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Post-Translational Proteolytic Cleavage of In Vitro-Synthesized Turnip Yellow Mosaic Virus RNA-Coded High-Molecular-Weight Proteins

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In a reticulocyte lysate, turnip yellow mosaic virus genomic RNA directs the synthesis of two proteins with molecular weights of 150,000 (150K) and 195K. We present evidence that the larger protein is processed in vitro, after its completion, in at least three fragments. The NH_2-terminal fragment (82K) and the COOH-terminal fragment (78K) have been well characterized by different methods. The fact that the 150K protein is not cleaved in vitro, although it contains the regions that are processed in the 195K protein, could be of fundamental biological significance for the expression of the viral genes: a single polypeptide chain could be processed in several ways, leading to different peptides with distinct biological activities.

In a nuclease-treated reticulocyte lysate, turnip yellow mosaic virus (TYMV) RNA, extracted from virion particles, directs, in addition to its own coat protein, the synthesis of two high-molecular-weight (HMW) proteins having molecular weights of 150,000 (150K) and 195K, respectively, and possessing common amino acid sequences (6) and the same NH_2-terminus (4). These two HMW proteins are synthesized from the genomic RNA (molecular weight, 2×10^6), which is infectious (27) and consequently contains the entire information of the viral genome. The coat protein is synthesized only from a subgenomic RNA molecule (molecular weight, 0.25×10^6) (4a, 14, 27, 30) corresponding to the 3′ region of the genomic RNA (7, 37).

We have recently shown that the 195K protein corresponds to the translation of the entire genomic RNA, except for the coat protein gene. Using yeast suppressor tRNA's in in vitro translation experiments, we have demonstrated that the silent region between the 195K protein and the coat protein genes is only 14 nucleotides long (5). Moreover, as in the case of tobacco mosaic virus RNA, which also directs the synthesis of two HMW polypeptides (21), the 195K protein results from a "readthrough" of the termination codon of the 150K protein gene (unpublished data). In addition, we have found that subgenomic RNAs of TYMV, other than the coat protein RNA, present in virus preparations (18) can be translated in vitro into polypeptides with molecular weights ranging from 10 to 140K, all possessing the same NH_2-terminal tryptic peptide and corresponding to the full translation of these incomplete RNA molecules (19).

In this paper, we present evidence that the 195K protein is processed in vitro in the reticulocyte lysate and gives rise to well-defined fragments whose biological roles have not yet been clarified. The fact that the 150K protein is resistant to proteolysis, even after several hours of incubation and although it contains the peptide bonds that are cleaved in the 195K protein, suggests that this process is catalyzed or induced by the 195K protein itself and, consequently, that the C-terminal region of this protein is directly or indirectly involved in the proteolysis, or that this specific proteolysis is due to differences in the secondary structures of 150 and 195K proteins.

MATERIALS AND METHODS

L-[^{35}S]methionine (over 500 Ci/mmol) and L-[^{35}S]-cysteine (over 500 Ci/mmol) were from New England Nuclear Corp. Tosylamido-phenylethyl chloromethyl ketone-treated trypsin (EC 3.4.21.4) was purchased from Worthington Biochemicals Corp., and micrococcal nuclease (EC 3.1.4.9) was from Boehringer Mannheim Corp. Canavanine, parafluorophenylalanine, puromycin, and edeine were from Sigma Chemical Co., and *N*-tosyl-lysyl-chloromethane was from Calbiochem. All other chemicals were from Merck Sharp & Dohme. Thin-layer cellulose plates (Chromogram) and X-O-Mat-R, RP-1 films were from Eastman Kodak Co. Partially purified yeast amber suppressor $tRNA^{Ser}$ and Su^- $tRNA^{Ser}$ (wild-type tRNA purified by the same procedure as suppressor tRNA) were a generous gift of R. Gesteland (University of Utah, Salt Lake City). Highly purified beef liver $tRNA_i^{Met}$ was

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kindly provided by O. Kellerman and J. P. Waller (Ecole Polytechnique, Palaiseau, France), and TYMV-infected Chinese cabbage leaves were cultivated and supplied by S. Astier, A. Masson, and P. Cornuet (Centre National de la Recherche Agronomique, Versailles, France).

Virus purification and RNA extraction. TYMV was purified from infected Chinese cabbage leaves by the method of Leberman (17). TYMV RNA was then extracted by the phenol-chloroform and sodium dodecyl sulfate (SDS) method (28). One volume of TYMV solution (5 mg/ml) was mixed with 0.5 volume of 100 mM Tris-hydrochloride (pH 7.5)-saturated phenol, 0.5 volume of chloroform, and 0.1 volume of 20% SDS and stirred at room temperature for 10 min. After centrifugation, the aqueous phase was stirred again with 1 volume of chloroform. The RNA solution was then brought to 0.2 M with sodium acetate (pH 5.0) and precipitated with ethanol. After centrifugation, the RNA pellet was dried, dissolved in 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)-KOH (pH 7.5), and stored at −70°C.

Reticulocyte lysate preparation and incubation conditions. Rabbits were made anemic by injections of *N*-acetylphenylhydrazine by the method of Villa-Komaroff et al. (41); the mRNA-dependent reticulocyte lysate was prepared as described by Pelham and Jackson (25) with minor modifications (6) and stored in small samples in liquid nitrogen. Incubations were performed in 50 μl containing 25 μl of reticulocyte lysate and 1 mM magnesium chloride, 100 mM potassium chloride, 19 amino acids (except methionine or cysteine) at 20 μM each, 3 μg of rat liver tRNA, 3 μg of TYMV RNA, and 25 μCi of L-[^{35}S]methionine or L-[^{35}S]cysteine. Incubations were usually performed at 30°C for 90 min. After incubation, 2-μl samples were removed, spotted on Whatman 3MM disks, and treated as previously described (3). Analysis of the in vitro translation products was performed by electrophoresis on polyacrylamide-SDS slab gels prepared by the method of Laemmli (15). After electrophoresis, the gel was fixed with a methanol-acetic acid-water solution (30:7.5:62.5), dried under vacuum, and autoradiographed. When necessary, the corresponding autoradiogram was scanned on a Joyce-Loebl microdensitometer. The molecular weights indicated in the figures have been determined by comparison with well-characterized proteins: myosin, 210K; β and β' subunits of *Escherichia coli* RNA polymerase, 165 and 155K; β-galactosidase, 130K; phosphorylase *a*, 94K; bovine serum albumin, 68K; ovalbumin, 43K; carboxypeptidase A, 34.6K; and TYMV coat protein, 20K.

