Complete uncoating of the 5' leader sequence of tobacco mosaic virus RNA occurs rapidly and is required to initiate cotranslational virus disassembly in vitro

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Destabilizing events required for subsequent cotranslational disassembly of tobacco mosaic virus (TMV) particles in vitro were studied. Brief treatment of U-32P-labelled TMV (strain vulgar or U2) with 1% SDS exposed only 2.5% of the RNA (160 5' nucleotides) in a susceptible subpopulation of virions. Limited uncoating occurred almost immediately and appeared to be synchronous because the amount of 5' oligonucleotide marker (t2) recovered remained constant throughout a 15 min period in SDS. Additional RNase T1-sensitive oligonucleotides were exposed only after 1 to 2 min in SDS. Coat protein (CP) subunits released from virions 'destabilized' by ultracentrifugation at between pH 7-2 and 9.2 were quantified using L-[35S]methionine-labelled particles of TMV strain U2. CP recovery and virus particle translation results were consistent with increasing numbers of virions uncoating for approximately 200 nucleotides. In the presence of sparsomycin (SPN), the TMV strain vulgar 5' leader and the first AUG codon can bind two 80S ribosomes. Electron microscopy of pH 7.5-treated TMV particles incubated in SPN-treated wheatgerm extract or rabbit reticulocyte lysate, showed that approximately 10% of virions complexed with one ribosome and approximately 10% with two bound ribosomes, confirming that Ω at least had been uncoated. Nucleocapsids in these complexes were shorter than untreated TMV by 9 to 10 nm (i.e. equivalent to 192 to 217 nucleotides exposed). The template activities of virions pretreated at pH 7-2 to 9-2 were destroyed by RNase H when short cDNAs were hybridized to sequences at, or immediately 3' to, the first AUG codon. We propose that the complete 5' leader of TMV RNA interacts weakly with CP subunits and that this micro-instability is due to the absence of G residues and is essential for initiation of cotranslational virus disassembly.

Introduction

Studies with tobacco mosaic virus (TMV) in vitro and in vivo have revealed a plausible mechanism to account for the extensive disassembly of stable ribonucleocapsids under physiological conditions. RNA uncoating is thought to occur cotranslationally (Wilson, 1984a,b; Shaw et al., 1986; Wilson & Shaw, 1987). A similar phenomenon has also been demonstrated in vivo with other rod-shaped and icosahedral plant RNA viruses: soil-borne wheat mosaic virus (Shirako & Ehara, 1986), potato virus Y (T isolate), beet necrotic yellow vein virus, barley stripe mosaic virus (Y. Shirako, personal communication), southern bean mosaic virus, brome mosaic virus, alfalfa mosaic virus, carnation mottle virus (Brisco et al., 1985, 1986; Shields et al., 1989; S.-A. Harbison, personal communication) and cowpea chlorotic mottle virus (Brisco et al., 1986; Roenhorst et al., 1989). Thus, interactions with the cellular protein synthetic machinery may be involved in uncoating most of the positive-strand RNA viruses of plants.

Events that destabilize virus structure and trigger the disassembly process in vitro remain largely unknown (reviewed in Wilson, 1988) and it is more straightforward to investigate how the process might be initiated in vitro. This information would then be of value for further studies on the early events of virus infection. Most likely, events leading to cotranslational virus disassembly initiate at the 5' leader sequence (Kozak, 1989), where 40S ribosomal subunits scan 5' to 3' for an AUG codon in good context.

In vitro it has been shown that many agents (alkali,
urea, DMSO and SDS) cause 300 nm TMV particles to disassemble progressively from the 5′ end of the RNA (Perham & Wilson, 1976; Wilson et al., 1976, 1981; Blowers & Wilson, 1982), implying that protein–RNA interactions are intrinsically relatively weak at this end. Until now, only fairly extensive uncoating of TMV RNA (for 2 kb or more) has been detected. Thus, a nested set of five 3′-coterminal rodots of 200 nm or less were isolated after treatment of TMV (strains *vulgare*, U4, U6 or U7) at pH 9-0 or above, or with urea (Perham & Wilson, 1978; Pelcher & Halasa, 1979a, b; Pelcher et al., 1980; Hogue & Asselin, 1984); TMV strain U2 was more resistant to extensive uncoating (Perham, 1969; Hogue & Asselin, 1984). Less extreme conditions are required to ‘destabilize’ or ‘activate’ TMV particles for cotranslational disassembly (Wilson, 1984a, b; Roenhorst et al., 1989).

