TRANSLATION OF POTATO VIRUS X RNA INTO HIGH MOLECULAR WEIGHT PROTEINS

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1. Introduction

Potato virus X (PVX) is a flexous rod-shaped virus containing a single molecule of genomic RNA of 2×10^6 mol. wt [1]. Recently some data on the structure and messenger activity of PVX RNA has become available: this RNA has a m^7 GpppG cap at the 5′-terminus and its 3′-end is not polyadenylated [2]. Furthermore PVX RNA is an active template for in vitro protein synthesis in wheat germ extract; the largest translation product coded by PVX RNA was found to have an app. mol. wt 110 000 [3].

We have used an mRNA-dependent reticulocyte lysate and a wheat germ extract to study the polypeptides coded by PVX RNA. In both systems PVX RNA was translated into two high molecular weight proteins of 180 000 and 145 000. Experimental evidence indicates that these two proteins share common amino acid sequences and that their synthesis may occur by a mechanism similar to that of TMV RNA or TYMV RNA-coded polypeptides.

2. Materials and methods

TMV RNA was prepared as in [4]. TYMV RNA was a kind gift of Dr A. L. Haenni, Paris. m^7GTP was kindly synthesized for us by Dr J. Kuśmierek of this Institute. Trypsin and rabbit liver tRNA were obtained from Sigma and Gibco, respectively. [^{35}S]-Methionine (spec. act. 1000 Ci/mmol) was from Amersham. Medical X-ray films were from Foton (Poland).

Abbreviations: PVX, potato virus X; TMV, tobacco mosaic virus; TYMV, turnip yellow mosaic virus; m^7GTP, 7-methylguanosine-5′-triphosphate; SDS, sodium dodecyl sulphate

2.1. *Preparation of potato virus X and its RNA*

PVX^n strain, isolated from potato variety Bintje, originated from the collection of the Potato Institute, Młochów. The virus was purified from infected leaves of *Nicotiana tabacum* var. Samsun essentially as in [5]. Briefly, leaves were homogenized in 0.1 M sodium citrate (pH 7.5) containing 10 mM cysteine (2 ml buffer/g leaves). The homogenate was centrifuged for 15 min at 10 000 × *g* and the virus purified from the supernatant by 2 cycles of precipitation with polyethylene glycol 6000 (6%, Koch-Light) and NaCl (0.25 M). The precipitate was suspended in 10 mM sodium citrate (pH 7.5) and spun at 100 000 × *g* for 2 h. Finally, the viral particles suspended in 10 mM sodium citrate, 5 mM EDTA (pH 7.5) were sedimented at 150 000 × *g* for 2 h through a 30% sucrose cushion made in the same buffer. The purified virus preparations had an A_{260}/A_{280} ratio of 1.20 and were free of non-viral components as judged by electron microscopy.

RNA was extracted from the purified virus with phenol–SDS [6]. The alcohol pellet of PVX RNA was dissolved in H_2O and stored at −70°C. Analysis of this RNA preparation in a 4% polyacrylamide gel containing 98% formamide [4] indicated a single component migrating as a sharp band with a mobility identical to that of TMV RNA (mol. wt 2.0×10^6).

2.2. *Cell-free protein synthesis*

Translation conditions in exogenous mRNA-dependent rabbit reticulocyte lysates [7] were as in [4]. Assays contained 100 μg rabbit liver tRNA/ml. Potassium acetate was 200 mM, except when indicated otherwise. Incubations were carried out at 25°C for 90 min. Translation in a wheat germ system was as in [4]. Assays were incubated for 120 min at 30°C in the presence of 100 mM K^+. Viral RNAs used in both

systems were 50 μg/ml. For translation assays [^{35}S]-methionine diluted with unlabeled methionine was added at 1 μM (70 Ci/mmol) to reticulocyte lysates and at 10 μM (20 Ci/mmol) to wheat germ extracts.

2.3. *Polyacrylamide gel electrophoresis and tryptic peptide analysis*

Aliquots (1 μl for assays with PVX or TMV RNAs and 2 μl with TYMV RNA) of the in vitro translation mixtures were analysed by electrophoresis in 10% or 8% polyacrylamide–SDS slab gels [8]. Dried gels were subjected to autoradiography.

