Vol. 99, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 16, 1981 Pages 89-94

COMPARISON OF IN VIVO AND IN VITRO TRANSLATION OF COWPEA MOSAIC VIRUS RNAs

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Received January 9,1981

SUMMARY. The translation products from Cowpea Mosaic Virus (CPMV) RNAs obtained in two different cell-free systems were compared with the viral polypeptides synthesized in CPMV-infected cowpea protoplasts. It was shown that in both the wheat germ system and the rabbit reticulocyte lysate CPMV M component RNA was translated into two polypeptides of 105,000 and 95,000 dalton, which were not detected in CPMV-infected protoplasts. B component RNA however, gave different products depending on the system used. In the reticulocyte system this RNA was translated into a 200,000 dalton polypeptide which was further cleaved to give 170,000 and 32,000 dalton polypeptides. In the wheat germ system this processing step was lacking as only the 200,000 dalton product was formed. Since the 170,000 and 32,000 dalton polypeptides were also found in CPMV-infected protoplasts the two *in vitro* systems used apparently represent different stages of the expression of the B component RNA, thus providing a tool to study the mechanism of CPMV gene expression *in vivo*.

INTRODUCTION. The single-stranded RNA genome of Cowpea Mosaic Virus (CPMV) type member of the comovirus group, is divided between two separate (middle and bottom) nucleoprotein particles (1, 2). Both middle (M) and bottom (B) component are necessary for infection (3, 4) indicating that the genetic information is distributed between both M and B RNAs (mol. wts. respectively 1.37×10^6 and 2.02×10^6 (5)). The RNAs both possess a 3'-terminal poly A tail (6) and a protein covalently attached to their 5'-termini (7, 8).

We have previously shown (9, 10) that CPMV infection of mesophyll protoplasts isolated from the natural host cowpea (*Vigna unguiculata* L.) results in the synthesis of at least eight virus-specific polypeptides of mol. wts. 170,000, 110,000, 87,000, 84,000, 60,000, 37,000, 32,000 and 23,000. Apart from the capsid proteins (mol. wts. 37,000 and 23,000) all virus-specific polypeptides are formed in protoplasts inoculated with purified B component,

Abbreviations: CPMV, Cowpea Mosaic Virus; mol. wt., molecular weight; SDS, sodium dodecyl sulfate.

0006-291X/81/050089-06$01.00/0

suggesting that they are all coded by B RNA. Peptide fingerprint experiments using *Staphylococcus aureus* V8 protease, have shown that the 110,000, 87,000 84,000 and 60,000 dalton polypeptides have amino acid sequences in common with the 170,000 dalton polypeptide, strongly suggesting that they are derived from the 170,000 dalton polypeptide by post-translational cleavages. To verify this we have compared these viral polypeptides with the translation products of both B and M RNA obtained in two different cell-free systems, the wheat germ system and the rabbit reticulocyte lysate.

MATERIALS AND METHODS. *Virus and RNA.* CPMV was grown in *Vigna unguiculata* L. 'Blackeye early Ramshorn' and purified as previously described (11, 12). The M and B components were separated by threefold centrifugation in linear (15-30%) sucrose gradients (Beckman Ti 15 rotor, 16 hours, 23,000 rpm at 10°C). CPMV RNAs were extracted from separated components as described (13).

Translation in wheat germ extracts. Commercial wheat germ (General Mills Inc., Vallejo, Calif.) was extracted as described by Davies *et al.* (14). RNA (1 - 2 µg) was added to 30 µl reaction mixtures containing 15 µl wheat germ extract, 20 mM Hepes-KOH pH 7.5, 2.9 mM Mg-acetate, 90 mM K-acetate, 0.4 mM spermidine-HCl, 2.5 mM ATP, 0.375 mM GTP, 10 mM creatine phosphate, 10 µg/ml creatine kinase, 2 mM dithiothreitol, 5 µg/ml human placental RNase inhibitor (15, 16), 37.5 mM of each amino acid (except methionine) and 5 to 10 µCi [^{35}S]methionine (1000-1100 Ci/mmole, Radiochemical Centre, Amersham). Incubation was for 1 hour at 30°C.

Translation in reticulocyte lysates. Translation in rabbit reticulocyte lysates (a generous gift of Dr. H.R.B. Pelham) was performed as described in detail (17). In all experiments the dithiothreitol concentration was 2 mM and CPMV RNAs were added to 60 µg/ml. [^{35}S]methionine was used as radioactive amino acid. Incubation was for 1 hour at 30°C.