N-terminal labeling of HMW polypeptides. *N*-Formyl-[^{35}S]methionyl-tRNA$_i^{Met}$ was prepared by the method of RajBhandary and Ghosh (30). The level of formylation and the incubation conditions were as previously described (4).

Analysis of tryptic peptides of in vitro translation products. Tryptic digestion of labeled proteins (N-terminal, C-terminal, or uniform labeling) was performed essentially as described by Morrison and Lodish (20).

Radioactive polypeptides were separated by electrophoresis on polyacrylamide-SDS slab gels and localized by autoradiography. The corresponding parts of the gel were excised and incubated in 1 ml of 1% ammonium bicarbonate (pH 8.6) containing 100 μg of tosylamido-phenylethyl chloromethyl ketone-treated trypsin. After 4 h at 37°C with shaking, the supernatant was removed, kept at −20°C, and replaced by 1 ml of a fresh trypsin solution. The incubation was continued overnight. After digestion, the supernatants were pooled, filtered through membrane filters (0.45 μm; Millipore Corp.), and dried at 45°C. Tryptic peptides were dissolved in a minimum volume of pyridine-acetic acid-water buffer, pH 4.75 (5:5:190), and spotted on a cellulose plate. Electrophoresis was performed in the same buffer for 120 min at 450 V. The plates were then submitted to ascending chromatography in an *n*-butanol-pyridine-acetic acid-water solvent (37.5:25:7.5:30). After drying, they were plunged into a PPO (2,5-diphenyloxazole)-ether solution as described by Randerath (31) and autoradiographed at −70°C.

RESULTS

Kinetics of HMW protein synthesis. We followed the synthesis of TYMV RNA-coded HMW proteins, using the mRNA-dependent reticulocyte lysate system. Samples were removed at different times and analyzed by electrophoresis on a 12.5% polyacrylamide-SDS slab gel. The autoradiogram of the gel presented in Fig. 1 shows that the 150K protein appeared 30 min after the beginning of incubation and that the 195K protein was detectable after 45 min.

Apart from the HMW proteins and the coat protein, many polypeptides with molecular weights ranging from 10 to 140K were synthesized. They can be generated by premature termination during genomic RNA translation, but also by the complete translation of smaller RNA molecules (19).

After 60 min of incubation, an intermediate-sized polypeptide with a molecular weight of 78K could be detected. This polypeptide did not appear with the unfinished products originating from subgenomic RNAs or from premature termination during genomic RNA translation. Rather, it appeared once the HMW proteins had been synthesized, suggesting that it might have resulted from the cleavage of HMW proteins. At the same time, a 120K protein was also detectable.

Proteolytic cleavage of an HMW polypeptide. Since the analysis of post-translational modifications was rendered difficult by the abundance of unfinished polypeptides, we designed experiments in which such polypeptides were not visible by autoradiography.

Since the elongation rate of protein synthesis is constant for at least 60 min, and since 30 min is necessary to synthesize the 150K protein (Fig. 1), if, after a few minutes of incubation, translation of TYMV RNA is synchronized by prevent-

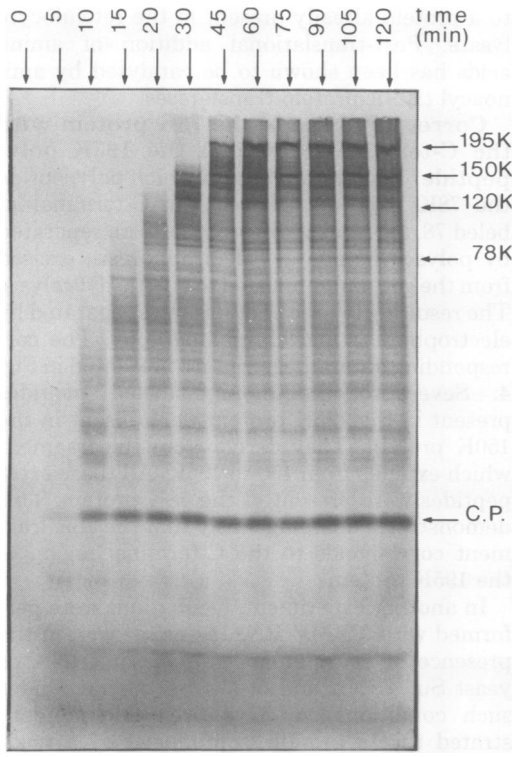

FIG. 1. *Kinetics of TYMV RNA-coded protein synthesis in the reticulocyte lysate. Incubation was performed under standard conditions as described in the text, in the presence of* L-[^{35}S]*methionine. At different times, 5-μl samples were removed from the incubation mixture and denatured. The samples were then analyzed by electrophoresis on a 12.5% polyacrylamide–0.1% SDS slab gel and autoradiography.*

ing the formation of new initiation complexes by addition of edeine, and if L-[^{35}S]methionine is added only 25 min after this synchronization, we would expect to find the radioactivity in polypeptides with molecular weights larger than 150K and only in their C-terminal region. On the other hand, if formyl-[^{35}S]methionyl-tRNA$_i^{Met}$ is used as the only source of radioactivity in the translation experiments, the N-terminal methionyl residue will be the only one to be labeled in the translation products.

These procedures were used to label the C-terminal or the N-terminal regions of the viral proteins. The results are shown in Fig. 2. Lane 1 shows the pattern observed when the nuclease-treated reticulocyte lysate was incubated in the absence of exogenous mRNA. In addition to residual globin synthesis, a strong band, with an apparent molecular weight of 45K (Endo), is visible. In lane 2, the experiment was performed in the presence of TYMV RNA under standard conditions (see above), except that L-[^{35}S]methionine was omitted at zero time. After 5 min, 20 μM edeine was added; 25 min later, L-[^{35}S]methionine was introduced, and the incubation was pursued for 60 min. The autoradiogram (lane 2) shows that most of the unfinished polypeptides and the coat protein which were visible in the uniform labeling experiment (lane 4) disappeared. As expected, the two HMW proteins (150 and 195K) are visible, as well as the endogenous polypeptide (Endo) and a peptide with a molecular weight of 78K. This suggests that the 78K protein results from the cleavage of one of the HMW polypeptides and corresponds to the

