Expression of the Middle Component RNA of Cowpea Mosaic Virus in vivo

By JOAN WELLINK, * MARTINE JAEGLE, † HEINRICH PRINZ, AB VAN KAMMEN AND ROB GOLDBACH

1Department of Molecular Biology, Agricultural University, De Dreejen 11, 6702 BC Wageningen, The Netherlands, 2Institut für Genetik, Universität Köln, D-5000 Köln 41, F.R.G. and 3Department of Virology, Binnenhaven 11, 6709 PD Wageningen, The Netherlands

(Accepted 25 June 1987)

SUMMARY

Upon infection of cowpea mesophyll protoplasts with cowpea mosaic virus (CPMV), the only M RNA-encoded proteins detected so far have been the two capsid proteins VP37 and VP23. We now report the detection of a M, 60000 (60K) precursor to both capsid proteins in infected protoplasts cultured in the presence of zinc ions. Furthermore a M RNA-encoded 48K protein was detected in the membrane fraction of infected cells using antisera raised against synthetic peptides. The results obtained indicate that, just like the bottom component RNA, the M RNA of CPMV is translated in vivo into a polyprotein from which proteins are derived by proteolytic cleavage.

INTRODUCTION

The genome of cowpea mosaic virus (CPMV) consists of two polyadenylated plus-strand RNAs which are separately encapsidated. Previous studies on the viral proteins encoded by the larger bottom (B) component RNA, 5889 nucleotides long excluding the poly(A) tail (Lomonossoff & Shanks, 1983), have revealed that the expression of this RNA involves translation into a M, 200000 (200K) polyprotein which upon proteolytic processing gives rise to six final cleavage products (for a recent review, see Goldbach & Van Kammen, 1985). The expression of the smaller middle (M) component RNA, 3481 nucleotides long excluding the poly(A) tail (Van Wezenbeek et al., 1983), is less well understood. Although a translation model for this RNA has been proposed, this model (Fig. 1) is almost entirely based on translation studies in vitro.

In both the wheat germ system and rabbit reticulocyte lysates, M RNA is translated into two overlapping proteins of 105K and 95K (Pelham, 1979; Franssen et al., 1982), the 105K protein being initiated at an AUG codon at position 161 and the 95K protein at an AUG codon at position 512 and/or 524 (Vos et al., 1984; Van Wezenbeek et al., 1983). Both primary translation products are processed by a B RNA-encoded protease to give 58K and 48K proteins respectively, together with a 60K precursor to both capsid proteins VP37 and VP23 (Fig. 1; Pelham, 1979; Franssen et al., 1982, 1984). All attempts to achieve cleavage of the 60K precursor into mature capsid proteins have so far failed. Since the mature capsid proteins are the only M RNA-encoded proteins detected in infected cells (Goldbach et al., 1980) it is not known whether the translation model presented in Fig. 1 is also valid in vivo. In this paper we report the occurrence of a 60K capcid precursor protein and an M RNA-encoded 48K protein in CPMV-infected cells. These results definitely show that the M RNA is also expressed in vivo by polyprotein processing.

METHODS

Virus and plants. CPMV (Sb isolate) was propagated in cowpea plants (Vigna unguiculata L. ‘California Blackeye’) as described by Klootwijk et al. (1977) and Van Kammen (1967), and B and M components were purified as described by Rezelman et al. (1980).

* Present address: Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, U.K.
Inoculation of protoplasts and labelling of proteins. Mesophyll protoplasts were prepared from 10-day-old primary cowpea leaves and inoculated and incubated as described by Rezelman et al. (1980). When labelled proteins were required, protoplasts were supplied with \(^{35}S\)methionine (1000 Ci/mmol: New England Nuclear) as described by Franssen et al. (1984). Protoplasts were collected by centrifugation and frozen. Frozen pellets, corresponding to portions of 10^6 protoplasts, were resuspended in 100 µl of HB buffer (50 mM-Tris-acetate pH 8.2, 10 mM-potassium acetate, 1 mM-EDTA, 5 mM-dithioerythritol, 1 mM-phenylmethylsulphonyl fluoride, 10% sucrose) and centrifuged at 4 °C for 30 min at 30000 g yielding a supernatant (S30) fraction and a pellet fraction. In some cases the medium in which the protoplasts had been incubated was also analysed.