In this paper we report that ≤200 nucleotides, including the 5′ leader sequence, are easily and rapidly uncoated *in vitro* in approximately 20% of TMV particles. The resulting metastable intermediates are the active templates for further extensive cotranslational disassembly (Wilson, 1984a; b; Roenhorst et al., 1989).

**Methods**

*Virus propagation and labelling.* TMV strains *vulgare* and U2 were isolated from systemically infected tobacco (*Nicotiana tabacum* L. cv. Samsun or Xanthi) by the methods of Leberman (1966) or Mundry (1969). Uniformly 32P-labelled TMV strain *vulgare* or U2 was grown and isolated as described by Mundry & Priess (1971). L-[35S]Methionine-labelled TMV strain U2 (generously provided by Professor M. Zaitlin, Cornell University, Ithaca, N.Y., U.S.A.) was grown in detached, infected tobacco leaves by petiole feeding and isolated as described by Bruening et al. (1976).

**SDS-stripping of 31P-labelled TMV and exposure of the 5′ leader.** U-31P-labelled RNA (specific activity 0.2 μCi/μg RNA) was uncoated by incubation of TMV strains *vulgare* or U2 at 37 °C in 100 μl ‘stripping mix’ (1 mg TMV particles, 1 mg SDS and 0.1 mg bentonite in 20 mM-sodium cacodylate, 2 mM-disodium EDTA pH 7.5). At intervals between 15 s and 15 min, 10 μl samples were diluted in 80 μl ice-cold 10 mM-Tris-HCl pH 7.4, 1 mM-disodium EDTA containing 0.5 mg/ml yeast RNA. After addition of 20 μl of the same buffer containing 0.1 μg RNase T1 (chromatographically pure; Sanko) in place of carrier RNA, the sample was incubated for 40 min at 37 °C at an enzyme : total RNA (w/w) ratio of about 1:20. In control experiments with RNase A (Warthington), this ratio was 1:1 to ensure fast complete hydrolysis of the RNA. Exposure and recovery of the 5′-proximal RNA T1-resistant marker oligonucleotide [70 residues (0) in strain *vulgare* or 55 residues in strain U2 (Kukla et al., 1979; Sotis & Garcia-Arenal, 1990)] was quantified by electrophoresis in 15% (w/v) polyacrylamide gels at 4 to 5 mA/lane for 4 to 5 h at 4 °C, followed by counting Cerenkov radiation in gel slices. The *vulgare* and U2 strain oligonucleotide markers migrated with Rn values (relative to bromophenol blue) of 0.48 and 0.65, respectively.

**Pretreatment of TMV for translation.** Virus particles were diluted to 0.1 to 2 mg/ml in 10 ml 0.1 M-Tris–HCl buffer of the stated pH at 0 to 4 °C, centrifuged (Beckman type 40 rotor, 36000 r.p.m., 4 °C for 90 min) and resuspended gently in sterile water to about 20 mg/ml and used immediately (Wilson, 1984a).

In experiments with L-[35S]Methionine-labelled TMV strain U2, coat protein (CP) was recovered from the supernatant fractions (10 ml) by adding 0.5 mg bovine serum albumin, 10 ml 25% (w/v) TCA and 0.65, respectively.

**In vitro translation.** Message-dependent rabbit reticulocyte lysate (RRL) and wheatgerm (WG) extract (a gift from General Mills) were prepared and used as described (Pelham & Jackson, 1976; Davies, 1979). After incubation, duplicate 3 μl samples were removed from standard translation reactions (25 μl) to assess the extent of incorporation of L-[35S]Methionine (>1100 Ci/mmol; NEN DuPont) into TCA-insoluble polypeptides. Polypeptide products were also fractionated by SDS-PAGE (Laemmli, 1970) and visualized in fixed dried gels by autoradiography.