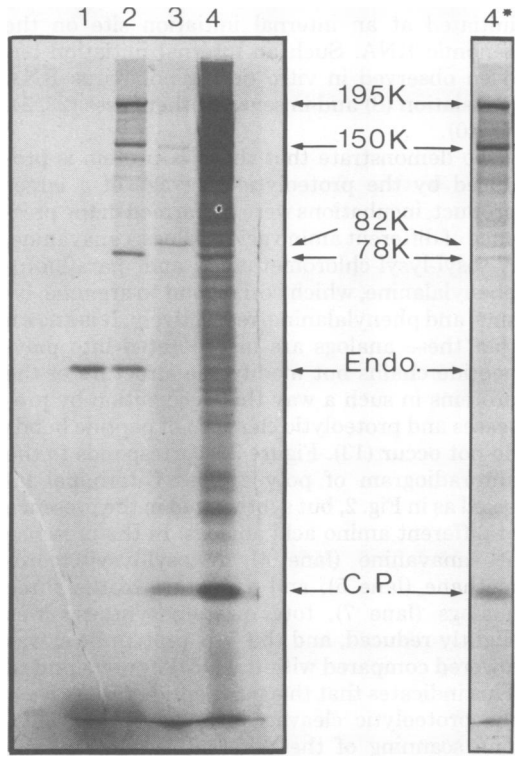

FIG. 2. *C-terminal and N-terminal labeling of TYMV RNA-coded proteins in the reticulocyte lysate. Lane 1 shows the pattern of proteins labeled in the mRNA-dependent reticulocyte lysate in the absence of added TYMV RNA. In lane 2, the experiment was performed under standard conditions, except that* L-[^{35}S]*methionine was omitted at zero time. After 5 min of incubation, edeine was added at 20 μM. At 25 min,* L-[^{35}S]*methionine was added at 500 μCi/ml, and the incubation continued for 60 min. Lane 3 represents the pattern of TYMV RNA-directed proteins synthesized in the presence of formyl-[^{35}S]methionyl-tRNA$_i^{Met}$ as the only source of radioactivity. Lane 4 corresponds to uniformly labeled TYMV RNA-coded proteins synthesized under standard conditions in the presence of* L-[^{35}S]*methionine, and lane 4* shows a shorter exposition by autoradiography of lane 4.*

C-terminal part of this molecule. However, one can also suppose that it is not related to the HMW proteins but is synthesized at a much lower rate and consequently contains labeled amino acids.

When the incubation was performed in the presence of formyl-[^{35}S]methionyl-tRNA$_i^{Met}$, the coat protein and the two HMW polypeptides were labeled (see Fig. 7), and a protein with a molecular weight of 82K was visible. It can also be detected among the uniformly labeled TYMV RNA-coded products (lanes 4 and 4*). This 82K polypeptide could originate either from the cleavage of premature termination of HMW proteins or from the synthesis of a protein that was initiated at an internal initiation site on the genomic RNA. Such an internal initiation has been observed in vitro during poliovirus RNA translation (8) and in several other cases (23, 24, 34, 40).

To demonstrate that the 78K protein is produced by the proteolytic cleavage of a larger product, incubations were performed in the presence of different amino acid analogs: canavanine, *N*-tosyl-lysyl-chloromethane, and parafluorophenylalanine, which correspond to arginine, lysine, and phenylalanine, respectively. It is known that these analogs are incorporated into polypeptide chains but modify the structure of the proteins in such a way that recognition by proteases and proteolytic cleavage of peptide bonds do not occur (13). Figure 3A corresponds to the autoradiogram of polypeptides C-terminal labeled as in Fig. 2, but synthesized in the presence of different amino acid analogs. In the presence of canavanine (lane 4), *N*-tosyl-lysyl-chloromethane (lane 5), and a mixture of the three analogs (lane 7), total protein synthesis was slightly reduced, and the 78K protein level was lowered compared with the HMW polypeptides. This indicates that this polypeptide results from the proteolytic cleavage of a larger molecule. The scanning of the different lanes (Fig. 3B) permits a better visualization of the influence of the incorporated analogs on the appearance of the 78K protein.

In addition, in the presence of *N*-tosyl-lysyl-chloromethane (lanes 5 and 7), the Endo protein was not labeled. At the present, we have no rational explanation for this observation. Nevertheless, it is interesting to note that the radioactivity of the Endo protein increased for several hours after addition of edeine and puromycin (see Fig. 8). We have, moreover, observed that the labeling of the Endo protein also occurs in the presence of sparsomycin (data not shown). It is most likely that it corresponds to the post-translational enzymatic addition of methionine or of one of the methionine degradation products to a protein already present in the reticulocyte lysate. Post-translational addition of amino acids has been shown to be catalyzed by aminoacyl tRNA-protein transferases.

Correspondence of the 78K protein with the C-terminal region of the 195K polypeptide. To determine from which polypeptide the 78K molecule derives, the C-terminal-labeled 78, 150, and 195K proteins were separated by polyacrylamide gel electrophoresis, excised from the gel, and submitted to tryptic hydrolysis. The resulting tryptic peptides were separated by electrophoresis and chromatography. The corresponding autoradiograms are presented in Fig. 4. Several [^{35}S]methionine-labeled peptides present in the 195K protein were absent in the 150K protein. They derive from the fragment which extends from 150 to 195K. All these extra peptides were present in the 78K protein. This demonstrates unambiguously that the 78K fragment corresponds to the C-terminal region of the 195K protein.

In another experiment, incubations were performed with TYMV RNA as messenger, in the presence of yeast amber suppressor tRNA or yeast Su$^-$ tRNA and of L-[^{35}S]cysteine. Under such conditions we have previously demonstrated that a protein is produced by a read-through of the amber termination codon of the 195K protein gene. When L-[^{35}S]cysteine was used as the labeled amino acid, most of the unfinished polypeptides did not appear (Fig. 5). This is probably due to the fact that cysteine codons of the HMW protein genes are located in the 3′ region of TYMV genomic RNA. Under standard conditions (lane 1) and in the presence of yeast Su$^-$ tRNA (lane 2), the two HMW proteins were synthesized, and the 78K polypeptide was formed. When yeast amber suppressor tRNA was added to the incubation (lane 3), the 210K protein appeared, as well as an extra peptide that migrated more slowly than the 78K fragment (78^{sup}) and that was probably produced by the proteolytic cleavage of the 210K protein.