Tryptic peptide mapping of PVX RNA-coded proteins was performed by the two-dimensional electrophoretic method [9]. A 10 mm-wide lane of the first gel (8%) was excised and polymerized horizontally into the stacking gel of the second gel containing 12.5% acrylamide–SDS as the separating gel. The stacking gel was overlayed with trypsin (30 μg, dissolved in 0.4 ml sample buffer) and electrophoresis carried out at 10 mA for 15 h. The gel was processed for fluorography as in [10].

3. Results

Addition of PVX RNA into an mRNA-dependent reticulocyte lysate resulted in an ~50-fold stimulation of [^{35}S]methionine incorporation into proteins. When translation products coded by PVX RNA were analysed by polyacrylamide–SDS gel electrophoresis, two predominant polypeptides were observed. Their molecular weights were estimated as 180 000 and 145 000 (fig.1(1)). Kinetic experiments excluded the possibility that 180 000 mol. wt protein is the precursor of the shorter 145 000 mol. wt polypeptide (results not shown). The electrophoretic pattern of proteins coded by PVX RNA in a reticulocyte lysate resembles those of products coded by genomic TMV and TYMV RNAs. TMV and TYMV are translated into pairs of high molecular weight products of 165 000 and 110 000, and 195 000 and 150 000, respectively (fig.1(2,3) and [4,7,11–13]).

Wheat germ extracts also supported the translation of PVX RNA into 180 000 and 145 000 mol. wt proteins (fig.1(4)). However, in this system larger amounts of polypeptides of lower molecular weights, most likely the products of premature termination [14], were also synthesized. TMV RNA did not code in a wheat germ extract for appreciable amounts of

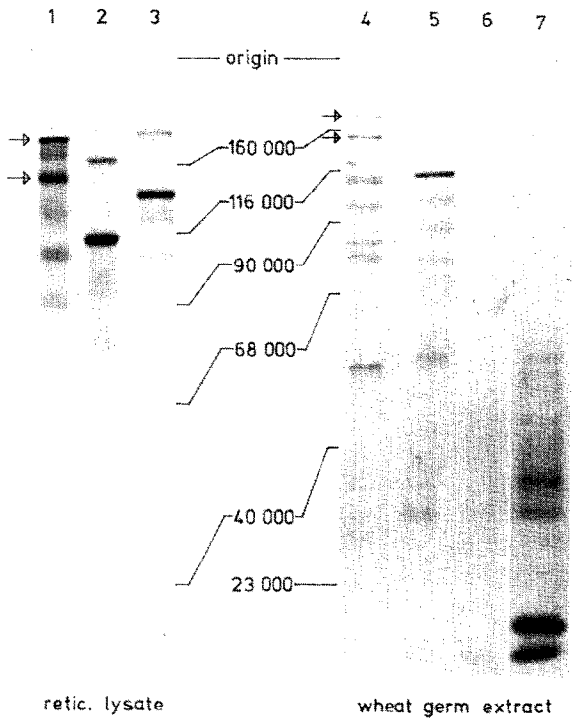

Fig.1. Translation products synthesized in vitro in the reticulocyte lysate (slots 1–3) and wheat germ (4–7) systems in response to PVX (1,4), TMV (2,5) and TYMV (3,7) RNAs. In slot 6 no RNA was added. Electrophoresis was run in an 8% (1–3) or 10% (4–7) polyacrylamide gel. Positions of marker proteins (*E. coli* RNA polymerase polypeptides, mol. wt 160 000, 90 000 and 40 000; β-galactosidase, 116 000; bovine serum albumin, 68 000; PVX coat protein subunit, 23 000) are indicated. PVX RNA-coded proteins of mol. wt 180 000 and 145 000 are marked with arrows. According to our estimate the shorter TYMV RNA-coded protein has mol. wt 140 000.

the larger, 165 000 mol. wt protein (fig.1(5) and [4,14,15]), while TYMV RNA did not stimulate the synthesis of either the 195 000 or the 150 000 mol. wt polypeptides (fig.1(7) and [16]). (The main product directed by TYMV RNA in a wheat germ extract was the virus-specific coat protein, the product of translation of the subgenomic coat protein mRNA present in the preparation of TYMV RNA [17,18]. This protein was also synthesized in a reticulocyte lysate but it has run off the 8% acrylamide gel.)