To isolate and examine initiation complexes, cell-free reaction mixtures were preincubated at 30 °C for 15 min with 0.3 mM-sparsozyme (SPN; a gift from Dr John Douros, Natural Products Branch, Division of Cancer Treatment, NCI, Bethesda, Md., U.S.A.). TMV strain *vulgare* particles, washed in 0.1 M-Tris–HCl pH 7.5, were then added and incubation was continued for 10 min. After 20-fold dilution with 5 mM-MgCl2, complexes were adsorbed onto carbon-coated rhodium-plated copper grids, negatively stained with 1% (w/v) uranyl acetate and viewed in a Jelel 1200EX electron microscope. The lengths of nucleocapsids were measured directly in 200 of these complexes using the internal scale-bars, checked against virus particle diameter (18 nm). Comparable experiments were performed with virions pretreated with buffers of pH 8.2 or 9.2.

**Oligonucleotide-targeted RNase H digestions.** Seven oligodeoxyribonucleotides (cDNAs; Table 1) were provided by Dr Andrew Northrop (AFRC IAPGR, Babraham, Cambridge, U.K.). Hybridization of

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<th>Table 1. Details of cDNAs used for RNase H-targeted cleavage of TMV RNA</th>
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* Partial (redundant) complementary target sequences.
cdNA to unencapsidated TMV RNA or to buffer-washed TMV particles, and subsequent digestion with RNase H (Pharmacia), were performed as described by Minshull & Hunt (1986) or Atabekov et al. (1988). Each lyophilized cdNA was dissolved in water to a concentration of 50 to 450 μM and added to the appropriate TMV template at cdNA:genome ratios between 100:1 and 1000:1. In one experiment (not shown), the cdNA:TMV genome ratio was varied from about 200:1 to 0:2:1 to confirm saturated binding and complete RNA digestion under standard conditions (Table 1; 175:1).

To assess whether all virus particles had exposed 5' sequences, cdNA3, or cdNA5, and RNase H were used to inhibit completely the template activity of a TMV preparation washed at pH 8-2. Residual encapsidated RNA was then extracted with phenol, precipitated with ethanol and translated in vitro. To detect cdNA carried through the RNA extraction procedure, RNase H was added once again to a sample of each RNA recovered prior to translation.

Results

Immediate exposure of the 5' leader of TMV RNA by SDS

The kinetics of uncoating by SDS and recovery of long and short oligonucleotides from TMV strain vulgare or U2 by RNase T1 were determined. Average results from five independent experiments (Fig. 1a) showed that within 15 s of addition, 1% SDS uncoated enough RNA for RNase T1 (or RNase A; Fig. 1c) to release 32P-labelled oligonucleotides equivalent to 2.5% of the total

Fig. 1. (a). Release of RNase T1-sensitive radioactivity from 32P-labelled TMV strain vulgare as a function of time of exposure to 1% SDS. Averaged data from five independent experiments are included, expressed relative to the amount of radioactivity released from particles treated with SDS for 3 min (taken as 1:0). Symbols refer to averages of five (●), four (■), three (△) or two (△) experiments; (○) represents a single experimental value only. (b). Recovery of 32P-labelling as a function of time of exposure of 32P-labelled TMV strain vulgare to 1% SDS. Averaged data from five independent experiments are included, expressed relative to the recovery of Ω from particles treated with SDS for 3 min (taken as 1:0). Symbols refer to averages from five (●), three (△) or two (△) independent experiments; (○) represents a single experimental value only. (c) Release of RNase-sensitive radioactive material from 32P-labelled TMV strain vulgare as a function of time of exposure to 1% SDS. At the times indicated, aliquots were taken and incubated with either RNase A (△) or RNase T1 (△). Free oligonucleotides were separated from RNase-resistant material by PAGE. Samples were not taken at time 0 in this experiment.
amount of RNA exposed and released after 15 min (2.5% is equivalent to 160 nucleotides per virion, provided susceptible virions were completely uncoated by 15 min).