Fractionation of cowpea leaves. CPMV-infected and non-infected cowpea leaves were fractionated into nuclear, membrane and cytoplasmic fractions, as described by Zabel et al. (1982).

Preparation of antiserum against synthetic peptides. Two peptides (of nine and 30 amino acids) were synthesized by the solid-phase method (Barany & Merrifield, 1980). The peptides were characterized by amino acid analyses and high performance liquid chromatography. The shorter peptide, peptide S, was coupled to keyhole limpet haemocyanin using glutaraldehyde (Baron & Baltimore, 1982), and the longer peptide, peptide L, was not coupled to a carrier protein. To obtain monospecific antibodies the coupled peptide S and free peptide L were injected into New Zealand white rabbits at 14 day intervals (Muller et al., 1986). Serum was collected 8 to 10 days after the third, fourth and fifth injections.

Translation in vitro. CPMV M RNA was translated in rabbit reticulocyte lysates (a generous gift from Dr R. J. Jackson) at 30 °C for 1 h as described by Pelham (1979). The 105K and 95K translation products were proteolytically processed into 58K, 48K and 60K proteins by the addition of 1 vol. of an S30 fraction from B RNA-inoculated protoplasts as described by Franssen et al. (1982).

Immunological methods. For immunoprecipitation, samples were either directly adjusted to 1 × RIA buffer (50 mM-Tris–HCl pH 7.5, 150 mM-NaCl, 5 mM-EDTA, 0.1% SDS), or first brought to 2% SDS, 5% 2-mercaptoethanol, boiled for 1 min, diluted at least 10-fold and then adjusted to 1 × RIA buffer. Immunoprecipitation was as described by Franssen et al. (1982). For protein blot analysis, fractions from cowpea protoplasts or leaves were separated in 12.5% SDS–polyacrylamide gels, blotted onto nitrocellulose filters and probed with antisera (1500-fold dilution in RIA buffer) and ^{125}I-labelled Protein A as described by Zabel et al. (1982).

**RESULTS**

Detection of a M, 60K precursor to the capsid proteins

According to the *in vitro* translation model for M RNA (Fig. 1) the two capsid proteins VP37 and VP23 are generated from a 60K precursor protein. To verify the existence of such a precursor *in vivo*, immunoprecipitations using anti-VP23 serum were carried out in extracts from CPMV-infected[^35]Smethionine-labelled cowpea protoplasts. However, only mature VP23 was detected in such extracts (Fig. 2, lane 3). In an attempt to accumulate possible precursor proteins by blocking or slowing down the proteolytic processing, CPMV-infected protoplasts were labelled in the presence of ZnCl₂, since zinc ions are known to inhibit *in vitro* the processing of both the M RNA-encoded polyproteins (Pelham, 1979; Franssen et al., 1982) and the B RNA-encoded 170K protein (Peng & Shih, 1984). Sixteen h after inoculation, a solution of ZnCl₂ was added to infected protoplasts to a final concentration of 2 mM-Zn²⁺ and 1 h later[^35]Smethionine (15 µCi/ml) was added. After a further 7 h (i.e. 24 h after inoculation) the protoplasts were collected by centrifugation and extracts were prepared for immunological analysis. In protoplasts incubated in the presence of ZnCl₂, but not in untreated ones, a 60K protein was precipitated with anti-VP23 serum (Fig. 2, lanes 4 and 5). This protein comigrated with the 60K capsid precursor protein generated *in vitro* by processing of the *in vitro* translation products from M RNA with viral protease (Fig. 1) (Franssen et al., 1982). The 60K protein was also precipitated with anti-VP37 serum (data not shown) and was only detectable in protoplasts inoculated with B and M components and not in protoplasts inoculated with B component alone (Fig. 2, lanes 7 and 9). These data are consistent with the idea that the 60K protein detected *in vitro* contains the sequences of both M RNA-encoded capsid proteins and represents a precursor to these products.

Detection of an M RNA-encoded 48K protein in infected protoplasts

To enable the detection of M RNA-encoded 48K and/or 58K proteins in infected cells, antisera were prepared against two peptides corresponding to the theoretical carboxy-terminal
Expression of CPMV M RNA in vivo

Fig. 1. Model for the expression of CPMV M RNA. The long open reading frame of M RNA is indicated with an open bar on which the positions of the initiation codons used for translation are shown. The proteins are represented by single lines and the cleavage sites indicated by ©, glutamine-methionine and \( \nabla \), glutamine-glycine.

