel. The antisera used were to PMoV CP (26), PMoV CI (26), TEV 49K NI protein (11), TEV 54K NI protein (11), tobacco vein mottling virus helper component (TVMV HC) (17,24), and papaya ringspot virus type W (PRSV) amorphous inclusion (AI) protein (7). All the antisera with the exception of TVMV HC (supplied by D. Thornbury, KY) were prepared in our laboratory. The protein bands were visualized by fluorography.

RESULTS

RNA isolation. Purified virus preparations, dissociated at pH 9.0, revealed sharp, nearly symmetrical RNA peaks (39S) after linear-log sucrose density gradient centrifugation. When purified RNA preparations were denatured by formamide and analyzed by sedimentation in linear-log sucrose density gradients, PMoV RNA sedimented as a single, sharp peak with a small, slower sedimentating shoulder.

Analysis of the in vitro translation products. The in vitro translation of PMoV RNA in RRL and WG systems stimulated incorporation of ^{35}S-methionine into proteins 10–20-fold and 30–50-fold higher than the endogenous controls, respectively. The major translation products in the RRL ranged in size from 22K to 110K (Fig. 1). The major translation products in the WG ranged in size from 15K to 90K (Fig. 2).

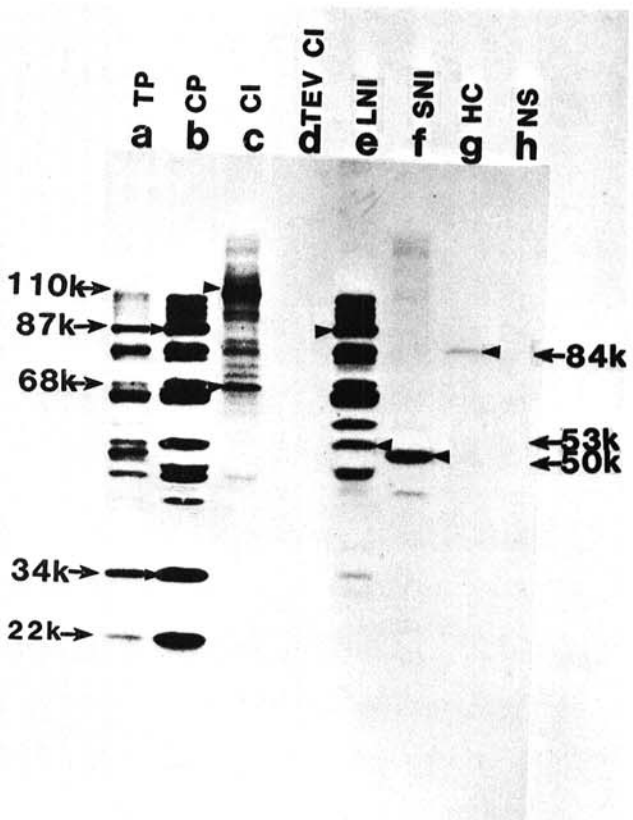

Fig. 1. Analysis of the in vitro translation products of peanut mottle virus RNA in the rabbit reticulocyte lysate (RRL) system with antisera to potyviral proteins. Products were separated on a sodium dodecyl sulfate gradient polyacrylamide gel (7.5–15%) and detected by fluorography. ^{14}C-labeled molecular weight markers (not shown) used to estimate molecular weights of the products were myosin 200,000 (200K), phosphorylase (93K), bovine serum albumin (67K), ovalbumin (46K), and carbonic anhydrase (29K). Lane a) total products (TP). Products in other lanes were precipitated by antisera to: b) peanut mottle virus (PMoV) coat protein (CP); c) PMoV cylindrical inclusion (CI) protein; d) tobacco etch virus (TEV) CI protein; e) TEV 54K nuclear (large) inclusion (LNI) protein; f) TEV 49K (small) NI(SNI) protein; and g) tobacco vein mottling virus helper component (HC) protein. Normal serum (NS) did not precipitate any protein (lane h). Some important proteins are marked with arrows and their molecular weights are indicated at the sides of the figure.

