

Expression of Bottom Component RNA of Cowpea Mosaic Virus in Cowpea Protoplasts

GEERTJE REZELMAN, ROB GOLDBACH,* AND ALBERT VAN KAMMEN

Department of Molecular Biology, Agricultural University, 6703 BC Wageningen, The Netherlands

Upon inoculation of cowpea protoplasts with the bottom component of cowpea mosaic virus, at least six virus-induced proteins (with sizes of 170, 110, 87, 84, 60, and 32 kilodaltons) are synthesized, but not the capsid proteins (37 and 23 kilodaltons). These bottom-component-induced proteins were studied with respect to their genetic origin and mode of synthesis. The analyses were based on their electrophoretic peptide patterns resulting from partial digestion with *Staphylococcus aureus* protease V8. Comparison of the peptide patterns of the virus-induced proteins with those of the cowpea mosaic virus RNA-coded polypeptides produced in rabbit reticulocyte lysate showed that the 170- and 32-kilodalton polypeptides, which are the first viral products in cowpea mosaic virus-infected cells, were actually coded by the bottom component RNA of the virus. The 110-, 87-, and 84-kilodalton polypeptides, and possibly the 60-kilodalton polypeptide, appeared to have amino acid sequences in common with the 170-kilodalton polypeptide, demonstrating that they were virus coded as well. The results indicated that cowpea mosaic virus bottom component RNA was translated in vivo into a single 200-kilodalton polyprotein from which probably all bottom-component-specific proteins arose by three successive cleavages.

The single-stranded RNA genome of cowpea mosaic virus (CPMV), type member of the comovirus group, is divided between two separate (bottom [B] and middle [M]) nucleoprotein particles (1, 10, 19). Both B and M particles are necessary for virus multiplication (5, 18), indicating that the genetic information is distributed between both B and M RNAs (molecular weights, respectively: 2.02×10^6 and 1.37×10^6 [14]). The RNAs both possess 3'-terminal polyadenylate tails (7) and single proteins covalently attached to their 5' termini (3, 16). Little is known about the location of genes on both RNAs. Infection of cowpea mesophyll protoplasts with the natural mixture of CPMV components results in the synthesis of at least eight virus-induced polypeptides, of 170, 110, 87, 84, 68, 37, 30, and 23 kilodaltons, the 37-kilodalton (37K) and 23K polypeptides representing the capsid proteins (15). Moreover, 130K and 112K polypeptides are sometimes found in very small amounts (P. J. M. Rottier, G. Rezelman, and A. van Kammen, *J. Gen. Virol.*, in press). We have previously shown (8a) that in cowpea protoplasts the B RNA can be replicated and expressed independently, i.e., in the absence of M component. Upon inoculation of protoplasts with the CPMV B component alone, at least the 170K, 110K, 87K, 84K, and 30K virus-specific polypeptides are synthesized, but not the coat proteins. It has therefore been proposed that B

RNA encodes the early functions (e.g., the replicase) and that M RNA encodes the late functions (e.g., the coat proteins) during viral infection (8a). As the B RNA has only a coding capacity for polypeptides totaling approximately 250 kilodaltons, the question arises, however, of whether all B-component-induced polypeptides are actually virus coded and how B RNA is translated. To study this we have compared the peptide maps (2) from the virus-induced polypeptides formed in CPMV-infected protoplasts and from the B-RNA-coded polypeptides produced in the rabbit reticulocyte system. The results indicate that B RNA is translated in vivo into one large polyprotein from which probably all B-RNA-specific proteins arise by three post-translational cleavages.

MATERIALS AND METHODS

Virus purification and separation of M and B components. CPMV (yellow strain) was grown in *Vigna unguiculata* L. (Blackeye Early Ramshorn) and purified as previously described (11, 17). The B and M virion components were separated by three cycles of centrifugation in a linear 15 to 30% zonal sucrose gradient (Beckman Ti 15 rotor, 16 h, 23,000 rpm at 10°C). By means of the local lesion infectivity assay (9) it was determined that M components were free of B and that B components contained less than 0.2% M.

Preparation of CPMV RNA. CPMV RNAs were extracted from separated components as described by

Davies et al. (4). Purity and quality of isolated RNAs were tested by electrophoresis in a 1% agarose gel (8).

Preparation of cowpea protoplasts and inoculation with CPMV. Cowpea mesophyll protoplasts were isolated as previously described in detail (9, 15) and inoculated by resuspension at a concentration of 5×10^6 protoplasts per ml in 0.6 M mannitol–0.01 M potassium citrate, pH 5.2, containing 7 μ g of B component per ml or 14 μ g of a 1:1 (wt/wt) mixture of B and M components per ml. The suspension was incubated for 15 min at room temperature. Protoplasts were then collected by centrifugation for 2 min at $600 \times g$ and washed three times with 0.6 M mannitol–10 mM CaCl_2 .