Similarly to the results in [3], no band corresponding in mobility to that of the PVX coat protein subunit was observed among the proteins coded by PVX RNA either in a wheat germ extract (fig.1(4))

or in a reticulocyte lysate (results not shown). This was also true when translation conditions (K^+ and Mg^{2+} concentrations, amount of RNA template and temperature of incubation) were varied (results not shown). If synthesized, the coat protein (mol. wt

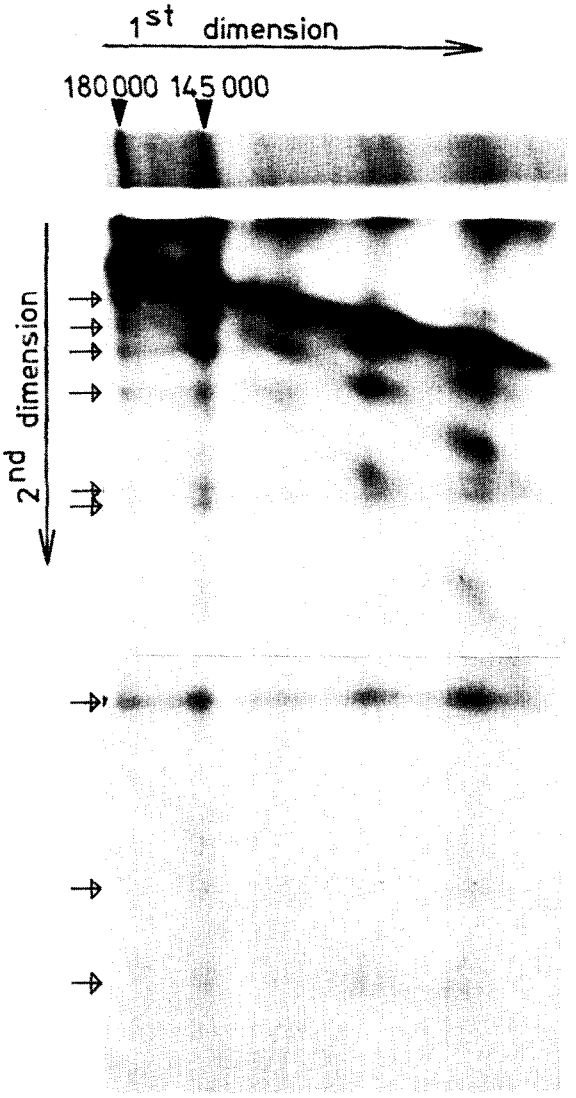

Fig.2. Analysis of trypsin digestion products of proteins coded by PVX RNA in a reticulocyte lysate. The two-dimensional gel electrophoresis was performed as in section 2. The autoradiogram of one-dimensional pattern of PVX RNA-coded proteins is shown at the top of the two-dimensional gel. Only the upper part (containing all the translation products; see fig.1(1)) of the first dimension gel was submitted to electrophoresis in the second dimension. Horizontal arrows indicate tryptic peptides common to mol. wt 180 000 and 145 000 polypeptides which are marked with triangles.