These two experiments are strong arguments in favor of the presumption that the 78K peptide corresponds to the C-terminal region of the 195K protein.

Correspondence of the 82K polypeptide with the NH_2-terminal region of the HMW proteins. To distinguish between the two possibilities concerning the origin of the 82K product, 82, 150, and 195K proteins labeled at their NH_2-terminus were isolated and, after tryptic digestion, analyzed as described for the 78K protein studies. The autoradiograms of the initiator tryptic peptides presented in Fig. 6 show that these peptides are identical in all three

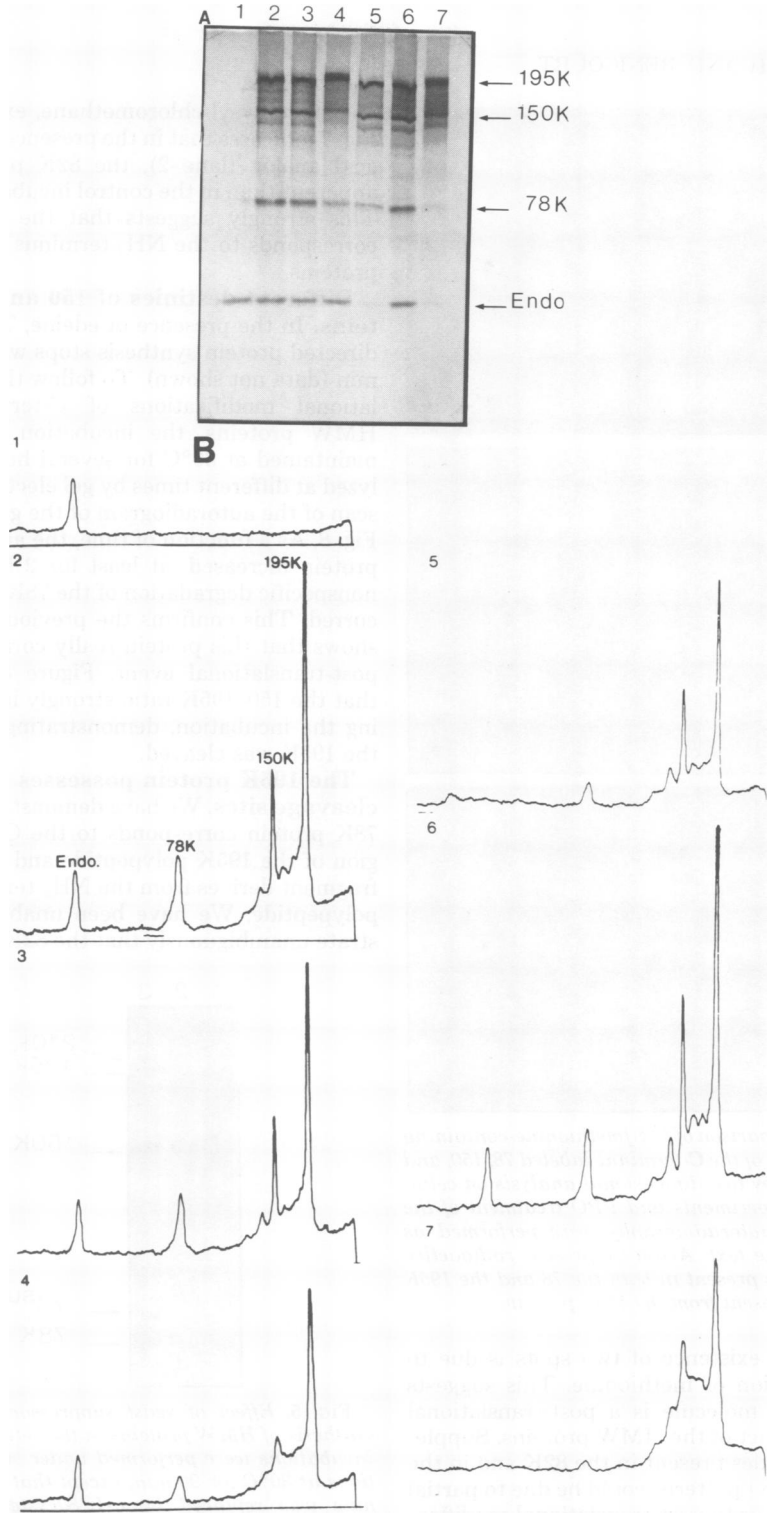

FIG. 3. *Effect of amino acid analogs on the synthesis, in the reticulocyte lysate, of TYMV RNA-coded proteins, separated by electrophoresis on a 12.5% polyacrylamide–0.1% SDS slab gel. (A) Autoradiogram of the gel; (B) microdensitometer tracing of the different lanes of the autoradiogram. Lane 1 corresponds to the proteins labeled in the absence of TYMV RNA. Lane 2 shows the TYMV RNA-coded polypeptides that have been C-terminal labeled as in Fig. 2. In lane 3, incubation was performed as in lane 2, except that arginine, lysine, and phenylalanine were excluded from the incubation mixture. In lanes 4 through 7, incubations were identical to that of lane 3, but the following analogs were added at 1 mM: lane 4, canavanine; lane 5,* N *-tosyl-lysyl-chloromethane; lane 6, parafluorophenylalanine; and lane 7, a mixture of the three analogs. All incubations were performed at 30°C for 90 min.*