Incubation of protoplasts and labeling of proteins. Washed protoplasts were incubated at a concentration of 5×10^6 cells per ml in culture medium as previously described in detail (15). Neither actinomycin D nor any other inhibitor of host protein synthesis was added. For labeling of proteins, 5-ml portions of protoplasts were labeled with 150 μ Ci of [^{35}S]methionine (Radiochemical Centre, Amersham, England; 1,380 Ci/mmol) between 20 and 25 h postinfection.

Subcellular fractionation of protoplasts. At the end of the incubation period, protoplasts were collected by centrifugation and stored frozen at -80°C . For subcellular fractionation, frozen protoplast samples were thawed and disrupted by homogenization for 5 min with a small potter with 0.5 ml of homogenization buffer (HB), containing 50 mM Tris-hydrochloride (pH 7.4), 10 mM KCl, 5 mM MgCl_2 , 1 mM EDTA, 60 mM β -mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 0.4 mM methionine, and 10% (wt/vol) sucrose. Intact cells were removed by low-speed centrifugation (30 s, $500 \times g$, 4°C). The homogenate was centrifuged for 30 min at $30,000 \times g$ and 4°C to give the $30,000 \times g$ supernatant. The $30,000 \times g$ pellet was extracted with (per 2.5×10^6 protoplasts) 0.125 ml of HB lacking MgCl_2 to which 0.2% (wt/vol) digitonin had been added. Centrifugation of this suspension for 30 min at $30,000 \times g$ gave the $30,000 \times g$ pellet extract.

In vitro translation. Translation in an mRNA-dependent rabbit reticulocyte lysate (a generous gift of H. R. B. Pelham, Department of Embryology, Carnegie Institution of Washington, Baltimore, Md.) was performed as described by Pelham (13). In all experiments the dithiothreitol concentration was 2 mM, and CPMV RNA was added at 60 μ g/ml. [^{35}S]methionine was used as the radioactive amino acid, and incubations were at 30°C for 1 h.

Sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis. Equal amounts of labeled proteins (as measured by acid-precipitable radioactivity) were mixed with 0.5 volume of a threefold-concentrated solution of sample buffer (final concentrations: 10 mM Tris-hydrochloride [pH 8.0], 1 mM EDTA, 10% [vol/vol] glycerol, 2% [wt/vol] sodium dodecyl sulfate [SDS], 5% [vol/vol] β -mercaptoethanol, and 0.001% [wt/vol] bromophenol blue). After heating samples for 3 min at 100°C , they were analyzed in polyacrylamide gels containing 12.5% acrylamide with 0.09% bisacrylamide as previously described (12), using spacers of 4% acrylamide with 0.1% bisacrylamide. Molecular weight markers used were myosin (210,000), *Escherichia coli* RNA nucleotidyltransferase (165,000

and 155,000), *E. coli* β -D-galactosidase (116,200), phosphorylase b (100,000 and 92,500), bovine serum albumin (68,000), ovalbumin (46,000), carbonate dehydratase (30,000), soybean trypsin inhibitor (20,100), and α -lactalbumin (14,400). Gels were dried and autoradiographed with Kodak Royal X-Omat film.

Peptide mapping. Polypeptides, localized by autoradiography, were eluted from dried, unstained gels by incubating gel slices in small volumes of 30 mM Tris-hydrochloride (pH 6.8)–0.25 mM EDTA–0.025% sodium dodecyl sulfate for 16 to 64 h at 4°C . The volume of the eluted protein samples was reduced four times by vacuum evaporation, and the sodium dodecyl sulfate concentration was raised to 0.5%. Partial digestion with *Staphylococcus aureus* V8 protease (Miles Laboratories, Inc., Elkhart, Ind.) or chymotrypsin (Worthington Biochemicals Corp., Freehold, N.J.) was performed at 30°C for 30 min with various enzyme concentrations as indicated in the figure legends. Peptide patterns were compared in 17.5, 20, or 22.5% polyacrylamide gels containing 0.07, 0.07, or 0.06% bisacrylamide, respectively.