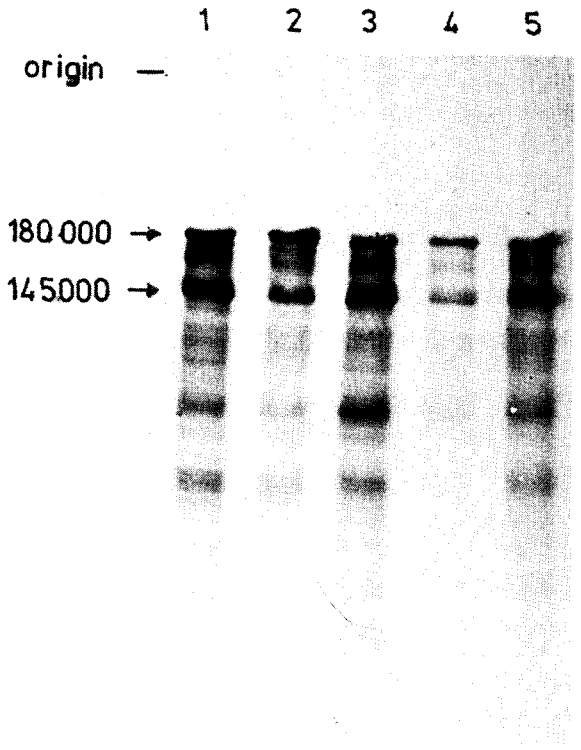

Fig.3. The effect of m^7GTP on translation of PVX RNA in a reticulocyte lysate. Assays were carried out at 40 mM (slots 1,2) or 200 mM (3–5) K^+ in the presence of 0.2 mM m^7GTP (2,4) or 0.2 mM GTP (5) or without nucleotide addition (1,3). Electrophoresis was run in 10% acrylamide gel. PVX RNA-coded proteins of mol. wt 180 000 and 145 000 are marked.

23 000) should be visible among the [^{35}S]methionine-labeled polypeptides as it contains 6 methionine residues [19].

Since the sum of molecular weights of the 180 000 and 145 000 polypeptides greatly exceeds the coding potential of PVX RNA, the nucleotide sequences coding for these two proteins are expected to overlap. Tryptic peptide mapping (fig.2) indicated that all PVX RNA-coded products generate many tryptic peptides with identical electrophoretic mobilities. Hence the 180 000 and 145 000 mol. wt proteins share common amino acid sequences. In fig.3 it is shown that at 200 mM K^+ the synthesis of the two PVX-specific high molecular weight proteins is inhibited by 0.2 mM m^7GTP. At low (40 mM) K^+, i.e., under conditions of low dependence of translation of 5′-terminal 7-methylguanosine [4,20], the effect of m^7GTP was much less pronounced. Addi-

tion of 0.2 mM GTP was without effect at either K^+ concentration. The requirement of 5′-terminal cap for the synthesis of the 180 000 and 145 000 mol. wt proteins may indicate that translation of both proteins starts near the 5′-terminus of RNA.

From the autoradiogram shown in fig.3 it is also evident that addition of a cap analog to the translation assay changes the ratio of 180 000/145 000 mol. wt protein in favor of the larger polypeptide. A similar change was observed when the amounts of 165 000 and 110 000 mol. wt proteins coded by TMV RNA in the presence of m^7GTP were measured (unpublished). The explanation of this phenomenon is not obvious at the moment. The presence of a cap analog may favor the synthesis of longer polypeptides in response to alfalfa mosaic virus RNA 1 [21].

4. Discussion

Translation of PVX RNA in a reticulocyte lysate or wheat germ extract results in the synthesis of 180 000 and 145 000 mol. wt proteins. The in vitro synthesis of PVX RNA-coded polypeptides of similar molecular weights was independently observed by U. Szybiak and A. B. Legocki (personal communication). Furthermore, two proteins of the same molecular weights represent the main translation products of PVX RNA in the S27 extract of yeast spheroplasts [22].

The results presented above could suggest that the strategy of translation of PVX genome RNA is similar to that of TMV and TYMV RNAs, two plant viral genomic RNAs of similar size. Each of these three viral RNAs codes in vitro for a pair of high molecular weight polypeptides sharing common amino acid sequences. In the case of TMV and TYMV RNAs it has been shown that two high molecular weight proteins are initiated at the same site on each RNA molecule [13,23]. For TMV RNA evidence was also presented that the larger protein is a read-through translation product of a leaky termination codon [13]. Although the exact mechanism of synthesis of the 180 000 and 145 000 mol. wt proteins coded by PVX RNA remains unknown, it is not unlikely that these polypeptides are also initiated at a single AUG codon positioned close to the 5′-terminus of the RNA. Sensitivity of translation of both PVX RNA-coded proteins to cap analogs offers some support to this notion.