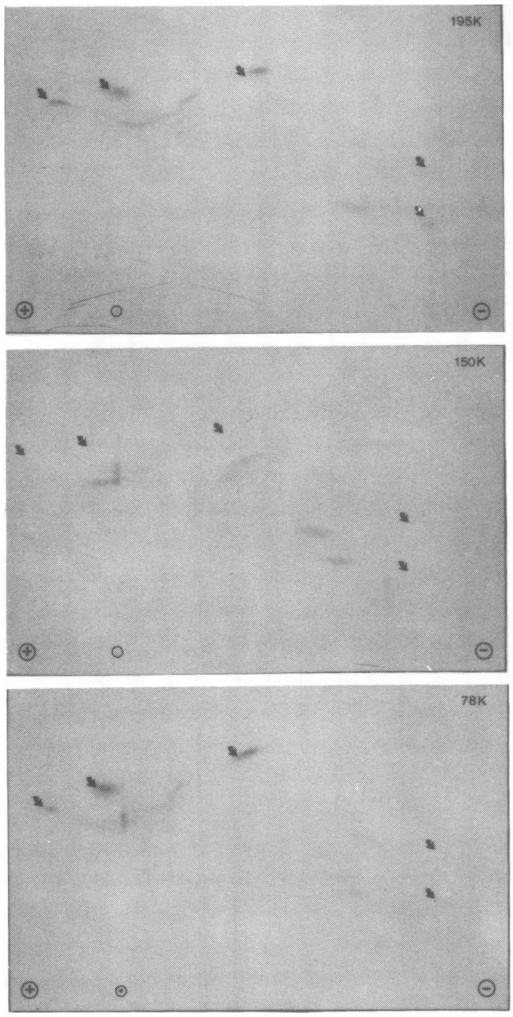

FIG. 4. *Comparison of [^{35}S]methionine-containing tryptic peptides of the C-terminal-labeled 78, 150, and 195K proteins by two-dimensional analysis on cellulose plates. Experiments and PPO treatment of the plates before autoradiography were performed as described in the text. Arrows represent radioactive tryptic peptides present in both the 78 and the 195K proteins and absent from the 150K protein.*

proteins. The existence of two spots is due to partial oxidation of methionine. This suggests that the 82K molecule is a post-translational cleavage product of the HMW proteins. Supplementary peptides present in the 82K and in the mix (82 + 195K) patterns could be due to partial deformylation or to post-translational modifications of the NH_2-terminus of the 82K protein. Nevertheless, confirmation that the 82K protein is produced by post-translational cleavage is presented in Fig. 7. In this experiment, incubations were performed in the absence or in the presence of *N*-tosyl-lysyl-chloromethane; examination of Fig. 7 indicates that in the presence of the amino acid analog (lane 2), the 82K protein is less apparent than in the control incubation (lane 1). This strongly suggests that the 82K product corresponds to the NH_2-terminus of the HMW proteins.

Different destinies of 150 and 195K proteins. In the presence of edeine, TYMV RNA-directed protein synthesis stops within 45 to 60 min (data not shown). To follow the post-translational modifications of C-terminal-labeled HMW proteins, the incubation mixture was maintained at 30°C for several hours and analyzed at different times by gel electrophoresis. A scan of the autoradiogram of the gel is shown in Fig. 8. As a function of time, the amount of 78K protein increased, at least for 2 h. After that, nonspecific degradation of the 78K probably occurred. This confirms the previous results and shows that this protein really corresponds to a post-translational event. Figure 8 also shows that the 150/195K ratio strongly increased during the incubation, demonstrating that mainly the 195K was cleaved.

The 195K protein possesses at least two cleavage sites. We have demonstrated that the 78K protein corresponds to the C-terminal region of the 195K polypeptide and that the 82K fragment derives from the NH_2-terminus of this polypeptide. We have been unable to demonstrate unambiguously that the 82K protein does

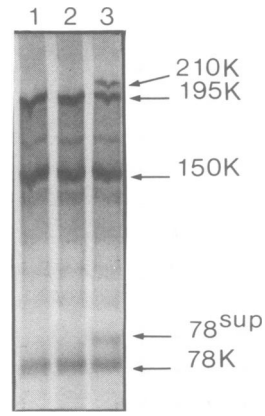

FIG. 5. *Effect of yeast suppressor tRNA on the synthesis of HMW proteins in the reticulocyte lysate. Incubations were performed under standard conditions at 30°C for 90 min, except that L-[^{35}S]methionine was replaced by L-[^{35}S]cysteine and yeast tRNA's were added as indicated. After appropriate treatment, the products were separated by electrophoresis on a 7.5% polyacrylamide–0.1% SDS slab gel as described in the text. Lane 1: without yeast tRNA; lane 2: with Su$^-$ tRNA^{Ser} at 20 μg/ml; lane 3: with amber suppressor tRNA^{Ser} at 20 μg/ml.*

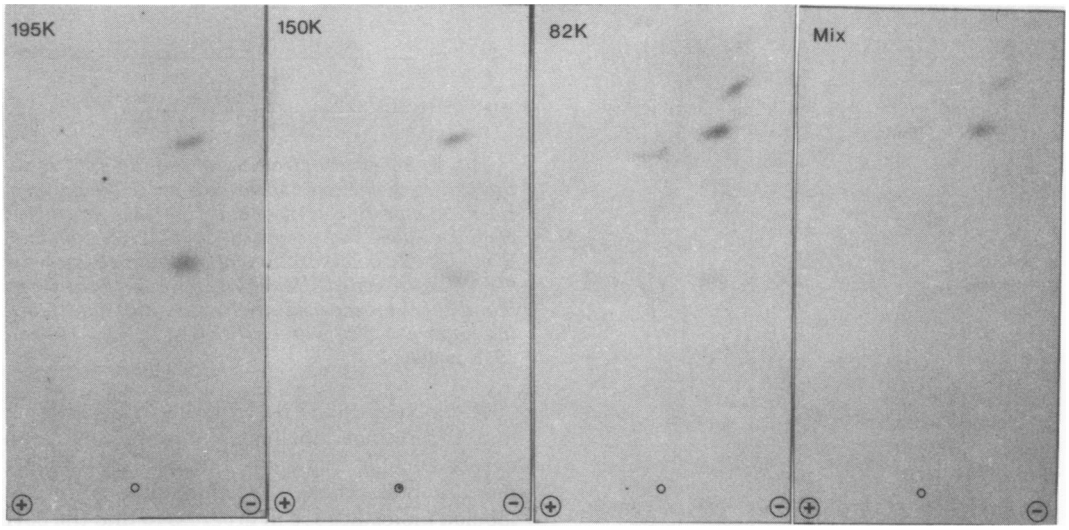

FIG. 6. *Comparison of the [^{35}S]methionine-labeled initiator tryptic peptides of the 195, 150, and 82K proteins. The proteins were labeled using formyl-[^{35}S]methionyl-tRNA$_i^{Met}$, and the resulting tryptic peptides were separated by electrophoresis and chromatography on cellulose plates as described in the text. From left to right, the different panels correspond to 195, 150, and 82K and a mixture of 195 and 82K, respectively. In this last experiment, the hydrolysis of both proteins was performed in the same tube.*