RESULTS

The 170K and 32K polypeptides produced in CPMV-infected protoplasts are virus coded. Infection of cowpea protoplasts with CPMV results in the synthesis of at least eight polypeptides, with estimated sizes of 170, 110, 87, 84, 68, 37, 30, and 23 kilodaltons (8a, 15), the 170K and 30K polypeptides being the first products synthesized (Rottier et al., *J. Gen. Virol.*, in press). These polypeptides were predominantly found in the $30,000 \times g$ supernatant fraction of the protoplasts (Fig. 1, lane 6), with the exception of the 68K polypeptide, which was only detected in the $30,000 \times g$ pellet fraction. The latter polypeptide is visible in lane 3 of Fig. 1 and has been indicated with 60 (recent estimates give a slightly different size for this polypeptide [formerly 68K, now 60K] as well as for the 30K polypeptide [now 32K]). All of these polypeptides except for the (37K and 23K) coat proteins, were also synthesized in protoplasts inoculated with the purified B component (Fig. 1, lanes 2 and 7; reference 8a). Pelham (13) has shown that in the reticulocyte system the primary translation product of B RNA is a polypeptide of approximately 205 kilodaltons which is cleaved into two polypeptides of 170 and 32 kilodaltons. We confirmed this result except that our calculations indicated an apparent molecular weight of 200,000 for the largest polypeptide. The 170K and 32K polypeptides produced in vitro coelectrophoresed with the 170K and 32K polypeptides produced in protoplasts infected with complete virus or with B component alone (Fig. 1, cf. lanes 6, 7, and 8), suggesting that these polypeptides were identical. To verify this, the proteins were eluted from the gel and subjected to partial proteolytic digestion with V8 protease

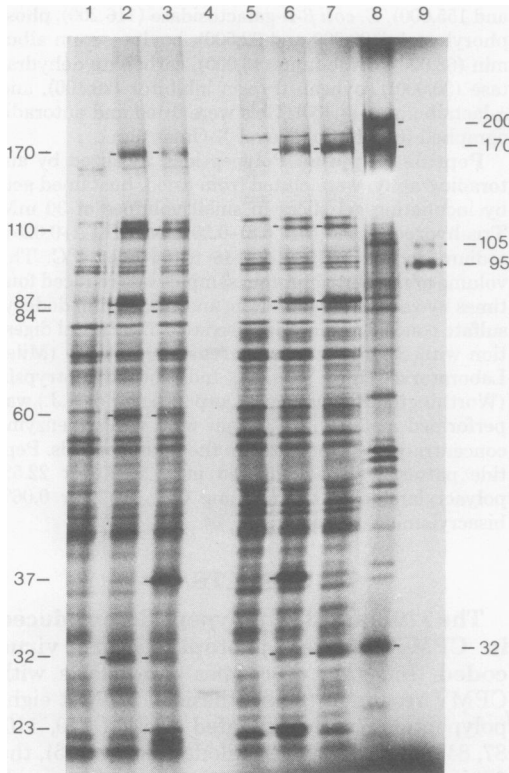


FIG. 1. Analysis of ^{35}S -proteins from *coupea* protoplasts infected with CPMV components on a 12.5% sodium dodecyl sulfate-polyacrylamide gel. Samples of protoplasts were inoculated 20 to 25 h postinfection in the presence of [^{35}S]methionine (for details, see the text). Protoplasts were either mock infected (lanes 1 and 5), inoculated with purified B components (lanes 2 and 7), or inoculated with complete virus (M and B components) (lanes 3 and 6). Lanes 1, 2, and 3 contained the proteins extracted from the $30,000 \times g$ pellets; lanes 5, 6, and 7 contained the proteins from the $30,000 \times g$ supernatant fractions (for details, see the text). Lane 4 contained ^{14}C -methylated marker proteins (Radiochemical Centre), only visible in longer-exposed autoradiograms. Lanes 8 and 9 contained the translation products of CPMV B and M RNA, respectively, synthesized in the rabbit reticulocyte cell-free system. The numbers at the left refer to the molecular weights ($\times 10^{-3}$) of the virus-induced polypeptides synthesized in the *coupea* protoplast system; the numbers at the right refer to the molecular weights ($\times 10^{-3}$) of the main products synthesized in the reticulocyte system. In the latter system M RNA was translated into two polypeptides, with molecular weights of 105,000 and 95,000, whereas the endogenous activity (no RNA added) of the reticulocyte lysate used was undetectable (data not shown).

from *S. aureus* or chymotrypsin. The digestion patterns of both the in vivo and the in vitro 170K polypeptides appeared to be identical and not significantly different from that of the 200K

in vitro polypeptide (Fig. 2). For instance, at the highest V8 protease concentration used (0.75 mg/ml), these three polypeptides had at least nine peptides in common. Protease V8 digestion of both 32K polypeptides also produced indistinguishable patterns, although the peptide bands became weaker at the higher protease concentrations used (Fig. 2). Cleavage with chymotrypsin (Fig. 2) provided additional evidence that these polypeptides were identical. However, the results shown in Fig. 2 did not decisively indicate whether the amino acid sequence of the 32K polypeptide was present in the 200K polypeptide and absent in both 170K polypeptides, as would be expected from the results of Pelham (13). This was mainly due to the large size difference between the 32K polypeptide and the other polypeptides, making a comparison by peptide mapping less suitable. Nevertheless, Fig. 2 shows conclusively that both the 170K and the 32K polypeptides produced in protoplasts infected with CPMV were coded by the B RNA of the virus.

