Cell-free Translation of Turnip Mosaic Virus RNA

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SUMMARY

RNA from the type strain of turnip mosaic virus (TuMV), a member of the potyvirus group, was translated in vitro in either rabbit reticulocyte lysate or wheat germ extract. We found no evidence for encapsidated, subgenomic TuMV RNA species. A broad size range of L-[35S]methionine-labelled polypeptides with molecular weights up to 200000 (200K) was observed in TuMV RNA-programmed reticulocyte lysates. In contrast, a single polypeptide of 44K predominated in the much narrower spectrum of labelled products, of 55K or less, seen in wheat germ extracts containing TuMV RNA. Time course experiments revealed that, in rabbit reticulocyte incubations, many of the low molecular weight TuMV RNA-encoded polypeptides accumulated only after synthesis of the largest polypeptides (i.e. those ≥100K). When the amino acid analogues p-fluorophenylalanine, L-canavanine and N-tosyl-lysyl-chloromethane were substituted for phenylalanine, arginine and lysine respectively, the major 44K product detected in wheat germ extracts diminished, to be replaced by a product of 92K which co-migrated with the major polypeptide normally observed in reticulocyte incubations. A rapid processing event may occur in standard wheat germ incubations to release the 44K polypeptide from a nascent, elongating precursor. The 44K product did not arise because of limiting amounts of a particular tRNA species. Reticulocyte incubations with or without additional reducing agent showed comparable levels of all the processed TuMV RNA-specific products. We could detect no serological cross-reaction between any of the TuMV RNA-encoded products and antiserum raised against HPLC-purified helper component of tobacco vein mottling virus, another potyvirus.

INTRODUCTION

Turnip mosaic virus (TuMV) is a member of the potyvirus group of plant pathogens with flexuous, rod-shaped particles 720 nm in length (Tomlinson, 1970; Hollings & Brunt, 1981). TuMV has a wide host range, infecting many economically important crop plants, particularly of the Brassica genus. It is sap-transmissible and is spread by over 40 species of aphids. Virus particles contain a single-stranded RNA molecule approximately 10 kb in length (39S), capable of directing TuMV-specific protein synthesis in vitro.

Recent controversies concerning the translation strategy of potyvirus RNAs (Dougherty & Hiebert, 1980b; Otal & Hari, 1983; Dougherty, 1983; Hellmann et al., 1983, 1985) have been more or less resolved in favour of the production of one (Dougherty, 1983; Vance & Beachy, 1984a, b; Yeh & Gonsalves, 1985; Allison et al., 1985) or more (Dougherty et al., 1985) high molecular weight precursor proteins or polyproteins which are proteolytically cleaved after synthesis. Potyviruses examined in these reports have included tobacco etch virus (TEV), pepper mottle virus, soybean mosaic virus, tobacco vein mottling virus (TVMV) and papaya ringspot virus (PRV). To extend these studies to an economically important virus infecting a wide variety of Brassica spp. and as a prelude to work on virus disassembly and early gene expression of TuMV RNA in vivo, we undertook the characterization of potential TuMV-specific gene products in vitro.

Our results demonstrate the post-translational cleavage of a large polyprotein precursor synthesized in a rabbit reticulocyte lysate, whereas rapid proteolytic degradation by an
endogenous protease in our wheat germ (WG) extract resulted in the appearance of a major product of only 44000 mol. wt. (44K), with no clear precursor–product relationship.