Fig. 2. Detection of the 60K precursor to both capsid proteins in CPMV-infected protoplasts. Protoplasts inoculated with whole virus (lanes 2 to 5, 7 and 8) or B component alone (lanes 9 and 10) or mock-inoculated (lane 6) were labelled with \[^{35}S\]methionine in the presence (lanes 4, 5, 7 and 9) or absence (lanes 2, 3, 6, 8 and 10) of 2 mM-ZnCl\(_2\). Immunoprecipitations were performed using 5 \( \mu l \) anti-VPg serum (lane 2, this serum also reacted with both capsid proteins, see Zabel et al., 1982) or 5 \( \mu l \) anti-VP23 serum (lanes 4 to 10). The precipitates were analysed in a 12.5\% polyacrylamide gel and the proteins visualized by fluorography. Lane 1 shows the products of translation in vitro of CPMV M RNA. The immunoprecipitation with anti-VPg serum was performed to show the difference between the B RNA-encoded 60K protein [(B)60K, VPg precursor] and the M RNA-encoded 60K capsid protein precursor.

Amino acid sequence of these proteins, as deduced from the M RNA nucleotide sequence (Van Wezenbeek et al., 1983). The primary structure of the peptides S and L is schematically shown in Fig. 3. The reactivity of the antisera obtained after the fourth injection was tested by immunoprecipitation of in vitro translation products of M RNA. Both antisera appeared to be able to recognize the 58K and 48K proteins as well as the 105K and 95K primary translation products (Fig. 4, lanes 1 and 2). The binding to the latter products was less pronounced probably because the antibodies bind to an internal region of these proteins. Preimmune sera did not react with any of the translation products of M RNA (data not shown). After verification of their reactivity and specificity, the two antisera were used to investigate the possible occurrence of M RNA-encoded 48K and 58K proteins in infected cells.

At first, antibodies against peptide S were used to detect these proteins. Two identical protein blots were prepared containing both cytoplasmic (S30) and pellet fractions from protoplasts inoculated with whole virus or with B component alone and from uninoculated protoplasts. The antiserum appeared to react with various proteins, mainly present in the cytoplasmic fraction.
Fig. 3. Structure of the synthetic peptides and their position in the M RNA-encoded proteins. The primary structure of the peptides is shown using the single letter code for amino acids. Prolines, thought to make a large contribution to the structure of the peptides, are underlined. The alanine at the carboxy terminus of peptide S is in parentheses since it is not present at the putative carboxy-termini of the 48K and 58K proteins. \( \triangle \), glutamine-glycine; \( \triangledown \), glutamine-methionine.

(Fig. 5, lanes 10 to 15). The reaction with a number of these proteins was non-specific as these proteins also reacted with antiserum pretreated with excess (100 \( \mu \)g) peptide S (Fig. 5, lanes 2 to 7). Further inspection of these blots revealed a protein reacting specifically with anti-peptide S serum and comigrating with the 48K product produced \textit{in vitro} by M RNA (compare lanes 7, 15 and 16 in Fig. 5). This 48K protein was only found in the pellet fraction of protoplasts inoculated with whole virus and was missing in a similar fraction from protoplasts inoculated with the B component alone (Fig. 5, lane 14). Furthermore a 90K protein, present in low amounts in the same fraction as the 48K protein, reacted specifically with the antiserum. Surprisingly, three B RNA-encoded proteins (170K, 110K and 87K) present in the cytoplasmic fraction also appeared to react specifically with the anti-peptides S serum (Fig. 5, lanes 3 and 11) although there is no amino acid homology between these proteins and the peptide used to prepare the antiserum.

The association of the 48K protein with the pellet fraction was rather strong as it was not possible to solubilize this protein by treatment of the pellet fraction with 1\% Triton X-100 (data not shown). Since one of the plant proteins that reacted non-specifically with the anti-peptide S serum had a size of approximately 60K (see Fig. 5) it was not possible to determine whether an M RNA-encoded 58K protein as predicted by the \textit{in vitro} translation model (Fig. 1) was present in CPMV-infected protoplasts as well. However, with the antiserum raised against peptide L, which did not show the non-specific reaction with a 60K plant protein, only a 48K protein could be detected in the pellet fraction of CPMV-infected protoplasts (see Fig. 6). Again a 90K protein also reacted with this antiserum (Fig. 6, lane 1). The anti-peptide L serum did not react with the B RNA-encoded proteins (data not shown).