Relationship between the 170K, 110K, and 87K polypeptides. The 170K and 32K in vivo polypeptides together represent approximately 85% of the coding capacity of B RNA. This raises the question of whether the other (110K, 87K, 84K, and 60K) B-component-induced polypeptides are also virus coded and, if this is the case, by which mechanism they arise. To answer these questions we made peptide maps of all B-component-induced polypeptides, using *S. aureus* V8 protease at three different concentrations. The 170K, 110K, and 87K polypeptides were closely related, as illustrated by a strong resemblance between their peptide maps (Fig. 3A). The most important peptides demonstrating the relationship between these three polypeptides are indicated with dots in Fig. 3. For instance, at the highest protease concentration used (in Fig. 3A, lane c) these proteins produced at least eight common peptides. The 170K polypeptide digest contained several (at least five) peptides which were lacking in both the 110K and the 87K peptide maps. Compared with the 87K polypeptide digest, the 110K polypeptide digest contained only two obvious extra peptides, whereas the 87K polypeptide pattern contained one small unique (possibly terminal) peptide. This close relationship was further supported by the peptide maps shown in Fig. 3B. In this experiment the digestion patterns of both proteins, after using $30 \mu\text{g}$ of protease V8 per ml (Fig. 3B, cf. lane b), showed at least 15 peptides in common within a size range of 50 to 14 kilodaltons.

The striking similarities within their peptide patterns indicated that the 170K, 110K, and 87K

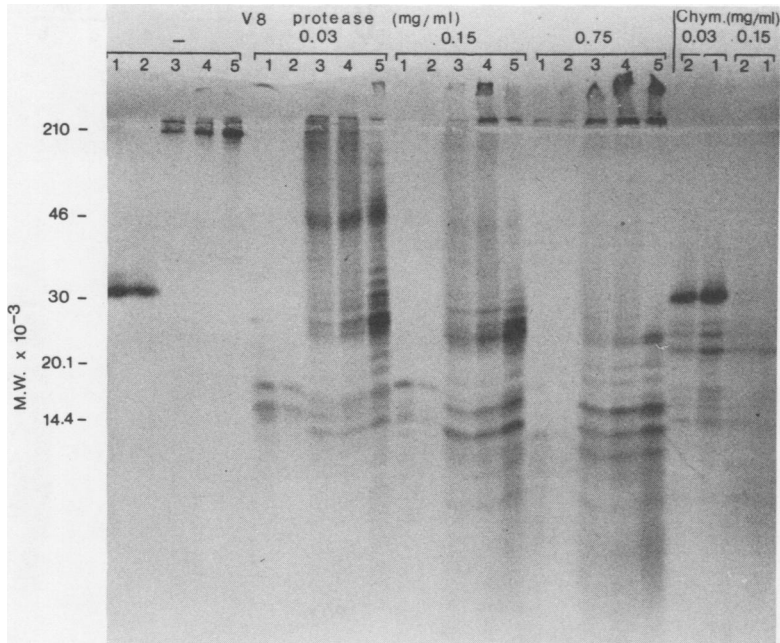


FIG. 2. Autoradiogram of the peptide maps of the *in vitro* B-RNA-coded 32K (lanes 1), 170K (lanes 4), and 200K (lanes 3) polypeptides and the 32K (lanes 2) and 170K (lanes 5) polypeptides from CPMV-infected protoplasts. The [³⁵S]methionine-labeled proteins were eluted from dried 12.5% acrylamide gels and digested with increasing amounts (indicated in the figure; -, no enzyme added) of *S. aureus* V8 protease or chymotrypsin (Chym.). Peptides generated by proteolysis were electrophoresed in a 22.5% acrylamide gel which was subsequently dried and autoradiographed. The molecular weights (M.W.) indicated refer to the positions of myosin (210,000), ovalbumin (46,000), carbonate dehydratase (30,000), soybean trypsin inhibitor (20,100), and α -lactalbumin (14,400) in the gel.

polypeptides had a major part of their amino acid sequence in common, suggesting that the 110K and 87K polypeptides were derived from the 170K polypeptide by posttranslational processing steps.