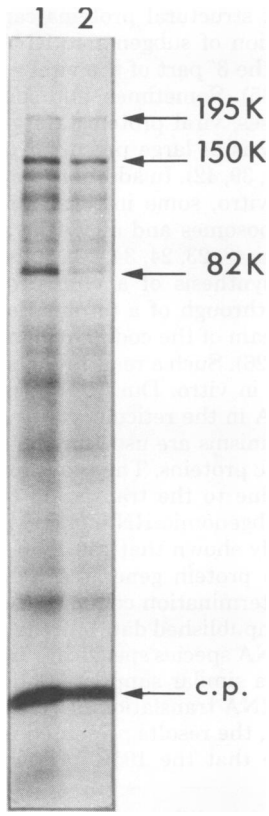

FIG. 7. *Effect of amino acid analog on the appearance of the TYMV RNA-coded 82K protein in a reticulocyte lysate. Incubations were performed using formyl-[^{35}S]methionyl-tRNA$_i^{Met}$ as the sole source of labeling, as described in the text, except that lysine was omitted from the amino acid mixture. After incubation, the proteins were separated by 12.5% polyacrylamide–0.1% SDS slab gel electrophoresis and localized by autoradiography. Lane 1 corresponds to an incubation performed for 90 min at 30°C without addition of amino acid analog. In lane 2, incubation was as in lane 1, except that N-tosyl-lysyl-chloromethane was added at 1 mM.*

not derive from the 150K protein, but the fact that the two HMW polypeptides do not follow the same degradation rates argues against this possibility. In addition, if the 150K protein had been cleaved at site B indicated in Fig. 9, a 33K fragment should have been generated and detected by C-terminal labeling. This was not the case (see Fig. 2, lane 2) and suggests that proteolytic cleavage does not occur at this site in the 150K protein. In the same way, if the 150K protein had been split at site A (Fig. 9) in the absence of cleavage at site B, a 68K fragment should have been detected in the C-terminal labeling experiment. Such peptides (33 and 68K) were undetectable in Fig. 2. All these observations suggest that the 150K protein is not cleaved in the reticulocyte lysate. As the 195K protein gives rise to the 78 and 82K fragments, it implies that another peptide with a molecular weight of 35K is also produced by proteolysis and that at least two cleavage sites are present along the

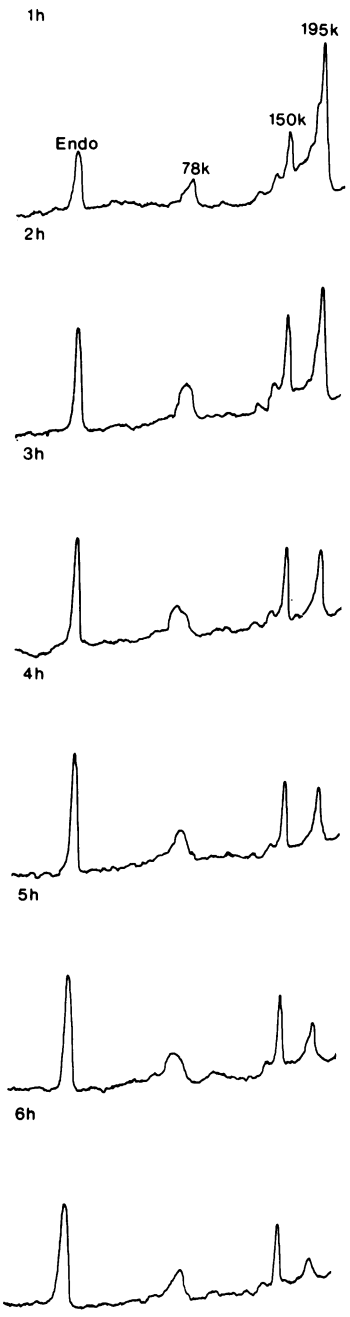

FIG. 8. *Effect of incubation time on the post-translational processing of the TYMV RNA-coded 195K protein in a reticulocyte lysate. Incubation and C-terminal labeling were performed as for Fig. 2, lane 2, in a final volume of 100 μl, except that puromycin was added at 200 μM after 1 h of incubation. Samples (15 μl each) were removed at different times and denatured before analysis by electrophoresis on a 12.5% polyacrylamide-0.1% SDS slab gel. The autoradiogram of the gel was analyzed by microdensitometer tracing of the different lanes as described in the text. From top to bottom, the patterns correspond to the samples removed after 1 to 6 h of incubation.*

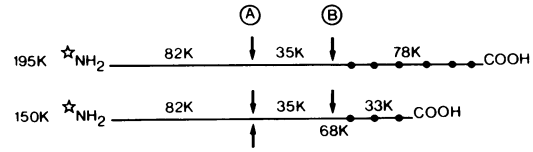

FIG. 9. *Schematic drawing of the different hypothetical cleavage sites of the 195 and 150K polypeptides. A and B correspond to the two proteolytic cleavage sites characterized on the 195K protein. Symbols: ●, incorporation of methionine into the TYMV RNA-coded HMW proteins during the C-terminal labeling experiments; ☆, NH_2-terminal labeling by formyl-[^{35}S]methionyl-tRNA$_i^{Met}$ of the 150 and 195K proteins.*

195K polypeptide (Fig. 9). Using NH_2-terminal and C-terminal labelings, we were unable to detect such a fragment. Further experiments using a pulse-chase procedure and uniformly labeled molecules are in progress to find the 35K fragment.

DISCUSSION

For several years evidence has accumulated that the genetic expression of RNA viruses might occur through different mechanisms. The synthesis of structural proteins can be due to the translation of subgenomic RNAs that correspond to the 3′ part of the viral genome (2, 9, 12, 27, 29, 35). Sometimes, and specifically for picornaviruses, viral proteins are generated by the proteolysis of large precursor polypeptides (1, 22, 23, 36, 39, 42). In addition, it appears that, at least in vitro, some internal initiation sites can bind ribosomes and allow the synthesis of viral proteins (8, 23, 24, 34, 40). Finally, in some cases, the synthesis of a viral protein results from a readthrough of a termination codon located upstream of the coding region of this protein (11, 21, 26). Such a readthrough occurs both in vivo and in vitro. During the translation of TYMV RNA in the reticulocyte lysate, most of these mechanisms are used for the synthesis of virus-specific proteins. The coat protein synthesis is only due to the translation of the corresponding subgenomic RNA (4a). Moreover, we have recently shown that the 195K protein is a readthrough protein generated by suppression of the opal termination codon of the 150K protein gene (unpublished data). In the reticulocyte lysate, a tRNA species specific for tryptophan is involved in a similar suppression during rabbit β-globin mRNA translation in vivo and in vitro (8a). Finally, the results presented in this paper demonstrate that the 195K protein is cleaved