Detection of the 48K protein in the membrane fraction of CPMV-infected cowpea leaves

Infected cowpea leaves were also tested for the presence of 48K and 58K proteins. CPMV-infected and uninfected leaves were fractionated into cytoplasmic and membrane fractions (Zabel \textit{et al.}, 1982) which were incubated with anti-peptide L serum. The immunoprecipitates were separated in a 12.5\% SDS-polyacrylamide gel and subsequently blotted onto nitrocellulose filters. When the blot was treated with anti-peptide L serum and \( ^{125}\text{I} \)-labelled Protein A (Fig. 7) a 48K protein was detected in the membrane fraction of infected leaves in agreement with the results obtained with protoplasts, but the 58K protein was again not found (Fig. 7, lanes 4 and 5). Proteins of about 90K and 130K, present in the same fractions as the 48K protein, also appeared to react with this antiserum. Neither the 48K nor the 58K protein could be detected in low-speed pellet fractions (nuclear fraction) or cell wall fractions prepared as described by Godefroy-Colburn \textit{et al.} (1986) of infected leaves (data not shown).
Fig. 4. Immunoprecipitation of \textit{in vitro} translation products of M RNA with anti-peptide sera. Immunoprecipitation with 10 \mu l anti-peptide L serum (lane 1) and 10 \mu l anti-peptide S serum (lane 2) were performed on products translated and processed \textit{in vitro} of CPMV M RNA (lane 3). Immunoprecipitates were separated in a 12.5\% SDS-polyacrylamide gel and the proteins visualized by fluorography.

Fig. 5. Detection of an M RNA-encoded 48K protein in CPMV-infected protoplasts. Protoplasts were inoculated with B and M components (lanes 4, 7, 12, 15), B component alone (lanes 3, 6, 11, 14) or were left uninoculated (lanes 2, 5, 10, 13). Forty h after inoculation, protoplasts were collected and fractionated into pellet fractions (lanes 5, 6, 7, 12, 14, 15) and cytoplasmic (S30) fractions (lanes 2, 3, 4, 10, 11, 12). Two identical protein blots were prepared containing these fractions (each isolated from 2 \times 10^5 protoplasts) separated in a 12.5\% SDS–polyacrylamide gel. Lanes 10 to 15 were incubated with anti-peptide S serum and 125I-labelled Protein A. Lanes 2 to 7 were incubated with the same antiserum, pretreated with excess (100 \mu g) peptide S, and 125I-labelled Protein A. Lanes 1 and 9 contain 35S-methionine-labelled CPMV-infected protoplasts and lanes 8 and 16 35S-methionine-labelled products of CPMV M RNA translated \textit{in vitro}.
Fig. 6. Protoplasts were inoculated with B and M component (lane 1) or B component only (lane 2) or were left uninoculated (lane 3). Forty h after inoculation protoplasts were collected and pellet fractions were isolated. A protein blot was prepared containing these fractions separated in a 12.5% SDS-polyacrylamide gel. The blot was incubated with anti-peptide L serum and 125I-labelled Protein A. Lane 4 contains [35S]methionine-labelled CPMV-infected protoplasts.

Fig. 7. Detection of the 48K protein in the membrane fraction of CPMV-infected cowpea leaves. A total leaf extract (lane 2) was fractionated into cytoplasmic (lane 3), membrane (lane 4), washed membrane (lane 5) and membrane wash fractions (lane 6). Immunoprecipitations with 10 µl of anti-peptide L serum were performed on these fractions which each contained approximately 50 µg of protein. The precipitates were separated in a 12.5% SDS-polyacrylamide gel and subsequently blotted onto nitrocellulose filters. The blot was incubated with anti-peptide L serum and 125I-labelled Protein A. Lane 1 contains the precipitate of the anti-peptide L serum and a membrane fraction of uninfected leaves. Lane 7 contains [35S]methionine-labelled products of CPMV M RNA translated in vitro.