Relationship between the 170K, 84K, and 60K polypeptides. At the highest V8 protease concentration used, the digestion pattern of the 170K polypeptide contained several peptides which were lacking in the 110K and 87K digests (Fig. 3A, lane c). Of these peptides the most pronounced (indicated with arrowheads in Fig. 3A) comigrated with the V8-derived peptides from the 84K polypeptide. In addition, the peptide patterns of the 170K and 84K polypeptides shared at least two other characteristic peptides (of higher molecular weight) when less protease was used (Fig. 3A, lane b). The relationship between the 170K and 84K polypeptides was further underlined by the results shown in Fig. 4. The peptide patterns of the 170K and 84K proteins again showed the same (at least seven) comigrating peptides, suggesting that the complete amino acid sequence of the 84K polypeptide was present in the 170K protein. However,

the relationship between the 60K polypeptide and the other virus-specific polypeptides was less clear. This polypeptide had only one pronounced protease V8-derived peptide (size, approximately 16 kilodaltons) in common with both the 170K and the 84K proteins (Fig. 4). Five other peptides appeared to comigrate in the peptide patterns of 60K and 84K, although they gave rise only to very faint bands. An extensive relationship between these two proteins was therefore less certain. Due to the relatively low yield of the 60K protein from infected protoplasts, this remains a point in question.

DISCUSSION

It has been previously shown (8a, 15; Rottier et al., *J. Gen. Virol.*, in press) that infection of cowpea protoplasts with CPMV induces the synthesis of at least eight virus-specific proteins, with apparent sizes of 170, 110, 87, 84, 60, 37, 32, and 23 kilodaltons. The 37K and 23K proteins represent the capsid proteins. Upon inoculation of protoplasts with B components alone, at least the 170K, 110K, 87K, 84K, 60K, and 32K proteins are synthesized, but not the capsid proteins