**METHODS**

*Purification and characterization of viral RNA.* TuMV (type strain) was kindly supplied by Dr R. Hull from the virus collection of the John Innes Institute. TuMV-infected, dried leaf material was ground in 10 ml sodium phosphate buffer pH 7-0, I = 0-1 and mechanically inoculated with Cellite onto 3-week-old plants of *Brassica campestris* var. rapiflora cv. Just Right. Twenty-eight days later, the systemically infected leaves were harvested, cooled to 4 °C and the virus was extracted by Method 1 of Moghal & Francki (1976). After centrifugation at 78000 g for 75 min, most of the virus failed to resuspend completely in 50 mM-borate buffer pH 8 (Moghal & Francki, 1976) and could be recovered by centrifugation at only 10000 g for 10 min. This pellet of partially pure TuMV was dispersed in 50 mM-borate pH 8 and used as the source of TuMV RNA. RNA was extracted by a modified version of the method of Brakke & van Pelt (1970), similar to that reported by Hellmann et al. (1980). Equal volumes of TuMV suspension and disrupting buffer [DB; 40 mM-Tris-HCl, 2 mM-EDTA, pH 9-0 containing 2% (w/v) SDS and 0-5 mg/ml bentonite] were gently mixed for 60 min at 30 °C before 1 ml samples were layered onto diethylpyrocarbonate-treated sucrose gradients [10 ml; 7-5 to 30% (w/v)] buffered with 10 mM-Tris-HCl pH 7-5 containing 1 mM-EDTA, 0-1 M-NaCl and 0-1% (w/v) SDS. After centrifugation at 38000 r.p.m. for 4 h at 10 °C in a Beckman SW41 rotor, gradients were fractionated and monitored continuously at 254 nm using an LKB Ulvacord S. Fractions containing TuMV RNA were pooled, diluted into 2 vol. 90% (v/v) ethanol and stored at −20 °C for 18 h. Precipitated RNA was recovered by centrifugation at 10000 g for 5 min at −5 °C in a Sorvall RC5B, washed twice with fresh ethanol (−20 °C), dried and dissolved in sterile, double-distilled water. Samples of RNAs were analysed on a 1% (w/v) agarose mini-gel after denaturation in 1 M-glyoxal, 50% (v/v) formamide at 55 °C for 15 min (McMaster & Carmichael, 1977). Co-electrophoresed tobacco mosaic virus (TMV) RNA (6395 nucleotides, *vulgare* strain; Goelet et al., 1982) served as a size marker.

In vitro translation and analysis of L-[35S]methionine-labelled polypeptides. Messenger-dependent rabbit reticulocyte lysate (MDL) was prepared according to Pelham & Jackson (1976). WG cell-free extract, prepared and used as in published procedures (Davies, 1979), was generously provided by Dr J. W. Davies, John Innes Institute. All incubations were at 30 °C for 90 min in a standard reaction volume of 25 μl, unless stated otherwise. Apart from the immunoprecipitation experiments (see below), all reactions contained either TuMV RNA at 40 μg/ml or TMV RNA at 50 μg/ml (final concentrations), and L-[35S]methionine [New England Nuclear; ≥1000 Ci/mmol, supplied in 10 mM-2-mercaptoethanol (2-ME) and stabilized in 50 mM-Tricine pH 7-4] at 0-5 μCi/μl. All other, unlabelled amino acids were added to 50 μM final concentration. In some experiments 25 μM-L-canavanine, 100 μM-N-tosyl-lysyl-chloromethane and 75 μM-p-fluorophenylalanine replaced arginine, lysine and phenylalanine, respectively. Duplicate 3 μl samples were removed from each incubation prior to adding 40 μl of SDS-containing gel-loading buffer (Laemmli, 1970) to the remainder (19 μl) and heating the mixtures at 100 °C for 3 min. The extent of protein synthesis in each 3 μl sample was measured as TCA-insoluble [35S]methionine (Pelham & Jackson, 1976).

The total, final cation concentrations for both the quality and quantity of [35S]-labelled products, were 100 mM-K* and 1-5 mM-Mg* for the MDL system, or 80 mM-K* and 3-0 mM-Mg* for the WG system. The total final concentration of reducing agents in all standard MDL incubations was ≤0-4 mM-dithiothreitol (DTT; contributed by the unlabelled amino acid stock solution) and 0-5 mM-2-ME (from the [35S]methionine). In time course experiments, samples of 3 μl (for measurement of [35S] incorporation) and 19 μl (for gel analysis) were withdrawn and treated as above at various intervals from parallel, large-scale (250 μl) incubations of either WG or MDL.

L-[35S]Methionine-labelled polypeptides were separated in 12-5% (w/v) polyacrylamide gels containing SDS (Laemmli, 1970) at 60 V for 18 h. Gels were fixed in methanol: acetic acid: water (50:10:50, by vol.) for 30 min, washed thoroughly in water and dried onto Whatman 3MM paper. Radiolabelled polypeptides were detected by autoradiography for 1 to 5 days at room temperature on Fuji RX X-ray film.