Preparation of cowpea protoplasts, inoculation with CPMV components and labelling of proteins. Cowpea mesophyll protoplasts were isolated and inoculated with CPMV components as described in detail elsewhere (10). For labelling of viral proteins, portions of protoplasts (2.5 x 10^6 in 5 ml culture medium) were incubated in the presence of 150 µCi [^{35}S]methionine between 20 and 25 hours after infection. At the end of the incubation 30,000 x*g* supernatant fractions suitable for gel electrophoresis were prepared as described (18).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Equal amounts of labelled proteins (as measured by acid-precipitable radioactivity) were mixed with a half volume of a three times concentrated solution of sample buffer (10) and heated for 3 min at 95°C. Samples were analysed in polyacrylamide gels containing 12.5% acrylamide and 0.09% bisacrylamide as previously described (19).

RESULTS AND DISCUSSION.

In the polyacrylamide gel of Fig. 1 the translation products of both CPMV M and B RNA as obtained in the rabbit reticulocyte lysate are electrophoresed along with the viral polypeptides from CPMV-infected cowpea protoplasts.

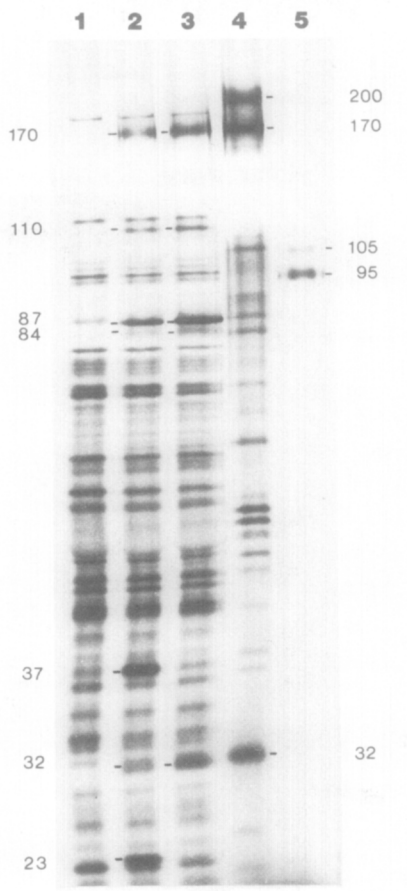

Fig. 1. Comparison on a 12.5% SDS-polyacrylamide gel of ^{35}S-labelled proteins from CPMV-infected cowpea protoplasts with the CPMV RNA *in vitro* products obtained in the rabbit reticulocyte system. Protoplasts were either mock-infected (lane 1), inoculated with purified B components (lane 3) or inoculated with complete virus (M and B components) (lane 2). Lanes 4 and 5 contain the translation products of CPMV B and M RNA respectively, synthesized in the rabbit reticulocyte system. The numbers indicated at the left side of the autoradiogram refer to the mol. wts. (x 10^{-3}) of the virus-specific polypeptides synthesized in protoplasts; the numbers indicated at the right side refer to the mol. wts. (x 10^{-3}) of the main products synthesized in the reticulocyte system. The endogeneous activity (no RNA added) of the reticulocyte lysate used was undetectable.

In the reticulocyte system B RNA is translated into a polypeptide with an estimated mol. wt. of 200,000, which is then cleaved into two polypeptides of mol. wt. 170,000 and 32,000 (ref. 17 and Fig. 1, lane 4). The latter two polypeptides appear to comigrate with the 170,000 and 32,000 dalton B component-specific polypeptides found in infected cells (cf. lanes 3 and 4