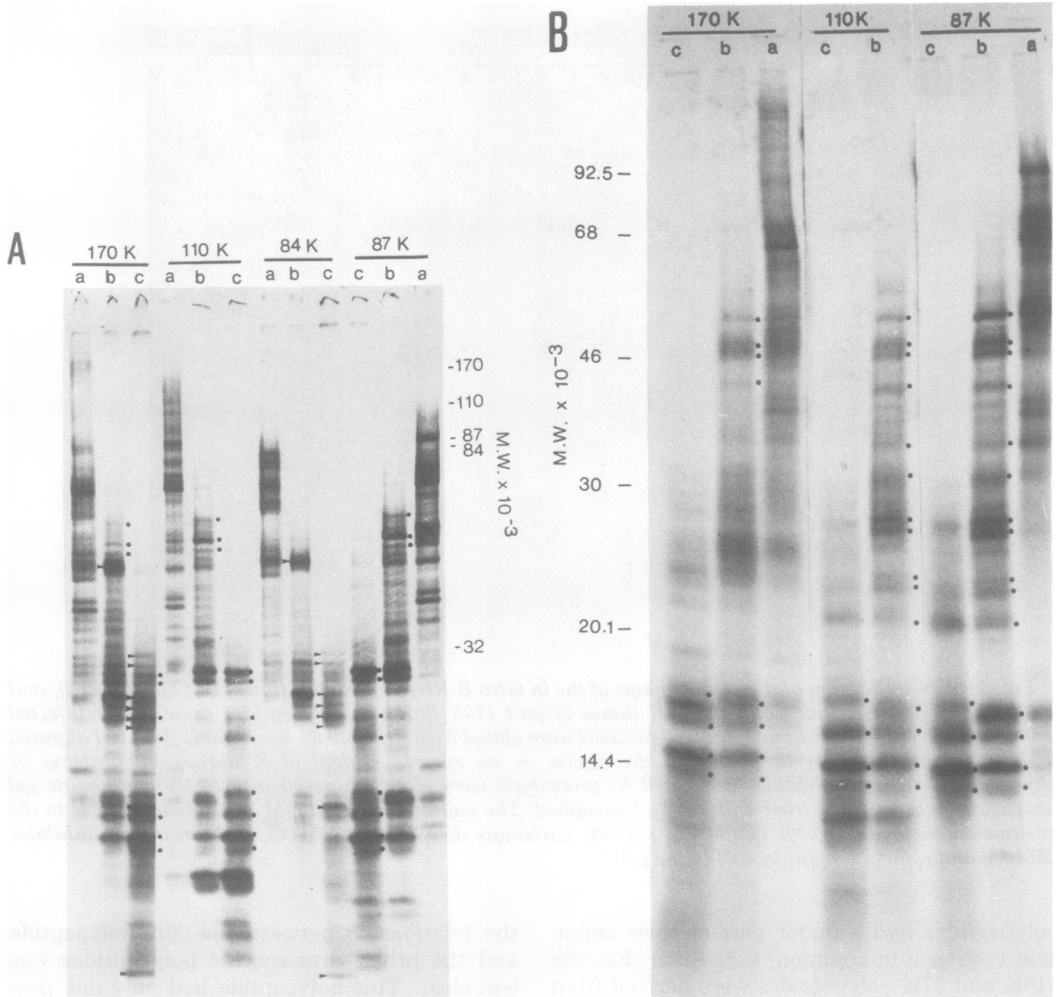


FIG. 3. Analysis of the peptide patterns obtained from the virus-specific 170K, 110K, 87K, and 84K polypeptides from CPMV-infected protoplasts. Polypeptides were eluted from a dried 12.5% acrylamide gel and incubated with 3 (a), 30 (b), or 150 (c) μg of V8 protease from *S. aureus* per ml (for details, see the text). The resulting peptides were analyzed in a 22.5% (A) or a 17.5% (B) acrylamide gel. Dots refer to protease-derived peptides demonstrating the relationship between the 170K, 110K, and 87K polypeptides. Peptides indicated with arrowheads demonstrate the relationship between the 170K and 84K polypeptides. M.W., Molecular weight.

(see Fig. 1 and reference 8a).

By comparison of the peptide fingerprints of the 170K and 32K polypeptides translated *in vitro* from CPMV B RNA with those of the equal-sized, virus-specific polypeptides from infected cells, it has now been demonstrated that the latter polypeptides are actually virus coded. In addition, these results indicate that the translation of B RNA in the rabbit reticulocyte system, as described in detail by Pelham (13), correctly produces proteins as seen *in vivo*. In this *in vitro* system CPMV B RNA is translated into a polypeptide of approximately 200 kilodaltons

which is then proteolytically cleaved to give the 170K and 32K polypeptides. A 200K polypeptide has not yet been found in infected cells. In view of the correct translation in the reticulocyte system, it is reasonable to assume that the same cleavage from a 200K primary precursor polypeptide also occurs *in vivo*. This remains to be verified, however.

By comparison of their proteolytic digestion patterns (using *S. aureus* V8 protease) on polyacrylamide gels, we have shown that the virus-specific 110K, 87K, 84K, and, possibly, 60K polypeptides have amino acid sequences in common