*Immunoprecipitation with antisera to HPLC-purified helper component (HC) of TVMV.* TuMV RNA or TVMV RNA (a generous gift from Dr J. G. Shaw, University of Kentucky, Lexington, Ky., U.S.A.) were present at 100 μg/ml (final concentration) in large-scale (65 μl) WG or MDL reactions incubated at 30 °C in the presence of 1-0 μCi L-[35S]methionine per μl. After 90 min, 3 μl and 20 μl samples were removed from each tube, as above. Additional duplicate 20 μl samples from each remaining reaction volume were diluted fivefold in 0-15 M-NaCl, 10 mM-Tris–HCl pH 7-0 (TBS) containing 1% (v/v) Triton X-100, 2 mM-EDTA (TBSTE) and 0-5 mg/ml chicken ovalbumin. Eight μl preimmune rabbit serum was added to each diluted sample and the tubes were mixed gently for 60 min at room temperature before addition of either 20 μl Protein A–Agarose (Bethesda Research Laboratories; 3-9 mg Protein A/ml gel, binding capacity 25 mg IgG/ml gel), or 50 μl Immunoprecipitin [Bethesda Research Laboratories; formalin-fixed *Staphylococcus aureus* cells, 10% (w/v)] to each pair of duplicate samples. After a further 60 min at room temperature, the immunoabsorbeds were recovered by centrifugation at 10000 g or
In vitro translation of TuMV RNA

2000 g, respectively. Each pellet was washed in TBSTE buffer (3 x 400 µl) and the supernatant fraction and the washings were pooled and incubated as above, but with 10 µl antiserum to HPLC-purified TVMV HC (Thornbury & Pirone, 1983; Hiebert et al., 1984; kindly supplied by Dr D. W. Thornbury, University of Kentucky) in place of the preimmune serum. All subsequent steps were as described above and are essentially similar to the protocol described by Dougherty & Hiebert (1980a) and used by Hiebert et al. (1984). Each preimmune or anti-TMVHC-specific immunosorbent pellet was resuspended in 50 µl gel loading buffer (Laemmli, 1970) containing 4% (w/v) SDS, and heated to 100 °C for 5 min. After centrifugation at 10000 g for 10 min, the supernatant solutions were carefully removed into clean polypropylene tubes and a 3 µl sample of each was spotted directly onto Whatman GF/C discs. These were then dried in air and the amount of L-[35S]methionine associated with each was measured as before (Pelham & Jackson, 1976). Preimmune and immunospecific, 35S-labelled polypeptides were resolved in 15% (w/v) polyacrylamide gels containing SDS and detected by autoradiography, as described above.

RESULTS AND DISCUSSION
Preparation and fractionation of TuMV RNA

Samples of material extracted from TuMV-infected turnip leaves and used as a source of TuMV RNA were negatively stained with uranyl acetate and viewed in an electron microscope. Large numbers of long, flexuous rod-shaped virus particles were clearly visible, with comparatively little contaminating cellular debris (K. Plaskitt, personal communication; data not shown). Potyvirus extraction Method 1 of Moghal & Francki (1976) thus appeared suitable for the partial purification of TuMV from infected Brassica spp. However, the virus repeatedly failed to resuspend in dilute (50 mM) borate buffer pH 8.0 containing 5 mM-EDTA either alone or with 1% (v/v) Triton X-100, and when followed to completion the published method (Moghal & Francki, 1976) resulted in such low yields of ‘highly-purified virus’ that none could be seen in the electron microscope. Other purification schemes (Choi et al., 1977) proved less successful.

Fig. 1 (a) shows the profile of u.v.-absorbing material separated on 7.5 to 30% (w/v) sucrose density gradients following disruption of partially pure TuMV particles. A single peak of rapidly sedimenting RNA is seen, together with some minor, more slowly migrating species. Gradient fractions corresponding to zones A, B and C (Fig. 1a) were pooled and the RNAs recovered. Two independent preparations of gradient-fractionated TuMV RNA were denatured and subjected to electrophoresis in a 1.0% (w/v) agarose gel (Fig. 1b). In both cases, gradient zone A (Fig. 1a) revealed a single major band of RNA (Fig. 1b, lanes 1 and 4) which migrated more slowly than TMV RNA (6.4 kb, Fig. 1b, lane 7). Gradient zones B and C (Fig. 1a) contained a broad size range of progressively shorter RNA molecules (Fig. 1b, lanes 2, 3, 5 and 6). The single major peak of TuMV RNA from gradient zone A [39S (10 kb); Hiebert et al., 1984] was used in the majority of translation experiments shown below (Fig. 3 to 6).