The similarity in strategy of gene expression of TMV, TYMV and PVX RNAs may extend to the mode of synthesis of viral coat proteins ([3] and this work). In the case of genomic TMV and TYMV RNAs the coat protein gene, positioned near the 3′-end of RNA molecule, is silent; its expression requires the formation of subgenomic coat protein mRNA [11,17,18].

The comparison of the translation products of three plant viral genomic RNAs in two different cell-free systems deserves some comment. While reticulocyte lysates were able to translate efficiently all viral RNAs into pairs of heavy molecular weight proteins, large differences in translability of individual RNAs were observed with wheat germ extracts. The most plausible explanation of this phenomenon may be the relatively high level of ribonucleases present in the wheat germ extract [14]. It is not unlikely that certain mRNAs (like e.g., TYMV or TMV genomic RNAs) are more accessible to the action of such nucleases and their translation into high molecular weight polypeptides is therefore less efficient.

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References

[1] Bercks, R. (1970) CMI/AAB Descriptions of Plant Viruses, no. 4, Commonwealth Mycological Institute, Kew, England.
[2] Sonenberg, N., Shatkin, A. J., Ricciardi, R. P., Rubin, M. and Goodman, R. M. (1978) Nucleic Acids Res. 5, 2501–2512.
[3] Ricciardi, R. P., Goodman, R. M. and Gottlieb, D. (1978) Virology 85, 310–314.
[4] Wodnar-Filipowicz, A., Szczęsna, E., Zan-Kowalczewska, M., Muthukrishnan, S., Szybiak, U., Legocki, A. B. and Filipowicz, W. (1978) Eur. J. Biochem. 92, 69–80.
[5] Skrzeczkowski, L. J. and Młotek, T. (1974) Zesz. Probl. Postępów Nauk Roln. 156, 13–23.

[6] Marcus, A., Efron, D. and Weeks, D. P. (1974) Methods Enzymol. 30, 749–754.
[7] Pelham, H. R. B. and Jackson, R. J. (1976) Eur. J. Biochem. 67, 247–256.
[8] Laemmli, U. K. (1970) Nature 227, 680–685.
[9] Bordier, C. and Crettol-Järvinen, A. (1979) J. Biol. Chem. 254, 2565–2567.
[10] Bonner, W. M. and Laskey, R. A. (1974) Eur. J. Biochem. 46, 83–88.
[11] Hunter, T. R., Hunt, T., Knowland, J. and Zimmern, D. (1976) Nature 260, 759–764.
[12] Benicourt, C., Péré, J. P. and Haenni, A. L. (1978) FEBS Lett. 86, 268–272.
[13] Pelham, H. R. B. (1978) Nature 272, 469–471.
[14] Hunter, A. R., Farrell, P. J., Jackson, R. J. and Hunt, T. (1977) Eur. J. Biochem. 25, 149–157.
[15] Bruening, G., Beachy, R. N., Scalla, R. and Zaitlin, M. (1976) Virology 71, 498–517.
[16] Benicourt, C. and Haenni, A. L. (1976) J. Virol. 20, 196–202.
[17] Klein, C., Fritsch, C., Briand, J. P., Richards, K. E., Jonard, G. and Hirth, L. (1976) Nucleic Acids Res. 3, 3043–3061.
[18] Pleij, C. W. A., Neeleman, A., Van Vloten-Doting, L. and Bosch, L. (1976) Proc. Natl. Acad. Sci. USA 73, 4437–4441.
[19] Goodman, R. M. (1975) Virology 68, 287–298.
[20] Weber, L. A., Hickey, E. D., Nuss, D. L. and Baglioni, C. (1977) Proc. Natl. Acad. Sci. USA 74, 3254–3258.
[21] Van Tol, R. G. L. and Van Vloten-Doting, L. (1979) Eur. J. Biochem. 93, 461–468.
[22] Szczęsna, E. and Filipowicz, W. (1979) submitted.
[23] Benicourt, C. and Haenni, A. L. (1978) Biochem. Biophys. Res. Commun. 84, 831–839.