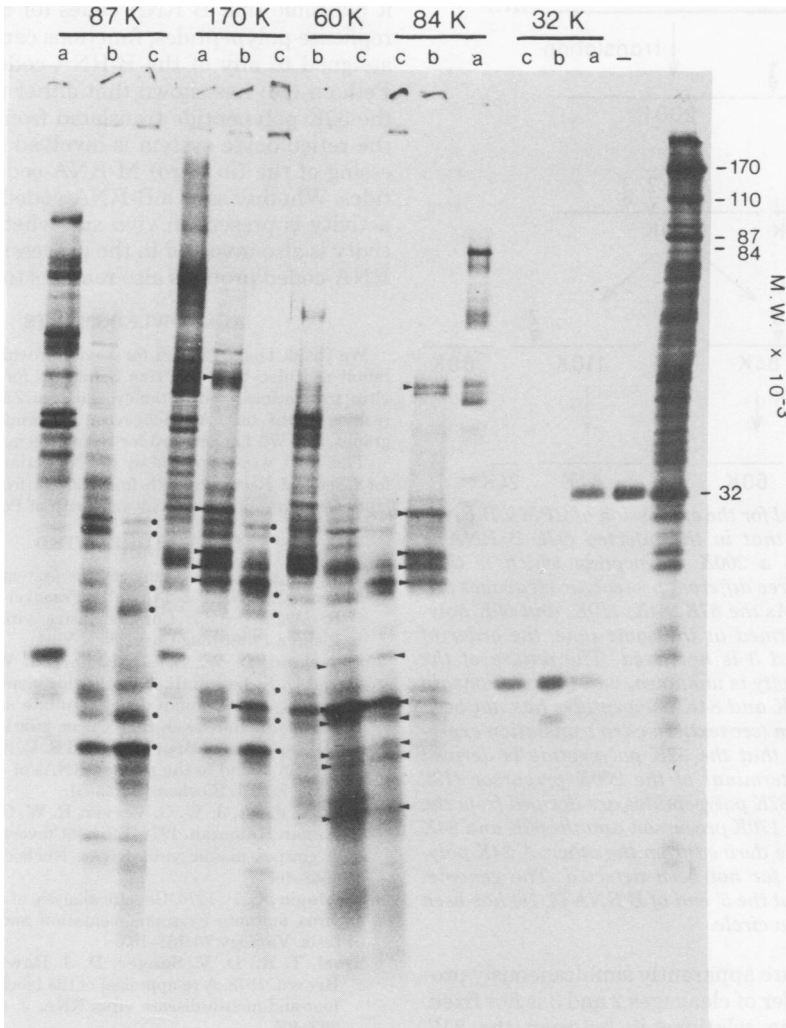


FIG. 4. Autoradiogram of the virus-specific 170K, 87K, 84K, 60K, and 32K polypeptides digested with different amounts of *S. aureus* V8 protease. Incubations were performed with 3 (a), 30 (b), or 150 (c) of protease per ml or without protease (-). The marker slot (right) contained the 30,000 \times g supernatant proteins of protoplasts inoculated with CPMV B component. Peptides marked with arrowheads indicate the relationship between the 170K, 84K, and 60K polypeptides; peptides marked with dots indicate the relationship between the 170K and 87K polypeptides. M.W., Molecular weight.

with the 170K polypeptide. Nearly all protease V8-derived peptides of the 87K polypeptide could be recovered in both the 110K and the 170K protease V8-peptide maps. This finding indicates that the 170K polypeptide contains two different cleavage sites, which are used for the derivation of the 110K and 87K polypeptides. However, the 87K and 84K polypeptides have totally different peptide patterns. Although other mechanisms cannot be completely ruled out, all results fit the model for the expression of B RNA presented in Fig. 5. The model proposes that all B-component-specific polypep-

tides (see Fig. 1, lanes 2 and 7) are cleaved from a single 200K polyprotein by three successive cleavages. It has been assumed that the primary cleavages of the 200K precursor to give 170K and 32K polypeptides as found in the rabbit reticulocyte system (13) also occurs in vivo. The fact that in infected cells the 170K and 32K polypeptides are the first detectable viral products is not inconsistent with this idea (Rottier et al., *J. Gen. Virol.*, in press). The 200K, 170K, 110K, and 84K polypeptides are precursors, and the others (87K, 60K, and 32K polypeptides) are end products. As the 110K, 87K, 84K, and 60K

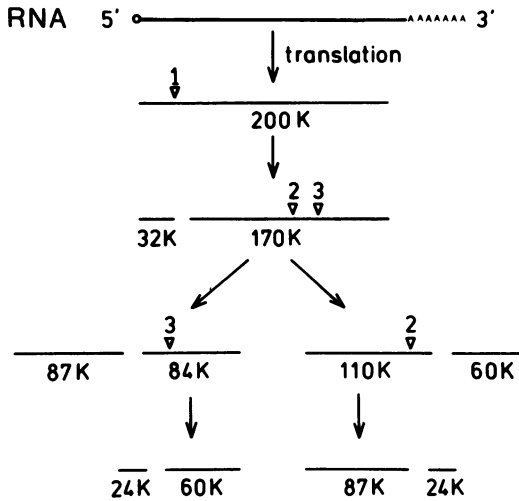


FIG. 5. Model for the expression of CPMV B RNA. It is proposed that in the infected cell, B RNA is translated into a 200K polypeptide which is then processed by three different proteolytic cleavages (indicated by ∇). As the 87K, 84K, 110K, and 60K polypeptides are formed at the same time, the order of cleavages 2 and 3 is not fixed. The nature of the proteolytic activity is unknown, and the relationship between the 60K and 84K polypeptides has not been definitely proven (see text). *In vitro* translation experiments suggest that the 32K polypeptide is derived from the NH₂-terminal of the 200K precursor (13). The 110K and 87K polypeptides are derived from the same end of the 170K precursor, and the 60K and 84K polypeptides are derived from the other. A 24K polypeptide has so far not been detected. The genome-linked protein at the 5' end of B RNA (3, 16) has been indicated with a circle.

polypeptides are apparently simultaneously produced, the order of cleavages 2 and 3 is not fixed. To explain the relationship between the 84K and 60K polypeptides on the one hand and that between the 110K and 87K polypeptides on the other, as suggested by the model, the presence of a polypeptide of approximately 24 kilodaltons must be envisaged. Such a protein has not been found. This may be due, however, to either a rapid turnover, a lack of methionine residues (by which the radioactive label was introduced in the procedures adopted), or masking by an equal-sized host protein. Alternatively, it is possible that the 24K polypeptide does not exist. This would be the case if the 110K and 84K polypeptides were not further cleaved, but were functional proteins. This would mean that the 170K polypeptide is processed in two alternative ways, resulting in four different proteins (110K, 87K, 84K, and 60K), having distinct functions. Such a translation strategy has been proposed for foot-and-mouth disease virus RNA (6).