In vitro translation of TuMV RNA

RNAs from sucrose density gradient zones A to C (Fig. 1a) were translated in vitro in both WG and MDL under standard conditions (see Methods). Fig. 2 demonstrates a significant qualitative difference in the overall size range of TuMV RNA-encoded products in the two cell-free translation systems (compare Fig. 2, lanes 4 and 9). A single major polypeptide of approximately 44K predominated among the few radiolabelled products of 14K to 55K observed in WG incubations (Fig. 2, lane 9). In contrast, MDL incubations revealed a much broader size range of TuMV RNA-encoded polypeptides between 35K and 200K, including more than eight major species, the origin of which is discussed below. For reference, we confirmed that the coat protein of TuMV migrated as a single major band of apparent size 27K with two minor species estimated to be approximately 25.5K and 24.9K (data not shown). The latter may be proteolytically degraded forms of the major species (Hiebert & McDonald, 1976). Fig. 2 also shows that the non-denatured RNA from sucrose gradient zones B and C did not contain any subgenomic TuMV RNAs. Fig. 2 lanes 5, 6, 10 and 11 did not reveal increased levels of any new L-[35S]methionine-labelled polypeptide(s). What appeared could be accounted for either by a declining contamination with genome-length TuMV RNA (Fig. 2, lanes 4 and 9), or by fortuitous 5' co-terminal fragments of TuMV RNA. In fact, several of the major bands detected in lane 4 did not appear in lanes 5 and 6.
Fig. 1. Purification and analysis of TuMV RNA. (a) Absorbance profile at 254 nm of TuMV RNA following particle disruption and fractionation on a linear 7-5 to 30% (w/v) sucrose gradient. Fractions corresponding to zones A, B or C were pooled and the RNA was recovered. Sedimentation was from right to left. (b) 1.0% (w/v) agarose gel electrophoresis of denatured samples from two independent preparations of TuMV RNA fractionated as in (a). Lanes 1 and 4 contained material from gradient zones A, lanes 2 and 5 from zones B, and lanes 3 and 6 from zones C. Lane 7 shows a TMV RNA size marker (6-4 kb). The gel was stained with ethidium bromide, viewed and photographed on u.v. transilluminator.

Originally it was proposed that the potyvirus TEV possessed seven subgenomic RNAs detected in TEV-infected tissue (Otal & Hari, 1983); however, these were soon shown to be electrophoretic artefacts (Dougherty, 1983). Our interpretation of the translation data in Fig. 2 leads us to conclude that TuMV does not encapsidate any subgenomic mRNAs, although such species may exist in TuMV-infected tissue.

The translation strategy of TuMV RNA

To investigate the origin of the major TuMV RNA-encoded polypeptides shown in Fig. 2, time course experiments were performed in both cell-free translation systems. The progress of incorporation of L-[35S]methionine into TCA-insoluble polypeptides in two parallel, large-scale incubations revealed a reproducible decline in the radioactivity incorporated at later times, particularly in the WG system (data not shown). This seems to indicate that extensive proteolytic degradation had occurred. Endogenous proteases have been characterized in both in vitro systems (Mumford et al., 1981). Alternatively, some proteolysis may be autocatalytic, as with many animal picornaviruses (Jackson, 1986) and several plant viruses, including members of the comovirus group (see references in Franssen et al., 1984). Qualitative analysis (Fig. 3a) of the products of MDL incubations revealed a linear, time-dependent elongation of nascent, 35S-labelled polypeptides up to approximately 200K during the first 40 min, with a steady accumulation of label in the 100K to 200K region of the gel. After this time, many polypeptides of lower molecular weight (20K to 92K) began to appear and accumulate as shown by the solid arrows to the right of Fig. 3(a). These species included at least six of the major TuMV-specific polypeptides routinely observed after the standard 90 min incubation (Fig. 2, lane 4). This result
In vitro translation of TuMV RNA