Following this rapid (immediate) phase of disassembly, stripping appeared to slow (Fig. 1a; strain vulgare) or to halt (Fig. 1c; strain vulgare) for about 2 min before uncoating proceeded further. Synchronous and immediate uncoating of susceptible TMV strain vulgare particles (from 15 s onwards, at least) was substantiated by quantifying the absolute amount of 70 nucleotide (Q) marker recovered at each time point (Fig. 1b). The amount of labelled Q exposed and recovered remained constant during 15 s to 15 min exposure to SDS (about $2 \times 10^3$ c.p.m.; expressed relative to the 3 min sample common to each of five independent experiments). Results like those shown in Fig. 1(a, b) were obtained with $^{32}$P-labelled TMV strain U2 (data not shown) and identical kinetics were observed with TMV strain vulgare when RNase A replaced RNase T1 (Fig. 1c).

From Fig. 1(b) it can be seen that most susceptible virus particles contained exposed Q at the earliest sampling point after the addition of SDS (i.e. at 0 min) and that by 15 s the absolute amount of Q exposed had reached the level that remained constant until 15 min. However, from the 15 min plateau value in Fig. 1(c) ($3.8 \times 10^5$ c.p.m) and the amount of starting virus used (1 μCi), it appears that only about 20% of all particles uncoated completely.

Release of TMV CP subunits during centrifugal washing

An alternative method to quantify the extent of RNA uncoating involved measuring the number of CP subunits released during ultracentrifugation of virus in 0.1 M-Tris–HCl of defined pH. Such an approach is not feasible with virions rendered translationally active by dialysis (Wilson, 1984a) or by direct addition of buffer (Roehorst et al., 1989). L-$^{35}$S-Methionine-labelled particles of the TMV strain U2 (Wittmann, 1965; TMV strain vulgare CP lacks methionine) were washed at pH 7.2, 8.2 or 9.2 and the resulting virus pellets were resuspended in water for incubation in RRL. As with a standard TMV RNA dose-response curve in RRL, the relative amounts of L-$^{35}$S-methionine incorporated into TCA-insoluble polypeptides (predominantly 126K protein; Fig. 2) suggested that the number of destabilized virus particles increased with increasing pH, rather than the extent of uncoating of a fixed fraction of virions in the population.

The amount of $^{35}$S-labelled CP released and recovered from each washing pH supernatant was expressed as a fraction of the total counts added as virus. Assuming 2130 uniformly labelled CP subunits per TMV strain U2 particle and given the template–dose effect in Fig. 2, these data are consistent with uniform exposure of about 200 nucleotides in 15, 30 or 78% of the virus particle population at pH 7.2, 8.2 or 9.2, respectively. Conversely, although less likely (Fig. 2; and data described below for TMV strain U1), if all virions were equally susceptible to uncoating, these data would correspond to the following average numbers of subunits released per virion: 10 at pH 7.2, 20 at pH 8.2 and 52 at pH 9.2 (i.e. uniform exposure of 30, 60 or 156 nucleotides per virus, respectively).

Formation of initiation complexes with TMV particles

To assess the percentage of virus particles engaged in translation and also the minimum amount of leader sequence exposed, SPN-arrested initiation complexes from WG and RRL programmed with pH 7.5-treated TMV strain vulgare particles were viewed in the electron microscope. Fully uncoated Q will bind two ribosomes (Tyc et al., 1984), one spanning the AUG codon for the 126K polypeptide (Goellet et al., 1982) and the other centred on AUU, residues 15 to 17, in TMV RNA (14 to
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16 in Ω). Selected initiation complexes are shown in Fig. 3. Scoring 200 clearly identifiable TMV rods from several independent samples showed 9-4% with one ribosome bound (monosomes), 8-3% with two ribosomes bound (disomes), 8-3% with three or more ribosomes attached (usually as a complex unresolvable cluster) and 74% with no ribosomes bound. Under conditions of SPN-inhibited elongation at low template RNA concentrations, the translation machinery should recruit all available virions. These values confirm that after washing at pH 7-5, only about 26% of virions participate in cotranslational disassembly (Wilson, 1984a; Roenhorst et al., 1989). This result is consistent with the 15, 30 or 78% of L-[35S]methionine-labelled TMV strain U2 particles estimated to uncoat for about 200 nucleotides after pH 7-2, 8-2 or 9-2 treatment (above and Fig. 2).