The translation products were analyzed by immunoprecipitations and SDS-PAGE (Figs. 1 and 2). Many of the translation products cross-reacted with two or more antisera in our immunoprecipitation analyses. Some of these products were similar in size to that predicted by the sum of the sizes of the viral-specified proteins. For example, antiserum to PMoV CP precipitated a number of translation products, including an 87K polyprotein and a 34K protein. The 34K protein was presumably the PMoV CP (Fig. 1, lane b). The 87K product also reacted with the antiserum to TEV 54K NI protein (Fig. 1, lane e) and presumably consists of a NI-CP polyprotein. Other products, some reactive with only one serum, i.e., the 22K protein in Figure 1, lane b, and others cross-reactive with more than one antiserum, i.e., two products greater than 87K in Figure 1 lanes b and e, did not correspond to predicted sizes of polyproteins made up of known viral-specific proteins. These products may be due to premature translation terminations and/or to degradation by nonspecific proteolysis. Antiserum to 54K NI protein precipitated a spectrum of proteins larger than 50K, which were similar in size to those precipitated by the CP antiserum except for a unique 53K protein (presumably a PMoV protein equivalent to the large TEV 54K NI protein). The possibility that the 87K polyprotein consisted of the 34K CP and 53K NI protein was tested in a sequential immunoprecipitation experiment. Cross absorption with either PMoV CP antiserum or TEV 54K NI protein antiserum before SDS-PAGE analysis eliminated the 87K protein band (see below). Antiserum to PMoV CI protein precipitated two predominant products of 110K and 68K (Fig. 1, lane c). The smaller product may correspond to the PMoV 68K CI protein (26,27). Antiserum to the small TEV 49K NI protein precipitated a 50K protein (Fig. 1, lane f). Antiserum to the 53K TVMV HC protein precipitated an 84K product (Fig. 1, lane g). Antiserum to PRSV amorphous inclusion protein, which is serologically related to TVMV HC protein (7,8), precipitated the 84K product more efficiently (data not shown).

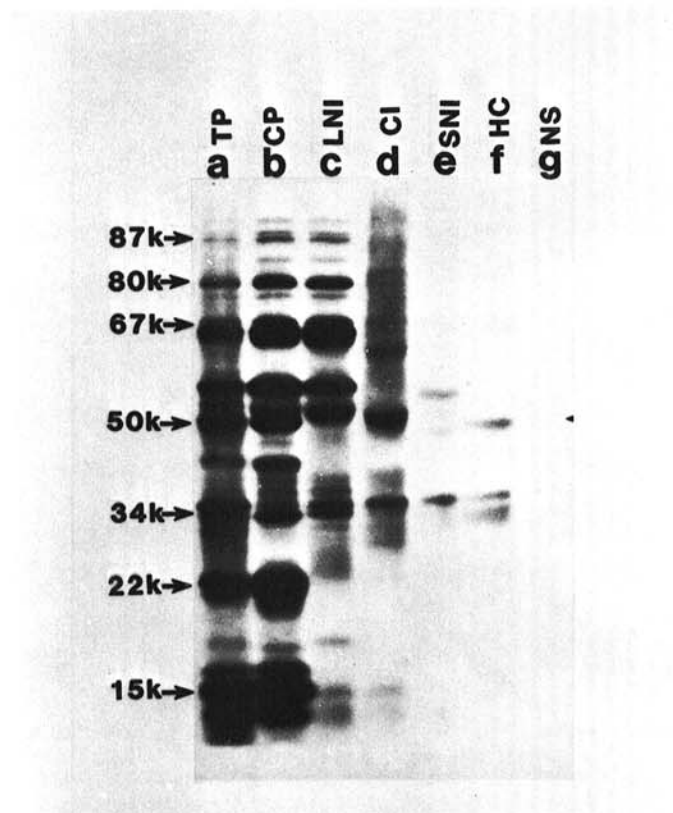

Fig. 2. Analysis of the in vitro translation products of peanut mottle virus RNA in the wheat germ system with antisera to potyviral proteins. Products were separated on a sodium dodecyl sulfate polyacrylamide gel gradient (7.5–15%) and detected by fluorography. ^{14}C-labeled molecular weight markers used were as described in Fig. 1. Lane a, total products. Products in other lanes were precipitated by antisera to: b) peanut mottle virus (PMoV) coat protein; c) tobacco etch virus (TEV) 54K nuclear inclusion protein; d) PMoV cylindrical inclusion protein; e) TEV 49K nulcear inclusion protein; and f) tobacco vein mottling virus helper component protein. Normal serum did not precipitate any protein (lane g).