Although recent experiments (8a) have made

it plausible that B RNA codes for one or more replicase polypeptides, functions can still not be assigned to any of the B-RNA-coded proteins. Pelham (13) has shown that either the 170K or the 32K polypeptide translated from B RNA in the reticulocyte system is involved in the processing of the (in vitro) M-RNA-coded polypeptides. Whether such a B-RNA-coded proteolytic activity is present *in vivo* and whether this activity is also involved in the processing of the B-RNA-coded proteins also remains to be verified.

ACKNOWLEDGMENTS

We thank Hugh Pelham for kindly providing samples of rabbit reticulocyte lysate, Han Schilthuis for help in the *in vitro* translations, John Stanley and Pim Zabel for critical reading of the text, Ab Hoogeveen for printing the photographs, and Wil Landeweerd for typing the manuscript.

This work was supported by the Netherlands Foundation for Chemical Research, with financial aid from the Netherlands Organization for the Advancement of Pure Research.

LITERATURE CITED

- Bruening, G. 1977. Plant covirus systems: two-component systems, p. 55-141. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), *Comprehensive virology*, vol. 11. Plenum Publishing Corp., New York.
- Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli. 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. *J. Biol. Chem.* **252**:1102-1106.
- Daubert, S. D., G. Bruening, and R. C. Najarian. 1978. Protein bound to the genome RNAs of cowpea mosaic virus. *Eur. J. Biochem.* **92**:45-51.
- Davies, J. W., J. W. G. Verver, R. W. Goldbach, and A. van Kammen. 1978. Efficient reverse transcription of cowpea mosaic virus RNAs. *Nucleic Acids Res.* **5**:4643-4661.
- De Jager, C. P. 1976. Genetic analysis of cowpea mosaic virus mutants by supplementation and reassortment tests. *Virology* **70**:151-163.
- Doel, T. R., D. V. Sanger, D. J. Rowlands, and F. Brown. 1978. A re-appraisal of the biochemical map of foot-and-mouth disease virus RNA. *J. Gen. Virol.* **41**:395-404.
- El Manna, M. M., and G. Bruening. 1973. Polyadenylate sequences in the ribonucleic acids of cowpea mosaic virus. *Virology* **56**:198-206.
- Goldbach, R. W., P. Borst, J. E. Bollen-de Boer, and E. F. J. van Bruggen. 1978. The organization of ribosomal RNA genes in the mitochondrial DNA of *Tetrahymena pyriformis* strain ST. *Biochim. Biophys. Acta* **521**:169-186.
- Goldbach, R., G. Rezelman, and A. van Kammen. 1980. Independent replication and expression of B-component RNA of cowpea mosaic virus. *Nature (London)* **286**:297-300.
- Hibi, T., G. Rezelman, and A. van Kammen. 1975. Infection of cowpea mesophyll protoplasts with cowpea mosaic virus. *Virology* **64**:308-318.
- Jaspars, E. M. J. 1974. Plant viruses with a multipartite genome. *Adv. Virus Res.* **19**:37-149.
- Klootwijk, J., I. Klein, P. Zabel, and A. van Kammen. 1977. Cowpea mosaic virus RNAs have neither m⁷GpppN... nor mono-, di-, or triphosphates at their 5'-ends. *Cell* **11**:73-82.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature (London)* **227**:680-685.
- Pelham, H. R. B. 1979. Synthesis and proteolytic proc-

- essing of cowpea mosaic virus proteins in reticulocyte lysates. *Virology* **96**:463-477.
14. **Reijnders, L., A. M. J. Aalbers, A. van Kammen, and R. W. J. Thuring.** 1974. Molecular weights of plant viral RNAs determined by gel electrophoresis under denaturing conditions. *Virology* **60**:515-521.
 15. **Rottier, P. J. M., G. Rezelman, and A. van Kammen.** 1979. The inhibition of cowpea mosaic virus replication by actinomycin D. *Virology* **92**:299-309.
 16. **Stanley, J., P. Rottier, J. W. Davies, P. Zabel, and A. van Kammen.** 1978. A protein linked to the 5' termini of both RNA components of the cowpea mosaic virus genome. *Nucleic Acids Res.* **5**:4505-4522.
 17. **Van Kammen, A.** 1967. Purification and properties of the components of cowpea mosaic virus. *Virology* **31**:633-642.
 18. **Van Kammen, A.** 1968. The relationship between the components of cowpea mosaic virus. I. Two ribonucleo-protein particles necessary for the infectivity of CPMV. *Virology* **34**:312-318.
 19. **Van Kammen, A.** 1972. Plant viruses with a divided genome. *Annu. Rev. Phytopathol.* **10**:125-150.