The fact that 50% of the initiation complexes contained only one ribosome probably reflects the high input level of pH 7-5-washed virions (100 μg/25 μl reaction), the inefficiency of the cell-free translation systems and sample damage during grid preparation, rather than a subpopulation of virus particles in which the leader is uncoated only to the first AUU sequence (nucleotides 15 to 17). This view is supported by data presented below and by earlier sucrose gradient analyses of SPN-arrested ribosomes complexed with TMV RNA (Filipowicz & Haenni, 1979) or Ω′-chloramphenicol acetyltransferase mRNA (Sleat et al., 1988a).

By measuring the length of nucleocapsid remaining in these mono- and disome complexes, it was clear that most had uncoated for 9 to 10 nm, thereby exposing 192 to 217 nucleotides [intact rods of TMV strain vulgare (295 to 300 nm) contain 6395 nucleotides (Goelet et al., 1982)]. After pretreatment of TMV at pH 8-2 to 9-2, increasing numbers of arrested complexes were seen, the majority with TMV rods shortened by 9 to 10 nm (data not shown).

Oligonucleotide-targeted TMV RNA cleavage by RNase H

Six short DNAs (cDNA1 to 6) complementary to portions of the 5′-proximal sequence of TMV RNA (residues 1 to 117), and one to the extreme 3′ end of TMV RNA (cDNA7), were used to target RNA digestion by RNase H (Minshull & Hunt, 1986; Atabekov et al., 1988) prior to incubation of virions or unencapsidated TMV RNA in RRL. Sequence details of the cDNAs, the genome coordinates of their target sequence(s) and the standard cDNA : TMV genome ratios used are given in Table 1. Their quantitative effects (with or without RNase H treatment) on RRL translation of pH 7-2-, 8-2- or 9-2-treated TMV particles or unencapsidated TMV RNA are summarized in Table 2. cDNA : TMV genome ratios varied between 100 : 1 and 1000 : 1 and, in some experiments, annealing was performed at 60 °C (Atabekov et al., 1988) rather than at 30 °C (Minshull & Hunt, 1986). Qualitative effects on the polypeptides encoded by unencapsidated TMV RNA or pH 7-2-washed virions are shown in Fig. 4(a, b and c). Because cDNA4 and cDNA5 severely inhibited cotranslational disassembly of TMV particles, we conclude that nucleotides 67 to 90 (at least) were exposed in all members of the translationally active subpopulation of virions.

The observation that cDNA6 (targeted to nucleotides 99 to 117) had less effect than cDNA4 and cDNA5 on translation of pH 8-2- or pH 9-2-washed TMV particles and unencapsidated TMV RNA (Table 2) was unexpected. Computer-predicted minimum free-energy structures for this region (Zucker & Stiegler, 1981; data not shown) suggested that extensive base-pairing occurs between genome coordinates 99 and 153 (Wilson et al., 1990). Increasing the cDNA : TMV genome ratio to 1000 : 1 improved the inhibitory effect of cDNA6 on pH 7-2-washed virions only slightly, and annealing cDNA6 to naked TMV RNA even at 60 °C had little beneficial effect (Table 2). Gel filtration of 32P-labelled cDNAs incubated with pH 8-2-treated TMV particles showed
Table 2. Effects of cDNAs on pH-washed virus-, or naked TMV RNA-directed RRL translation, with or without RNase H treatment