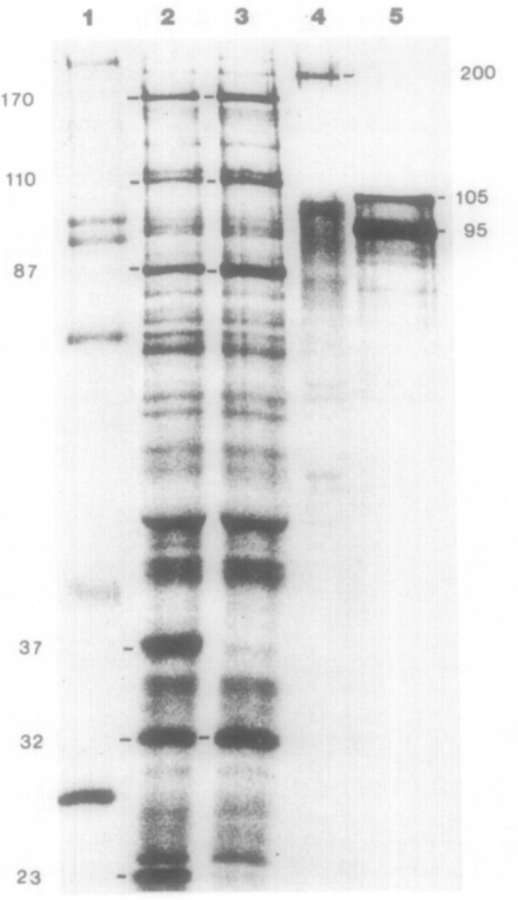

Fig. 2. Electrophoretic analysis of the CPMV RNA *in vitro* products obtained in the wheat germ cell-free system. Lane 1 contains ^{14}C-methylated marker proteins (see below); lanes 2 and 3 contain the ^{35}S-labelled polypeptides from protoplasts inoculated with complete virus (M + B) or B components respectively; lanes 4 and 5 contain the translation products of B and M RNA respectively obtained in the wheat germ system. The endogeneous activity of the wheat germ system was undetectable. The numbers indicated at the left side of the autoradiagram refer to the mol. wts. (x 10^{-3}) of the virus-specific polypeptides synthesized in cowpea protoplasts; the numbers indicated at the right side refer to the mol. wts. (x 10^{-3}) of the products synthesized in the wheat germ system. The ^{14}C-methylated marker proteins (The Radiochemical Centre, Amersham) used were myosin (210,000), phosphorylase b (100,000 and 92,500), bovine serum albumin (68,000), ovalbumin (46,000) and carbonic anhydrase (30,000).

in Fig. 1). By peptide fingerprint experiments it has been shown (10) that these polypeptides are indeed identical. On the other hand translation of M RNA in the reticulocyte system results in the synthesis of two polypeptides with mol. wts. of 105,000 and 95,000, which do not comigrate with any

of the virus-specific polypeptides synthesized in infected cells (cf. lanes 2 and 5 in Fig. 1). Apparently no mature coat proteins (mol. wts. 37,000 and 23,000; see Fig. 1 lane 2) are produced under the direction of CPMV RNAs. The same is true for the wheat germ cell-free extract. In this system M RNA is translated into the same two polypeptides (mol. wts. 105,000 and 95,000) as produced in the reticulocyte lysate (Fig. 2, lane 5). On the other hand translation of B RNA in the wheat germ system does not give the 170,000 and 32,000 dalton polypeptides as found in the reticulocyte lysate, but only the 200,000 dalton primary product (Fig. 2, lane 4). This difference can be explained by the processing mechanism supposed to be present in the reticulocyte system (17) and apparently absent in the wheat germ system. According to our model for the *in vivo* translation of B RNA (10) in a primary processing step the B RNA-coded 200,000 dalton polypeptide is cleaved to give a 32,000 and a 170,000 dalton polypeptide, the latter being further cleaved into at least four smaller polypeptides. The use of both the wheat germ system - in which no post-translational processing occurs - and the reticulocyte system - producing two correct *in vivo* proteins by post-translational processing - may therefore be of great value in determining the mechanism of CPMV gene expression *in vivo*. Early stages of the expression of at least the B component RNA, are represented in both *in vitro* systems, providing the means to study the initial products and the activity responsible for at least the primary cleavage of the 200,000 dalton protein. So far, the contribution of M RNA has remained the more obscure as *in vitro* translation does not give any viral protein as found in CPMV infected cells. In view of the faithful translation of B RNA, the M RNA-specific *in vitro* polypeptides probably represent correct primary translation products which remain undetectable in infected cells.

ACKNOWLEDGEMENTS. The authors wish to thank Dr. H.R.B. Pelham for the gift of rabbit reticulocyte lysates, Dr. P. Blackburn for the gift of human pla-

cental RNase inhibitor, Dr. J. Stanley for critical reading of the text, Dr. A. van Kammen for his advice and interest and Mrs. A. Bruins 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.).

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