Immunoprecipitation of the in vitro translation products from the WG system (Fig. 2) displayed many products reactive with antisera to CP and the 54K TEV NI protein and few products reactive with antisera to TVMV HC, 49K TEV NI protein, and CI. Antiserum to TVMV HC protein (Fig. 2, lane f) precipitated a product of 50K instead of the 84K polypeptide precipitated from the RRL system (Fig. 1, lane g).

Detection and analysis of proteins with high molecular weight. Dithiothreitol (DTT), a reducing agent and a normal component of the RRL system, is reported to promote the proteolytic cleavage of cowpea mosaic virus RNA translation products (20) and of PRSV RNA translation products (29). In the absence of DTT in the RRL system, a protein approximately 330K in size was detected from PRSV RNA translation products. Experiments were conducted to test the possible effect of DTT on the protein processing of PMoV RNA translation products. The reaction mixture was made as described before (10) except that no DTT was added. The reaction mixture was then divided into three aliquots. DTT was added to one aliquot as a control. After incubation at 30 C for 90 min, the reaction in the aliquot with DTT was terminated by addition of 3 volumes of Laemmli dissociation buffer. The reactions in the other two aliquots were terminated by adding RNase A to a final concentration of 0.5 mg/ml. To one of these aliquots, DTT was added to a final concentration of 4.8 mM. Both aliquots, one with added DTT and the other without, were further incubated at 30 C for 2 hr. The incubation was terminated by adding 3 volumes of Laemmli dissociation buffer. The translational products in all three aliquots were subjected to analysis as before.

Proteins of 210–250K were detected by SDS-PAGE only from the translations without DTT in the translation reaction and during subsequent incubation (Fig. 3, lanes b–h). The aliquots with DTT present either in the course of translation (Fig. 1) or during further incubation after translation without DTT yielded the same translation product profile (Fig. 3, lanes i–o) with no apparent high molecular weight protein. The 210–250K protein was precipitated by antisera to PMoV CP, PMoV CI protein, TEV 54 NI protein, and TEV 49K NI protein (Fig. 3, lanes c–f). A protein with a M_r of 137K was precipitated by antisera to PMoV CP, TEV 54K NI protein, and TEV 49K NI protein (Fig. 3, lanes c, d, and f), but not by antiserum to PMoV CI protein (Fig. 3, lane e). Antisera to TVMV HC protein (Fig. 3, land g) and to PRSV AI protein did not precipitate any of the high molecular weight proteins. It was apparent from these data that the 210–250K polyprotein was the precursor for the 34K CP, 53K NI, 50K NI, and 68K CI proteins, and that the 137K polyprotein was the precursor for 34K CP, 53K NI, and 50K NI proteins. This presumption was tested by preabsorption experiments described below. When the reaction mixture was cross-absorbed with any one of the four antisera, the 210–250K protein disappeared. When the reaction mixture was cross-absorbed with any of the CP and NI protein antisera, the 137K protein was not observed. These results indicated that the 210K and 137K polyproteins contained and were the precursor of the four (CI, 50K NI, 53K NI, and CP) and the three (50K NI, 53K NI, and CP) proteins, respectively.

Proteolytic processing of the translation products. Incubation with DTT abolished most of the high molecular weight proteins synthesized in the absence of DTT (Fig. 3). When abundant high molecular weight proteins were present, very small amounts of the viral-specified proteins of 34K (CP) (lane c), 53K (NI) (lane d), 68K (CI) (lane e), 50K (NI) (lane f) were observed. When the high molecular weight proteins disappeared upon further incubation with DTT, the viral-specified proteins increased significantly (Fig. 3, lanes i–o).