Fig. 8. Detection of the 48K protein in the culture medium of infected protoplasts. Protoplasts were inoculated with complete virus (B + M) (lanes 3, 5 and 7), B components only (lanes 2 and 4), or were left uninoculated (lanes 1 and 6) and collected 40 h later. Immunoprecipitations were performed on 300 µl of the culture medium that had contained about 3 x 10^5 protoplasts (lanes 1 to 5) and on a cytoplasmic fraction isolated from about 2 x 10^5 protoplasts (lanes 6 and 7) using 10 µl anti-peptide L serum (lanes 1, 2 and 3) or 5 µl anti-24K serum (lanes 4 to 7). The precipitates were separated in a 12.5% SDS-polyacrylamide gel and subsequently blotted onto nitrocellulose filters. The blot containing lanes 1 to 3 was incubated with anti-peptide L serum and 125I-labelled Protein A while that containing lanes 4 to 7 was incubated with anti-24K serum and 125I-labelled Protein A.
Expression of CPMV M RNA in vivo

Possible excretion of the 48K protein from cowpea protoplasts

It has been suggested that the 48K protein may have a function in the cell-to-cell transport of virus (or viral RNA) in cowpea leaves (Rezelman et al., 1982). Therefore we examined whether this protein was excreted by infected protoplasts. Considerable amounts of the 48K protein could indeed be precipitated by anti-peptide L serum from the culture medium (Fig. 8, lane 3). Some larger products of about 90K and 130K were also detected in this medium. To test for proteins in the medium originating from damaged protoplasts, the culture medium was tested for the presence of other viral proteins. No proteins were detected using the anti-24K serum which specifically reacts with B RNA-encoded 170K, 110K, 84K and 24K proteins (Wellink et al., 1987) (Fig. 8, lanes 4 and 5). It is possible that the 48K protein was much more stable in the medium than other viral proteins, but it appears more plausible that the 48K protein was specifically excreted by the protoplasts into the medium.

DISCUSSION

Of the two RNAs that make up the genome of CPMV, the expression of B RNA has been extensively studied in vivo as well as in vitro (Goldbach & van Kammen, 1985). Analyses of the in vivo translation of M RNA have been hampered because the only M RNA-specified products found in vivo were the two mature capsid proteins VP37 and VP23. As a result the expression of M RNA has almost exclusively been studied in in vitro translation systems. The in vitro data (Fig. 1) have now gained substantial support by the detection in infected cells of a CPMV M RNA-encoded 48K protein and a 60K precursor to both capsid proteins similar to the proteins produced in vitro by proteolytic processing of the M RNA-encoded 95K primary translation product.

The 60K precursor was only detectable in CPMV-infected cowpea protoplasts when they were incubated in the presence of 2 mM-ZnCl₂. It is possible that normally the 60K capsid precursor is rapidly cleaved in vivo into the mature capsid proteins VP23 and VP37, while in the presence of Zn²⁺ ions the protease (or alternatively the production of the protease) responsible for this cleavage is inhibited, thus causing the accumulation of the unstable precursor. Zn²⁺ ions are indeed known to interfere with the processing of the CPMV polyproteins by inhibiting the cleavage of the B RNA-encoded 170K precursor into 110K and 60K proteins (Peng & Shih, 1984; J. Wellink, unpublished results) as well as the cleavage of the M RNA-encoded polyproteins in vitro (Pelham, 1979; Franssen et al., 1982).