Fig. 2. In vitro translation of size-fractionated TuMV RNA. Rabbit reticulocyte lysate (lanes 2 to 6) or wheat germ extract (lanes 7 to 11) incubations were programmed with TMV RNA (lanes 3 and 8) or TuMV RNA fractions from sucrose gradient (Fig. 1a) zones A (lanes 4 and 9), B (lanes 5 and 10) or C (lanes 6 and 11). The activities of both cell-free translation systems in the absence of added template RNA are shown in lanes 2 and 7. 14C-labelled marker proteins (Amersham) are shown in lane 1 adjacent to their respective molecular weights ($\times 10^{-3}$). Where possible, equal quantities of TCA-precipitable, $^{35}$S-labelled polypeptides ($3 \times 10^5$ c.p.m.) were loaded on each lane. The dried gel was autoradiographed at room temperature for 3 days.

provides strong evidence for post-translational proteolytic processing in TuMV RNA-programmed MDL incubations. In contrast, time course experiments in WG extracts did not reveal any large, precursor form(s) of the predominant 44K product (Fig. 3b). The latter may therefore arise either by premature termination of translation, or by rapid and efficient proteolysis from a nascent precursor. Experiments to resolve these possibilities are described below. Pulse–chase time course experiments, in which an inhibitor of initiation (edeine) and an excess of unlabelled L-methionine were added after incubation for 10 to 15 min, produced patterns of TuMV-specific polypeptides identical to those shown in Fig. 3, but with less $^{35}$S-labelling of all products (data not shown).

Further support for the view that proteolytic processing is involved in the expression of TuMV-specific polypeptides, particularly in WG extracts, was obtained using several amino
Fig. 3. Time course of translations of full-length TuMV RNA in vitro. Qualitative analysis of L-[^35]S methionine-labelled polypeptides encoded by TuMV RNA in (a) rabbit reticulocyte lysate or (b) wheat germ extract incubated between 5 and 300 min at 30 °C under standard conditions. The positions of co-electrophoresed marker proteins are indicated in lanes M, with their molecular weights (× 10^-3) on the left. Where possible, equal amounts of labelled products (2 × 10^3 c.p.m.) were loaded on each lane. Dried gels were autoradiographed for 5 days at room temperature. TuMV RNA-encoded polypeptides which accumulated and later diminished are indicated in (a) by open triangles on the right. Products which accumulated disproportionately late during incubation are indicated by closed triangles.
Fig. 4. Effects of amino acid analogues or thermal denaturation of TuMV or TMV RNAs on the spectra of polypeptides encoded in vitro. Wheat germ extract (lanes 1 to 7) or rabbit reticulocyte lysate incubations (lanes 8 to 14) were programmed with TMV RNA (lanes 2 to 4 and 9 to 11) or TuMV RNA (lanes 5 to 7 and 12 to 14). The endogenous activities of both cell-free systems are evident in lanes 1 and 8, respectively. Incubations shown in lanes 3, 6, 10 and 13 contained amino acid analogues. Heat-denatured TMV or TuMV RNAs were added to the cell-free incubations shown in lanes 4, 7, 11 and 14. Control incubations under standard conditions are shown in lanes 2, 5, 9 and 12. 14C-labelled marker proteins were co-electrophoresed in lane M and their molecular weights (× 10^{-5}) are shown on the left. Equal volumes from each incubation (containing up to 3 × 10^5 c.p.m. of 35S-labelled polypeptides) were loaded on lanes 1 to 8 and 12 to 14. Lanes 9 to 11 each received 3 × 10^5 c.p.m. 35S-labelled material. The dried gel was autoradiographed for 4 days at room temperature.

acid analogues during in vitro translation experiments. Incorporation of analogues into translation products has been shown to block or slow proteolytic cleavage (Pelham, 1978, 1979; Vance & Beachy, 1984a). Our results are shown in Fig. 4, together with some preliminary evidence which suggests that extensive secondary structure in the RNA template was not responsible for the accumulation of lower molecular weight polypeptides, by ribosome arrest, in either cell-free translation system. Surprisingly, neither amino acid analogues nor heat denaturation of the RNA template (60 °C for 5 min, then rapid chilling on ice immediately before translation), had any marked effect on the polypeptides encoded by either TuMV or TMV RNAs in the MDL system (Fig. 4, lanes 9 to 14). However, in WG extracts the effect of amino acid analogues on both TuMV- and TMV-specific products was considerable (Fig. 4, lanes 3 and 6). This result provides further support for the conclusion that an endogenous protease in WG extracts is responsible for the rapid production of the 44K TuMV-specific
Fig. 5. Influence of DTT or tRNA on the *in vitro* translation of TuMV RNA. Full-length TuMV RNA (Fig. 1a) was added to rabbit reticulocyte lysate (lanes 2 and 3) or wheat germ extract (lanes 5 and 6) incubations, under standard translation conditions (lanes 2 and 5) or with additional DTT (5 mM) (lane 3) or calf liver tRNA (50 μg/ml) (lane 6). The endogenous activities of MDL and WG are evident in lanes 1 and 4, respectively. The positions and mol. wt. (×10⁻³) of ¹⁴C-labelled marker proteins are shown on the left. Equal amounts of ³⁵S-labelled products (1 × 10⁶ c.p.m.) were loaded on lanes 2, 3, 5 and 6. The dried gel was autoradiographed for 6 days at room temperature.