Sequential immunoprecipitation analysis of the polyproteins. To determine the homology and the precursor-product relationship of different in vitro translation products, sequential immunoprecipitations of the translation products with different

antisera were performed. The in vitro translation products were allowed to react first with one antiserum and then with *Staphylococcus aureus* cells. After centrifugation at ~3,000 *g* to remove any immunoprecipitated materials, the supernatant of the reaction mixture was mixed with a different antiserum. The supernatant of the second precipitation was sometimes subjected to another precipitation by adding a third antiserum. Absorptions with antiserum to CI protein and to TEV 54K and 49K NI proteins did not affect those M_r 47K or smaller proteins that were precipitated by antiserum to PMoV CP (Fig. 4, lanes c and f). The proteins of M_r larger than 47K were eliminated or reduced greatly. Absorption with PMoV CP antiserum nearly eliminated all proteins precipitated by antiserum to TEV 54K NI protein except for a few faint bands with M_r 67K, 53K, and 45K (Fig. 4, lanes i, m). Absorption with antiserum to PMoV CI and TEV 49K NI protein did not affect the proteins with M_r less than 100K that were precipitated by PMoV CP antiserum (Fig. 4, lane d). The proteins of M_r 140K or less precipitated by antiserum to PMoV CP remained unchanged after the absorption with antiserum to PMoV CI protein (Fig. 4, lane g), but the proteins with M_r of 210–250K were eliminated. Antiserum to PMoV CI precipitated 68K and 50K M_r proteins and reduced amounts of higher M_r products from the translation products previously absorbed by antiserum to 49K NI protein (Fig. 5, lane c), and by antisera to 54K NI protein and CP (Fig. 5, lane d). Antiserum to TEV 49K NI protein precipitated a faint band around 50K from the supernatant previously immunoprecipitated with antisera to CP and 54K NI (Fig. 5, lane h). The 100K protein precipitated by 49K NI antisera was eliminated by prior absorption with antisera to CP and 54K NI protein, but not by antiserum to CI protein (Fig. 5, lane k).

DISCUSSION

In this study, five viral-proteins translated from PMoV RNA were identified serologically. The 34K CP, 53K NI protein, 50K NI protein, 68K CI protein, and 50K HC protein account for approximately 80% of the estimated coding capacity of the RNA. The remainder was assigned to two unknown proteins of 34K and 42K, according to their intermediate product linkage to HC and CI proteins, respectively. The 34K unknown protein was mapped to the 5′ end of the genome on the basis of the wheat germ translation analysis (26). The 42K unknown protein was mapped next and 5′ to the CI coding region on the basis of the 110K product, which was serologically related only to the 68K CI protein. Antisera to CP, TEV 54K, and 49K NI proteins, and CI protein immunoprecipitated a common 210–250K product. This indicated that these products