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<th>pH 9-2 TMV</th>
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* See Table 1 for details of cDNA oligonucleotide sequences, genome target coordinates and standard cDNA:genome ratios used.
† Relative (%) incorporation of TCA-insoluble c.p.m./μl RRL compared to an RRL incubation programmed with the same TMV template, without cDNA but with RNase H (e.g., Fig. 4 a to c, lanes 4). Absolute levels (100%) of TCA-insoluble c.p.m./μl RRL were 0.65 × 10^5 (pH 7.2 TMV), 2.55 × 10^5 (pH 8.2 TMV), 3.25 × 10^5 (pH 9.2 TMV) and 2.29 × 10^5 (TMV RNA).
‡ Data are presented for two independent series of experiments with TMV RNA hybridized with cDNA1-cDNA6 at 630 °C or 60 °C.
§ All data refer to cDNA:genome ratios given in Table 1, except for one independent experiment with pH 7.2 washed TMV where all cDNAs were used at 1000-fold molar excess.
| ND, Not determined.

that cDNA1 and cDNA6 bound very inefficiently compared with cDNA2, -3, -4 and -5 (data not shown). These observations may reflect the high content of (U-dA) and (A-dT) pairs with cDNA1 and confirm the computer-predicted inaccessibility of genome co-ordinates 99 to 117 to cDNA6.

To confirm that Ω was exposed in only a minor subpopulation of TMV rods, residual RNA was phenol-extracted from pH 8-2-washed virions which had been rendered translationally inactive by binding cDNA3 or cDNA5 in the presence of RNase H (Fig. 5, lanes 3 and 8). The extracted TMV RNA was as active as untreated control TMV RNA when translated in RRL (final concentrations 25 to 125 μg/ml; data not shown). Approximately 60% of the template activity recovered by extracting total RNA (Fig. 5, lanes 4 and 9) was sensitive to further addition of RNase H (Fig. 5, lanes 5 and 10), confirming that some cDNA3 or cDNA5 had coprecipitated with intact TMV RNA extracted from the RNase H-inactivated virus population.

Discussion

Several independent lines of evidence are presented to indicate that some virus particles in a preparation of TMV strain vulgare or U2 exhibit micro-instability and are susceptible to rapid, but limited, disassembly for 200 nucleotides or less in SDS or mild alkali. In the mildest alkali (pH 7.2), we estimate this fraction to be between 10 and 20% of rods. The absence of G residues in the leaders of several strains of TMV may be significant for this behaviour (Mundry, 1965, 1967, 1969; Mandelès, 1968; Garfin & Mandelès, 1975; Richards et al., 1978; Kukla et al., 1979). Recently, refined X-ray fibre diffraction data have shown that G interacts especially strongly via two specific hydrogen bonds with Arg 122 and Asp 115 (Namba & Stubbs, 1986; Namba et al., 1989) when in the first (5') position of the conserved CP trinucleotide-binding site. The amino groups of G (but not those of A or C) are more protected against deamination by HNO2 in intact TMV particles than in isolated TMV RNA (Schuster & Wilhelm, 1963; Sehgal & Soong, 1972), supporting a strong involvement of G in TMV protein–RNA interactions. Therefore, functional modulation of TMV helix stability may occur by virtue of the distribution of G along TMV RNA.

As reported for a wide range of chimeric RNA constructs (Sleat et al., 1988a), Ω (or its derivative, Ω') has a low capacity to engage in base-pairing with a range of downstream coding sequences. In native TMV RNA, the first residue predicted to be involved in base-pairing is the G of the AUG at the 3' end of Ω (Wilson et al., 1990). We believe that this unstructured RNA leader recruits 40S ribosomal subunits very efficiently (Sleat et al., 1988a). However, lack of RNA folding is unlikely to be responsible for the ease of uncoating of Ω in virions treated with SDS or alkali because RNA–RNA interactions are precluded in helical virus particles. Although RNA uncoating appears to proceed beyond the 5' leader sequence, we believe that particle instability results primarily from the low G-content leader, a conclusion supported by recent X-ray fibre diffraction data (Namba et al., 1989).

Pseudovirus particles made in vitro (Sleat et al., 1986; Gallie et al., 1987; Jupin et al., 1989) or in vivo (Sleat et al., 1988b) which contain additional G residues upstream of Ω' and between Ω and the first open reading frame consistently failed to uncoat cotranslationally in vitro, even after washing at pHs up to 9.0 (D. E. Sleat, J. K. Osbourn & T. M. A. Wilson, unpublished results). We are presently engaged in experiments to produce pseudovirus particles containing a systematic series of synthetic leaders to investigate this concept further.