Product. It seems reasonable to deduce, particularly in view of the effects of analogues of both arginine and lysine, that the trypsin-like activity found in WG extracts but not in MDL (Mumford *et al.*, 1981) is the enzyme responsible. This enzyme also appears to degrade the 126K polypeptide encoded by TMV RNA (compare Fig. 4, lanes 2 and 3). It may be coincidental that the putative 'precursor form' of the 44K polypeptide revealed in Fig. 4, lane 6 co-migrated with one of the major, persistent polypeptides (that of 92K) observed in the proteolytically less active MDL system (Fig. 4, lanes 12 to 14).

Evidence that the 44K product in WG extracts did not arise by lack of a minor isoaccepting tRNA species, causing premature termination of translation, is shown in Fig. 5, lanes 5 and 6.
Addition of 50 μg/ml (final concentration) total calf liver tRNA (used in the preparation of MDL) to WG extract had no effect on the spectrum of TuMV-encoded polypeptides (Fig. 5, lane 6).

Fig. 5 also shows the minimal effect of additional exogenous DTT (added to 5 mM final concentration) on MDL incubations programmed with full-length TuMV RNA for 90 min. Additional DTT (lane 3) caused only a slight increase in the levels of all the short TuMV-specific polypeptides, previously seen to be derived by proteolytic processing from a larger precursor (Fig. 3a). In a previous report, using PRV RNA and MDL (Yeh & Gonsalves, 1985), a precursor polypeptide of 330K could be detected in the absence of added DTT. In the presence of 4-8 mM-DTT, an endogenous, sulphhydril (SH)-dependent protease was responsible for at least the primary cleavage of the PRV polypeptide. The incubation mixture analysed in Fig. 5, lane 2, contained less than 0.4 mM-DTT (from the amino acid stock solution); however, there was no increase in the stability or amount of any of the high molecular weight TuMV precursor polypeptides seen at earlier incubation times (Fig. 3a). This result could be interpreted either as evidence for an autocatalytic TuMV polypeptide processing mechanism or to indicate the involvement of a predominantly SH-independent reticulocyte protease, equally active at 5-4 mM- or below 0.4 mM-DTT. In all cases (Yeh & Gonsalves, 1985, and here), it would appear that approximately 0.5 mM-2-ME (from the [35S]methionine stock) was present during incubation.

Serological relationship of TuMV products with TVMV HC

Several potyvirus-coded proteins have been purified from virus-infected plants and used to raise antisera to assist in identifying precursor-product relationships among polypeptides synthesized in vitro. Antisera to partially purified or homogeneous potyvirus-coded HC [proteins involved in aphid transmission (Pirone, 1981)] have been used to delineate serological relationships among viruses of this group (Thornbury & Pirone, 1983; Sako & Ogata, 1981; Hiebert et al., 1984; Thornbury et al., 1985). Hiebert et al. (1984) translated the RNAs from two isolates of TuMV (TuMV 1, aphid-transmissible; and TuMV 31, non-transmissible) in their MDL system and observed, at most, three radiolabelled polypeptides, including a major product of 93K. In both isolates, this large TuMV-specific polypeptide was selectively immunoprecipitated with antiserum to the HPLC-purified HC of TVMV, but not with antiserum to the HC of potato virus Y. We attempted to extend this serological relationship to the in vitro translation products of the type strain of TuMV described above. Dr D. W. Thornbury kindly provided a sample of the HPLC-purified TVMV-HC antiserum used previously (Thornbury & Pirone, 1983; Hiebert et al., 1984). Immunoprecipitation experiments were repeated six times in all, using a variety of published protocols. Parameters which were varied included the time (1 to 18 h) or temperature (4 °C to 20 °C) of incubation, the buffer composition and the wash volumes. We also used Protein A-Sepharose (Pharmacia P-L Biochemicals) to precipitate the non-specific (preimmune) or immunospecific IgG complexes (Martin & Northcote, 1982).