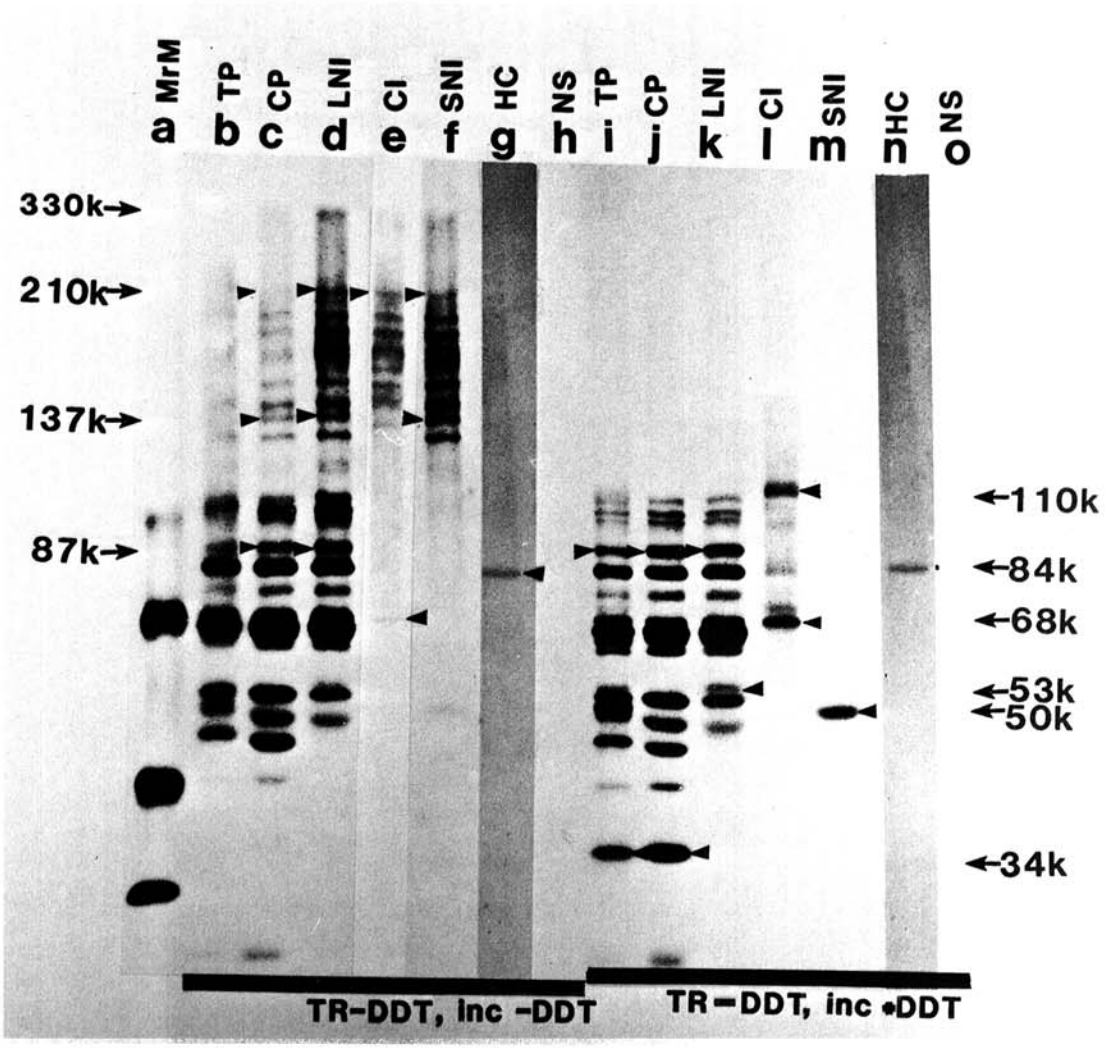

Fig. 3. Immunoprecipitation analysis of the in vitro products of peanut mottle virus (PMoV) RNA translated in the rabbit reticulocyte lysate (RRL) system in the absence of dithiothreitol (TR-DTT) and incubated for 2 hr without DTT (inc. −DTT) (lanes b–h) or with DTT (inc. +DDT) (lanes i–o). Products were separated on a sodium dodecyl sulfate polyacrylamide gel gradient (5–15%) and detected by fluorography. Lane a, ^{14}C-labeled molecular weight markers (MrM) as described in Fig. 1; lanes b and i, total products. Products were precipitated with antisera to: c and j, peanut mottle virus (PMoV) coat protein; d and k, tobacco etch virus (TEV) 54K nuclear inclusion protein (NI); e and l, PMoV CI; f and m, TEV 49k NI protein; g and n, tobacco vein mottling virus helper component protein. Normal serum did not precipitate any protein (lanes h and o). Some important proteins are marked with arrows and their molecular weights are indicated.