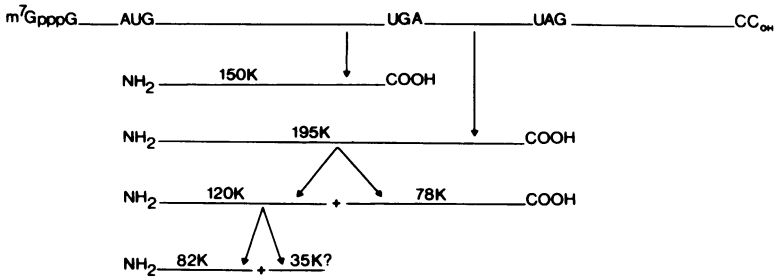

FIG. 10. *Organization of the TYMV genomic RNA and possible scheme of the post-translational modifications of HMW proteins coded by the viral RNA. The 35K fragment has not yet been characterized and therefore is designated as "35K?." AUG corresponds to the common initiator triplet of both 150 and 195K proteins, and UGA and UAG correspond to the terminator triplets of 150 and 195K protein genes, respectively.*

into at least three fragments. In Fig. 1, a 120K polypeptide is detected together with the 78K protein. The fact that the amount of the 120K polypeptide is constant between 60 and 120 min indicates that this polypeptide results from the primary cleavage of the 195K protein and that it must be the precursor of the 82K fragment. This is summarized in Fig. 10.

It is interesting to stress that the 150K product is not split in the reticulocyte lysate although it contains most of the amino acid sequences present in the 195K protein. One possibility could be that the 195K precursor possesses a proteolytic activity in its C-terminal region, directly involved in the processing. The 150K protein, which does not contain this C-terminal region, would be unable to catalyze its own cleavage. Such virus-specified proteolytic activities have been well characterized in the case of encephalomyocarditis virus (16), of adenovirus type 2 (32), and of Rous sarcoma virus (42). However, one cannot exclude that the conformation of the 195K protein is such that sites A and B are accessible for degradation and that this is not the case for the 150K protein.

Differential proteolytic cleavage of proteins possessing common features could be of major importance for the genesis of viral proteins. A single peptide may possess different biological roles depending on its folding and its location in the proteins. In the case of Qβ RNA bacteriophage, A1 protein is necessary for the infectivity of viral particles. This protein is a readthrough product of the coat protein gene (11). Consequently, addition of amino acids to the C-terminal region of the coat protein leads to a protein possessing a new biological function. Recently, in the case of the avian sarcoma virus B77 strain, it has been shown that the reverse transcriptase contains peptide sequences belonging to the *gag* proteins (10). This is probably due to incomplete processing of the precursor polypeptide by the virus-coded protease.

Nothing is known about TYMV RNA-coded products in vivo and their biological roles. Experiments with TYMV-infected Chinese cabbage protoplasts have been undertaken to detect virus-specific polypeptides in vivo and try to correlate both in vivo and in vitro observations.

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LITERATURE CITED

1. **Aliperti, G., and M. J. Schlesinger.** 1978. Evidence for an autoprotease activity of Sindbis virus capsid protein. Virology **90**:366–369.
2. **Beachy, R. N., and M. Zaitlin.** 1977. Characterization and *in vitro* translation of the RNAs from less-than-full-length, virus-related, nucleoprotein rods present in tobacco mosaic virus preparations. Virology **81**:160–169.
3. **Bénicourt, C., and A. L. Haenni.** 1976. *In vitro* synthesis of turnip yellow mosaic virus coat protein in a wheat germ cell-free system. J. Virol. **20**:196–202.
4. **Bénicourt, C., and A. L. Haenni.** 1978. Differential translation of turnip yellow mosaic virus mRNAs *in vitro*. Biochem. Biophys. Res. Commun. **84**:831–839.

4a. **Bénicourt, C., A. L. Haenni, and F. Chapeville.** 1978. Regulation of translation of the genome of plant RNA viruses; a model: turnip yellow mosaic virus, p. 95–102. *In* J. Zelinka and J. Balan (ed.), Proceedings of Third International Symposium of Ribosomes and Nucleic Acid Metabolism. Slovak Academy of Sciences, Bratislava.

5. **Bénicourt, C., and M. D. Morch.** 1979. Organization of the genome of TYMV deduced from its messenger properties. Biochem. Transact. **7**:1095–1097.
6. **Bénicourt, C., J. P. Pere, and A. L. Haenni.** 1978. Translation of TYMV RNA into high molecular weight proteins. FEBS Lett. **86**:268–272.
7. **Briand, J. P., G. Jonard, M. Guilley, K. Richards, and L. Hirth.** 1977. Nucleotide sequence (n = 159) of the amino-acid-accepting 3'-OH extremity of turnip yellow mosaic virus RNA and the last portion of its coat-protein cistron. Eur. J. Biochem. **72**:453–463.