Two initiation sites are used during the translation of M RNA in vitro resulting in primary translation products of 105K and 95K (Pelham, 1979; Franssen et al., 1982; Vos et al., 1984). These proteins are cleaved by a B RNA-encoded protease into 58K and 48K proteins together with the 60K capsid precursor protein discussed above (see Fig. 1; Pelham, 1979; Franssen et al., 1982, 1984). By employing antibodies raised against synthetic peptides the 48K but not the 58K protein has now been detected in infected cowpea leaves and protoplasts. Since the 58K and 48K protein have identical carboxy termini it is not very likely that only the carboxy terminus of the 58K protein is processed (this would make it impossible for our antisera to react with this protein). Therefore such hypothetical modification does not provide the reason for our failure to detect this protein in infected cells. This raises the question whether the AUG codon at position 161 at the beginning of the long open reading frame of M RNA is actually used for translation in vivo. When M RNA is translated in vitro, the 95K protein is always produced in greater quantities than the 105K protein, thus indicating that, in vitro, there is a preference for the internal initiation codons (at positions 512 and/or 524) over the 5' proximal AUG at position 161. Both internal initiation codons indeed conform better to the Kozak rule (Kozak, 1984; Van Wezenbeek et al., 1983). It is therefore possible that in vivo the AUG codon at position 161 is rarely or never used. Alternatively the 105K and 95K proteins may be produced in more or less the same proportion as in vitro, but upon processing the 58K protein is rapidly degraded, whereas the 48K protein is accumulated. This idea is supported by the amino-terminal rule described by Bachmair et al. (1986), if one assumes that the amino-terminal methionine, coded for by the initiation codon, is removed from the 58K and 48K proteins [as is the case with the amino-terminal methionine of the CPMV B RNA-encoded 200K polyprotein (Wellink et al.,]
This would result in a 58K protein with an amino-terminal phenylalanine (predicted to be unstable; Bachmair et al., 1986) and a 48K protein with an amino-terminal serine (predicted to be stable, Bachmair et al., 1986) when the AUG codon at position 524 is used as the initiation codon. Since all comoviruses tested so far produce two polyproteins upon in vitro translation of their M RNAs (Goldbach & Krijt, 1982), it is possible that they all follow this strategy. This idea is further supported by the sequence of the M RNA of red clover mottle virus (RCMV), another comovirus (Shanks et al., 1986). A considerable amount of amino acid homology was detected between the proteins encoded by the M RNAs of CPMV and RCMV, except for the region coding for the amino terminus of the CPMV 105K protein. In fact, the homology with the RCMV proteins starts just behind the AUG codon of the 95K protein (Shanks et al., 1986). Also for RCMV, the amino-terminal rule predicts an unstable 58K protein and a stable 48K protein. But why do comoviruses use two initiation sites on their M RNA resulting in a stable and an unstable protein with partially overlapping sequences? It may be possible that by the use of several initiation sites, the M RNA of these viruses is translated more efficiently, thus producing the large amounts of capsid proteins needed to encapsidate the viral RNA.

A 95K primary translation product has not been detected in cowpea leaves or in protoplasts, either with antisera against the capsid proteins or with antisera against the synthetic peptides, presumably because it is rapidly cleaved into the 48K protein and the 60K capsid precursor. In fractions that contained the 48K protein, proteins of 90K and 130K were also found to react specifically with the anti-peptide sera. Although their origin is unknown, their sizes suggest that they are dimers and trimers of the 48K protein.

A 95K primary translation product has not been detected in cowpea leaves or in protoplasts, either with antisera against the capsid proteins or with antisera against the synthetic peptides, presumably because it is rapidly cleaved into the 48K protein and the 60K capsid precursor. In fractions that contained the 48K protein, proteins of 90K and 130K were also found to react specifically with the anti-peptide sera. Although their origin is unknown, their sizes suggest that they are dimers and trimers of the 48K protein.

The 48K protein was detected by antisera directed against both peptide S and peptide L. Both antisera reacted non-specifically with several plant-encoded proteins. The anti-peptide S serum also reacted specifically with three CPMV B RNA-encoded proteins, but at the moment we cannot explain this reaction.

The 48K protein was found to be present in the membrane fraction of both infected leaves and protoplasts. The association with membranes was strong since treatment of the membranes with 1% Triton X-100 did not solubilize the protein. Additionally the 48K protein and its presumptive multimers were detected in medium in which infected protoplasts had been cultured, whereas no other non-structural proteins could be detected in this medium. The presence of free 48K protein seems not to be due simply to leakage of damaged protoplasts, but may be the result of specific excretion. It has been suggested that the 48K protein is involved in transport of virus particles or RNA throughout leaves (Rezelman et al., 1982). The presence of this protein in membranes of infected cells as well as in culture medium is consistent with this idea. Furthermore the 48K protein shows limited homology to the 30K protein of tobacco mosaic virus (Meyer et al., 1986) which is thought to be involved in cell-to-cell transport (Leonard & Zaitlin, 1982; Ohno et al., 1983; Zimmern & Hunter, 1983).

We thank Job Tielens for photography and artwork, and Gré Heitkönig for typing the manuscript. This work was supported by the Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from the Netherlands Organization for the advancement of Pure Research (Z.W.O.). M.J. was the recipient of a fellowship from the Commission of the European Communities in the molecular biology and radiobiology program.

REFERENCES


Expression of CPMV M RNA in vivo


(Received 27 April 1987)