were within 210–250K protein coding distance from the 3′ terminus since it is now known that the CP is located at the 3′ terminus of the potyviral genome (1–3,9,19). The 250K protein may be proteolytically processed into a 110K polyprotein and a 137K polyprotein (Fig. 6). The 110K polyprotein, serologically related only to CI protein, presumably consisted of the 68K CI protein and a 42K unknown protein, for which no immunological probe was available. The 137K polyprotein was serologically related to the 50K and 53K NI proteins and 34K CP. The 53K NI protein and the 34K CP were also related to an 87K polyprotein. The 84K polyprotein, immunoprecipitated by HC antiserum, presumably consisted of an unknown 34K protein (no serological probe was available for this protein) mapped at the 5′ terminus of the genome and a 50K HC protein. Some of the polyproteins identified on the basis of size and serological reactions are illustrated in Figure 6. Proteins thus mapped on the PMoV genome are from the 5′ terminus to 3′ terminus in order: 34K unknown protein, 50K HC protein, 42K unknown protein, 68K CI protein, 50K NI protein, 53K NI protein, and 34K CP (Fig. 6). The total molecular weight of the proposed polyprotein is approximately 330K.

The following were taken into account in proposing the PMoV genome organization and the proteolytic processing strategy of the in vitro translation products (Fig. 6): 1) the difference observed between the products synthesized in the presence or absence of DTT and between the non-DTT synthesized products obtained after subsequent incubation with or without DTT, 2) the analysis by immunoprecipitations, and 3) the cross absorption with different combinations of antisera. Results from this study indicated that PMoV RNA behaves as a monocistronic messenger in the RRL system. The viral RNA was presumably translated into a single polyprotein, estimated to be 330K in size. Trace amounts of product estimated to be 330K were detected (Fig. 3, lanes c–f). The 84K product, reactive with HC antiserum but not with antisera to CI, NI, and CP, and the 210–250K product, reactive with antisera to CI, NI proteins, and CP but not HC, may together represent all the protein coded for by the PMoV genome. The failure to detect large amounts of the full-length product (330K) may be due to cleavage occurring on the nascent polyprotein chains during synthesis and due to a thio-group-independent cleavage site at the 84K-250K junction.

The WG system in vitro translation analyses of PMoV RNA revealed numerous products reactive with the CP and 54K NI protein antisera but few products reactive with antisera to proteins mapped 5′ to these two. This is in marked contrast with the WG translations of PRSV and pepper mottle virus RNAs where most of the translational activity is confined to the 5′ end products (8). This selective expression of the different potyviral genomes in the WG system done under similar conditions is not understood.