As with pseudoviruses (above), potexviruses, in which 5' RNA–protein interactions are reputedly stronger...
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Fig. 4. The qualitative effects of cDNA1 to cDNA7, either alone or with RNase H, on L-[35S]methionine-labelled polypeptides encoded by unencapsidated TMV RNA (a) or pH 7-2-treated TMV strain vulgare (b and c). cDNA hybridizations were at standard (Table 1) cDNA:genome ratios (a and b) or at 1000:1 (c). Shown are autoradiographs of L-[35S]methionine-labelled polypeptides encoded in RRL, fractionated by SDS-PAGE in 12.5% (w/v) gels. Adjacent pairs (lanes 5 to 18) represent TMV templates incubated with cDNA1 (lanes 5 and 6) to cDNA7 (lanes 17 and 18) either alone (odd numbers) or with RNase H (even numbers). Common RRL control lanes were template-free RRL (lane 1), template alone preincubated on ice (lane 2), template alone preincubated at 30 °C (lane 3) and template preincubated at 30 °C only with RNase H (lane 4).

Fig. 5. Recovery of RNA template activity by phenol extraction of total RNA from pH 8.2-washed TMV particles pretreated with RNase H and cDNA3 (lanes 1 to 5) or cDNA5 (lanes 6 to 10). Shown is an autoradiograph of L-[35S]methionine-labelled polypeptides encoded in RRL, fractionated by SDS-PAGE in a 12-5% (w/v) gel. RRL incubations were supplemented with water only (lanes 1 and 6), pH 8-2-washed TMV (lanes 2 and 7), pH 8-2-washed TMV treated with RNase H and cDNA3 or cDNA5 (lanes 3 and 8), and total RNA phenol-extracted from the corresponding inactivated pH 8-2-washed TMV in the absence (lanes 4 and 9) or presence (lanes 5 and 10) of further added RNase H. From TCA-insoluble c.p.m., approximately 60% of the template activity recovered in lanes 4 and 9 was lost upon a second addition of RNase H (lanes 5 and 10). The position of the TMV strain vulgare RNA-encoded polypeptide of 126K is shown on the right.

(AbouHaidar & Erickson, 1985), also failed to act as efficient templates for cotranslational disassembly in vitro (Wilson, 1984a). All virus or pseudovirus nucleocapsids examined to date disassemble in vivo. Thus cotranslational disassembly of alkali-treated virions in vitro cannot explain fully the early events of virus infection.

We and others (Atabekov et al., 1988; Crum et al., 1988; Minshull & Hunt, 1986) have been able to inhibit expression of TMV RNA by targeting short or long, synthetic or cloned oligonucleotides to the region of RNA surrounding (or immediately downstream from) the first AUG codon. However, this paper reports the first attempt to use such an approach to map portions of RNA exposed in virions. Limited inhibition of expression of TMV RNA or washed virions by cDNA4, -5 and -6 in the absence of RNase H (Table 2) may simply reflect steric blockage of the coding region, as proposed by Crum et al. (1988).

All our data indicate that CP-stripping in 1% SDS or mild alkali occurs very rapidly in susceptible TMV strain vulgare or U2 particles and uncoats about 200 nucleotides. The sequences of TMV strain vulgare and U2 RNA around nucleotides 190 to 220 (Goel et al., 1982; Solis & García-Arenal, 1990) do not reveal especially G-rich or repetitive-G regions analogous to the origin-of-assembly sequence (Zimmern & Wilson, 1976), which might be expected to block uncoating by strong interactions with CP (Namba et al., 1989).

From these studies, we conclude that components of
destabilized the energy of 40S subunit 'scanning' (Kozak, 1989). The required (or likely, 200 residues) exposed by pretreatment with alkali or SDS.

Further in vivo studies are required to determine what subcellular site or component is exploited by TMV to trigger this intrinsic capacity to uncoat all of its leader sequence (Namba et al., 1989). Furthermore, how do more stable virus or pseudovirus particles become destabilized in vivo?

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References