8. **Celma, M. L., and E. Ehrenfeld.** 1975. Translation of poliovirus RNA *in vitro*: detection of two initiation sites. J. Mol. Biol. **98**:761-780.
8a. **Geller, A. I., and A. Rich.** 1980. A UGA termination suppression $tRNA^{Trp}$ active in rabbit reticulocytes. Nature (London) **283**:41-46.
9. **Glanville, N., M. Ranki, J. Morser, L. Kaaraiainen, and A. E. Smith.** 1976. Initiation of translation directed by 42S and 26S RNAs from Semliki Forest virus *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **73**:3059-3063.
10. **Hizi, A., M. A. McCrae, and W. K. Joklik.** 1978. Studies on the amino-acid sequence content of proteins specified by the *gag* and *pol* genes of avian sarcoma virus B77. Virology **89**:272-284.
11. **Hofstetter, H., H. J. Monstein, and C. Weissmann.** 1974. The readthrough protein A1 is essential for the formation of viable Qβ particles. Biochim. Biophys. Acta **374**:238-251.
12. **Hunter, T. R., T. Hunt, J. Knowland, and D. Zimmern.** 1976. Messenger RNA for the coat protein of tobacco mosaic virus. Nature (London) **260**:759-764.
13. **Jacobson, M. F., and D. Baltimore.** 1968. Polypeptide cleavages in the formation of poliovirus proteins. Proc. Natl. Acad. Sci. U.S.A. **61**:77-84.
14. **Klein, C., C. Fritsch, J. P. Briand, K. E. Richards, G. Jonard, and L. Hirth.** 1976. Physical and functional heterogeneity in TYMV RNA: evidence for the existence of an independent messenger coding for coat protein. Nucleic Acids Res. **3**:3043-3061.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680-685.
16. **Lawrence, C., and R. E. Thach.** 1975. Identification of a viral protein involved in post-translational maturation of the encephalomyocarditis virus capsid precursor. J. Virol. **15**:918-928.
17. **Leberman, R.** 1966. The isolation of plant viruses by means of "simple" coacervates. Virology **30**:341-347.
18. **Matthews, R. E. F.** 1974. Some properties of TYMV nucleoproteins isolated in cesium chloride density gradients. Virology **60**:54-64.
19. **Mellema, J. R., C. Bénicourt, A. L. Haenni, A. Noort, C. W. A. Pleij, and L. Bosch.** 1979. Translational studies with turnip yellow mosaic virus RNAs isolated from major and minor virus particles. Virology **96**:38-46.
20. **Morrison, T. G., and H. F. Lodish.** 1975. Site of synthesis of membrane and non-membrane proteins of vesicular stomatitis virus. J. Biol. Chem. **250**:6955-6962.
21. **Pelham, H. R. B.** 1978. Leaky UAG termination codon in tobacco mosaic virus RNA. Nature (London) **272**:469-471.
22. **Pelham, H. R. B.** 1978. Translation of encephalomyocarditis virus RNA *in vitro* yields an active proteolytic processing enzyme. Eur. J. Biochem. **85**:457-462.
23. **Pelham, H. R. B.** 1979. Synthesis and proteolytic processing of cowpea mosaic virus proteins in reticulocyte lysate. Virology **96**:463-477.
24. **Pelham, H. R. B.** 1979. Translation of fragmented viral RNA *in vitro*: initiation at multiple sites. FEBS Lett. **100**:195-199.
25. **Pelham, H. R. B., and R. J. Jackson.** 1976. An efficient mRNA-dependent translation system from reticulocyte lysate. Eur. J. Biochem. **67**:247-256.
26. **Philipson, L., P. Andersson, U. Olshevsky, R. Weinberg, D. Baltimore, and R. Gesteland.** 1978. Translation of MuLV and MSV RNAs in nuclease-treated reticulocyte extracts: enhancement of the *gag-pol* polypeptide with yeast suppressor tRNA. Cell **13**:189-199.
27. **Pleij, C. W. A., A. Neeleman, L. Van Vloten-Doting, and L. Bosch.** 1976. Translation of turnip yellow mosaic virus RNA *in vitro*. A closed and an open coat protein cistron. Proc. Natl. Acad. Sci. U.S.A. **73**:4437-4441.
28. **Porter, A., N. Carey, and P. Fellner.** 1974. Presence of a large poly (rC) tract within the RNA of encephalomyocarditis virus. Nature (London) **248**:675-678.
29. **Purchio, A. F., E. Erikson, and R. L. Erikson.** 1977. Translation of 35S and of subgenomic regions of avian sarcoma virus RNA. Proc. Natl. Acad. Sci. U.S.A. **74**:4661-4665.
30. **RajBhandary, U. L., and H. P. Ghosh.** 1969. Studies on polynucleotides. Yeast methionine transfer into nucleic acid: purification properties and terminal nucleotide sequences. J. Biol. Chem. **244**:1104-1113.
31. **Randerath, K.** 1970. An evaluation of film detection methods for weak β-emitters, particularly tritium. Anal. Biochem. **34**:188-205.
32. **Rashid Bhatti, A., and J. Weber.** 1979. Protease of adenovirus type 2: partial characterization. Virology **96**:478-485.
33. **Ricard, B., C. Barreau, H. Renaudin, C. Mouches, and J. M. Bove.** 1977. Messenger properties of TYMV-RNA. Virology **79**:231-235.
34. **Salomon, R., M. Bar-Joseph, H. Soreq, I. Gozes, and U. Z. Littauer.** 1978. Translation *in vitro* of carnation mottle virus RNA. Virology **90**:288-298.
35. **Shih, D. S., and P. Kaesberg.** 1973. Translation of brome mosaic viral ribonucleic acid in a cell-free system derived from wheat embryo. Proc. Natl. Acad. Sci. U.S.A. **70**:1799-1803.
36. **Shih, D. S., C. T. Shih, D. Zimmern, R. R. Rueckert, and P. Kaesberg.** 1979. Translation of encephalomyocarditis virus RNA in reticulocyte lysates: kinetic analysis of the formation of virion proteins and a protein required for processing. J. Virol. **30**:472-480.
37. **Silberklang, M., A. Prochiantz, A. L. Haenni, and U. L. RajBhandary.** 1977. Studies on the sequence of the 3'-terminal region of turnip yellow mosaic virus RNA. Eur. J. Biochem. **72**:465-478.
38. **Spector, D. H., and D. Baltimore.** 1975. The molecular biology of poliovirus. Sci. Am. **232**:25-31.
39. **Svitkin, Y. V., and V. I. Agol.** 1978. Complete translation of encephalomyocarditis virus RNA and faithful cleavage of virus-specific proteins in a cell free system for Krebs-2 cells. FEBS Lett. **87**:7-11.
40. **Van Vloten-Doting, L., J. Bol, L. Neeleman, T. Rutgers, D. Van Dolen, A. Castel, L. Bosch, G. Marbaix, G. Huez, E. Hubert, and Y. Cleuter.** 1977. *In vivo* and *in vitro* translation of the RNAs of alfalfa mosaic virus, p. 387-411. *In* Nucleic acids and protein synthesis in plants. NATO Advanced Study Institutes Series, series A, vol. 12. Plenum Publishing Corp., New York.
41. **Villa-Komaroff, L., M. McDowell, D. Baltimore, and H. F. Lodish.** 1974. Translation of reovirus mRNA, poliovirus RNA and bacteriophage Qβ RNA in cell-free extracts of mammalian cells. Methods Enzymol. **30**:709-723.
42. **Von der Helm, K.** 1977. Cleavage of Rous sarcoma viral polypeptide precursor into internal structural proteins *in vitro* involves viral protein p15. Proc. Natl. Acad. Sci. U.S.A. **74**:911-915.