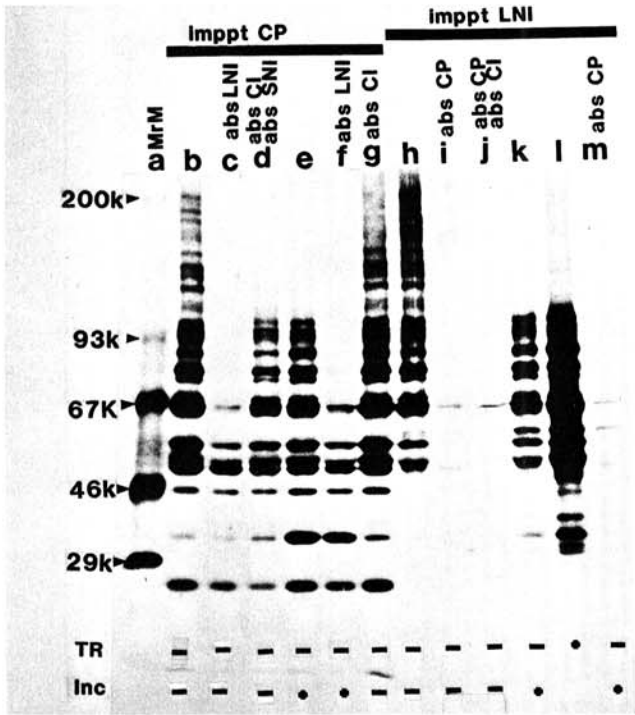

Fig. 4. Immunoprecipitation analysis of the in vitro translation products of peanut mottle virus (PMoV)RNA translated in the rabbit reticulocyte lysate (RRL) system. Products were separated on a sodium dodecyl sulfate polyacrylamide gel (7.5–15%) and detected by fluorography. Lane a) ^{14}C-labeled molecular weight markers as described in Fig. 1. Translation (TR) was carried out for 90 min without (−) the addition of dithiothreitol (DTT). The products were then incubated (Inc) without DTT (−) (lanes b, c, d, g, h, i, j,) or with DTT (lanes e, f, k, m) for another 2 hr. Translation in the normal reaction conditions (+) (DTT present) was done as a control (lane 1). Products were precipitated by antiserum to peanut mottle virus (PMoV) coat protein (CP) (imppt cp), either directly (lanes b and e) or after absorption with antisera to: c and f, tobacco etch virus (TEV) 54K nuclear inclusion (NI) protein; d, PMoV cylindrical inclusion (CI) protein and TEV 49K NI protein; g, PMoV CI protein. Products were precipitated by antiserum to TEV 54K NI protein (imppt LNI), either directly (lanes h, k, and l) or after absorption with antisera to: i and m, PMoV CP; PMoV CP and CI protein. No products were detected with normal serum immunoprecipitation (not shown).

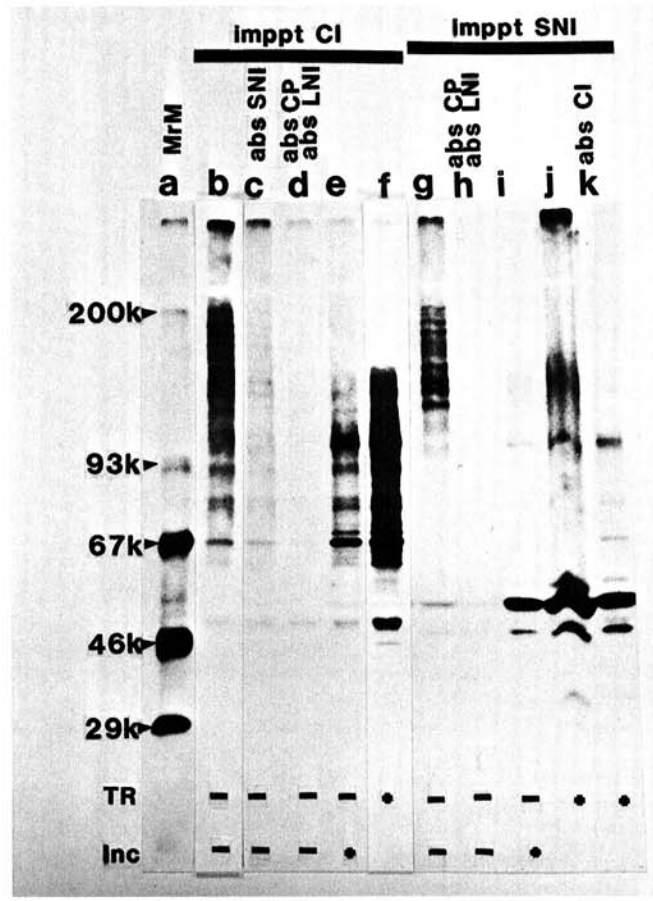

Fig. 5. Analysis of the in vitro translation products of peanut mottle virus (PMoV) RNA in the RRL system with antisera to potyviral proteins. Products were separated on a sodium dodecyl sulfate polyacrylamide gel (7.5–15%) and detected by fluorography. Lane a) ^{14}C-labeled molecular weight markers as described in Fig. 1. Translation (TR) was carried out for 90 min without (−) the addition of dithiothreitol (DTT). The products were then incubated (Inc) without (−) DTT (lanes b, c, d, g, and h) or with (+) DTT (lanes e and i) for another 2 hr. Translation with the usual reaction conditions (+) (DTT present) was done as a control (lanes f, j, and k). Products were precipitated by antiserum to PMoV cylindrical inclusion (CI) protein directly (lanes b, e, and f) or after absorption (abs) with antisera to: c, TEV 49K nuclear inclusion (NI) protein; d, PMoV coat protein (CP) and TEV 54K NI protein. Products were precipitated by antiserum to TEV 49K NI protein directly (lanes g, i, and j) or after absorption with antisera to: h, PMoV CP and TEV 54K NI protein; and k, PMoV CI protein. No products were detected with normal serum immunoprecipitation (data not shown).