In all cases, the results were identical to those shown in Fig. 6, lanes 2 to 7, where the particular conditions were extremely similar to those of Hiebert et al. (1984), with the exception that we applied preimmune and anti-TVMV HC sera sequentially to each diluted cell-free translation reaction. The immunoprecipitated products shown in Fig. 6, lanes 3, 4, 6 and 7 were detected even when the respective sera were omitted from the incubations (data not shown). This probably represents non-specific adsorption or entrapment of material by the immobilized form of Protein A. However, immunoprecipitation of p75 (Fig. 6, lane 13) did require addition of the anti-TVMV HC serum. Lane 13 received only 40% (v/v) of the equivalent amount of immunoprecipitated material loaded onto lanes 3, 4, 6, 7, 9, 10 and 12, and so the homologous reaction is stronger than it seems. In contrast to the previous study (Hiebert et al., 1984), the results in Fig. 6, lanes 2 to 7 suggest that none of our TuMV-specific products, from either the WG or MDL systems, was selectively immunoprecipitated with antiserum to HPLC-purified TVMV-HC. This result was unexpected since the same antiserum was used in both series of experiments, the homologous reaction (p75; lane 13) was as strong as previously reported (Hellmann et al., 1980, 1983), and our reaction conditions were extremely similar to those of Hiebert et al. (1984). Possible explanations for this discrepancy include the fact that we did not
Fig. 6. Immunoprecipitation of products encoded by TuMV RNA (lanes 2 to 7) or TVMV RNA (lanes 8 to 13) in wheat germ extract (lanes 2 to 4 and 8 to 10) or rabbit reticulocyte lysate incubations (lanes 5 to 7 and 11 to 13). Lanes 2, 5, 8 and 11 each received 2 × 10^5 c.p.m. of 35S-labelled products from the corresponding aliquot (20 µl) removed directly from each incubation. All other lanes, except 13, received 25 µl (50%) of the total immunoprecipitated material recovered from the corresponding Protein Agarose pellet. Lane 13 received only 10 µl (20%) of the total immunoprecipitate. Lanes 3, 6, 9 and 12 show the material recovered by preimmune rabbit serum from each of the parental reactions in the adjacent track to the left. Lanes 4, 7, 10 and 13 contained material precipitated by the anti-TVMV HC antiserum. In the homologous reaction (lane 13), the major polypeptide recovered was p75, as shown on the right. Immunoprecipitates loaded on lanes 3, 4, 9 and 10 represent products from volumes equivalent to the total reactions shown in lanes 2 and 8. Immunoprecipitates loaded on lanes 6, 7 and 12 represent products from five times (and in lane 13, from twice) the total reactions shown in lanes 5 and 11. 14C-labelled marker proteins were co-electrophoresed on lane 1; their molecular weights (×10^{-3}) are shown on the left. The dried gel was autoradiographed for 4 days at room temperature.

dissociate our cell-free reactions at 100 °C in SDS prior to adding the antisera. Conceivably, the TuMV RNA-encoded products may have remained in some aggregated form, unlike the TVMV products. Alternatively, by first adding and then recovering the preimmune serum, we may have removed a portion of radiolabelled, TuMV-encoded material which reacts non-specifically (see Fig. 6, lane 3). We loaded a substantially smaller proportion of each immunoprecipitate than did Hiebert et al. (compared with the total reaction products) and all our gel lanes were exposed to X-ray film for the same length of time; thus, we may simply have failed to see any weak cross-reaction with the anti-TVMV HC IgG. Further work is clearly necessary to resolve this point and to clarify any possible serological heterogeneity between products encoded by different strains or isolates of TuMV.
In vitro translation of TuMV RNA

The translational data reported here represent the first study on the polypeptide-coding properties of TuMV RNA. The complexity of products, particularly in MDL incubations, seems common among potyviruses. We have presented further evidence to support a post-translational proteolytic processing strategy of gene expression, which is now considered the favoured mechanism among this important group of plant viruses.

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REFERENCES


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