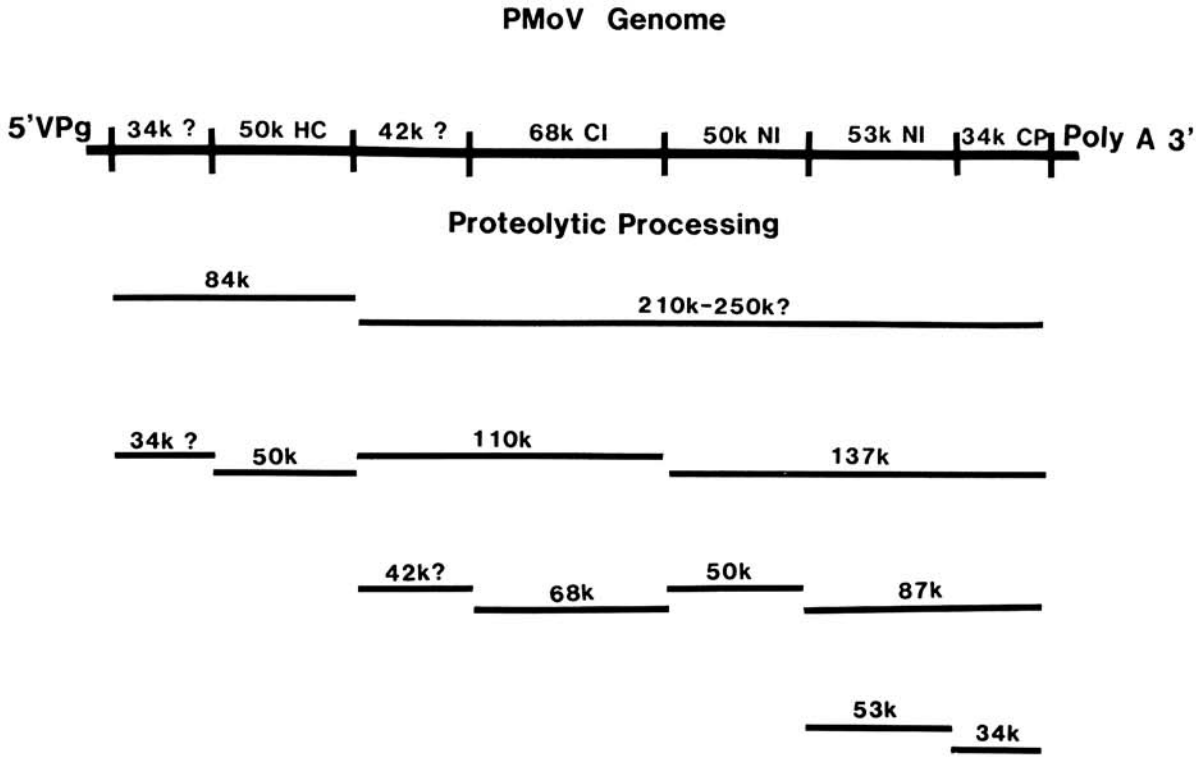

Fig. 6. Proposed peanut mottle virus (PMoV) genome map and proteolytic processing pathway of its translation products. The proteolytic processing pathway of peanut mottle virus (PMoV) RNA in vitro translation products was based on the experimental data (see text). The precursor and product relationship was indicated by placing the smaller products immediately below the precursors. CI, cylindrical inclusion protein; CP, coat protein; HC, helper component protein; NI, nuclear inclusion proteins; ?, unknown proteins; Poly A, poly A tail at the 3'-terminus of potyviral genome; VPg, genome-linked protein at the 5-terminus of potyviral genome, proposed on the basis of tobacco etch virus.

This study has demonstrated the proteolytic processing of PMoV RNA translation products in an in vitro translation system. The genomic organization of the PMoV, based on in vitro translation analysis, is similar to the organization resolved by sequencing the genomes of two other potyviruses, TVMV (9) and tobacco etch virus (2).

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