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DIFFERENTIAL TRANSLATION OF TURNIP YELLOW MOSAIC VIRUS mRNAs IN VITRO

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SUMMARY

Total TYMV RNA was incubated in a reticulocyte lysate, and the initiation peptides of the main proteins synthesized in vitro (195 K, 150 K and 20 K daltons) analyzed after tryptic digestion. The 195 K and the 150 K dalton proteins present analogous patterns, different from the one obtained with the 20 K dalton protein (coat protein), suggesting that only one initiation site exists on the genomic RNA for the synthesis of the two high molecular proteins. The results of competition experiments between genomic and coat protein mRNA indicate that the ribosomes have a much greater affinity for the coat protein mRNA. This may represent a regulatory mechanism for the preferential amplification of coat protein synthesis in the infected cells.

INTRODUCTION

The multiplication of an RNA virus in a cell requires at least two types of proteins : structural proteins, and a replicating enzyme (or a subunit thereof) which allows the polymerization of new viral RNA molecules. This replicating enzyme, which presumably catalyzes the synthesis of several RNA molecules, needs to be produced only in small amounts in the infected cells. In contrast the structural proteins must be synthesized in large quantities to encapsidate the nascent viral RNA molecules. This raises the question of the differential synthesis of these two types of polypeptides, especially when their information is encoded by the same RNA molecule. This is the case of several plant RNA viruses such as Tobacco Mosaic Virus (TMV) and Turnip Yellow Mosaic Virus (TYMV).

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TYMV has long been considered to contain a unique RNA molecule of 2 x 10^6 daltons within a capsid composed of 180 identical coat protein molecules. More recently, it was shown that TYMV contains two main RNA species : the genomic RNA of 2 x 10^6 daltons which is infectious and therefore contains all the genetic information of the virus (1), and an RNA deriving from the genomic RNA which codes for the viral coat protein and whose size varies from 0.2 to 0.3 x 10^6 daltons depending on the authors (1-4). We have recently shown that, in a reticulocyte cell-free system, total TYMV RNA is translated into coat protein as well as into two high molecular proteins (HMW) of 195 000 and 150 000 daltons with common amino acid sequences (5). As these two large proteins are not precursors of the coat protein and as genomic RNA does not direct any synthesis of coat protein (4), it implies that the genomic RNA probably possesses only one initiation site accessible for the ribosomes *in vitro*.

The existence of an RNA species of low molecular weight coding for structural proteins and deriving from a larger one, appears to be very general both among animal and plant viruses (6-8). Hypotheses involving a specific amplification of this type of RNA molecule have been proposed to explain the synthesis in large amounts of structural proteins during viral infection (6,9).

In this paper, experiments are presented suggesting that such an amplification process is not necessary to explain the differential synthesis of viral proteins, since *in vitro* the affinity of ribosomes for the two RNA species of TYMV seems to be the most important mechanism involved in this regulation.

MATERIALS AND METHODS

1. Materials

TYMV infected Chinese cabbage leaves were a kind gift of

S. Astier and P. Cornuet (INRA, Versailles, France) and the virus was extracted as described by Leberman (10). Highly purified beef liver $tRNA_i^{Met}$ was kindly supplied by O. Kellerman and J.P. Waller (Ecole Polytechnique, Palaiseau, France). ^{35}S-L-Methionine (700 to 1000 Ci/mmole) was purchased from Amersham, N_5-formyltetrahydrofolate (leucovorin) from Serva, and TPCK-treated trypsin from Worthington. Polygram 300 cellulose plates were from Macherey-Nagel and Co. and RP-Royal-X-Omat films from Kodak.

2. Methods

TYMV RNA was extracted from the virion using the phenol-chloroform-SDS-method described by Porter et al. (11).

Preparation of the mRNA-dependent reticulocyte lysate was according to Pelham and Jackson (12) with minor modifications (5) and the assay for protein synthesis performed as previously described (5). Analysis of the translation products was carried out on 15 % polyacrylamide-0.1 % SDS slab gels (13) and the in vitro synthesized proteins detected by autoradiography using RP-Royal-X-Omat films.

Preparation of f-^{35}S-Met-$tRNA_i^{Met}$ was performed as described by RajBhandary and Ghosh (14) using beef liver $tRNA_i^{Met}$ and an E. coli S_{100} extract in the presence of 30 µM ^{35}S-L-methionine (100 Ci/mmole) and 100 µM leucovorin. The level of formylation was checked by analysis of KOH-hydrolyzed f-^{35}S-Met-$tRNA_i^{Met}$ on a cellulose plate submitted to electrophoresis at 600 volts for 45 minutes in a pyridine-acetic acid-water pH 6.5 buffer (25:1:474) and autoradiographed. The extent of formylation was always over 95 %.

Initiation of protein synthesis in the reticulocyte system was performed in the same conditions as protein synthesis except that ^{35}S-L-methionine was replaced by 1 µg of f-^{35}S-Met-$tRNA_i^{Met}$

(0.5 μCi/μg) and 100 μM unlabelled methionine. Incorporation was stopped after 90 min and the samples incubated 20 more min at 30° C with pancreatic RNase (100 μg/ml) in the presence of 5 mM EDTA. The samples were then boiled for 3 min in an appropriate buffer (13) prior to electrophoresis on a 12.5 % polyacrylamide-0.1 % SDS slab gel. After electrophoresis, the gel was fixed with acetic acid, washed with water and dried. The radioactive proteins were located by autoradiography, excized from the gel and incubated in 1 % ammonium bicarbonate containing 100 μg/ml of TPCK-treated trypsin as described by Morrison and Lodish (15). The ammonium bicarbonate solutions were then dried at 37° C. The residue was redissolved in 5 μl formic acid and spotted on a cellulose plate (20 x 20 cm) prior to electrophoresis and pH 4.7 (pyridine-acetic acid-water, 25:25:950) at 500 volts for 130 min. Electrophoresis was followed by ascending chromatography (n-butanol-acetic acid-pyridine-water, 75:15:50:60) as described by Chen (16). The cellulose sheet was then treated with PPO-ether as described by Randerath (17) and autoradiographed at - 70° C using a flash-activated film (18).

RESULTS

1. Initiation of protein synthesis with TYMV RNA

Previously we have shown that the genomic RNA directs the synthesis of two HMW proteins of 195 K and 150 K daltons bearing common amino acid sequences (5). It remained to be determined whether these two proteins are initiated at the same site or at two different sites on the same RNA molecule. To this end, incubation in the reticulocyte system was performed using f-^{35}S-Met-$tRNA_i^{Met}$ and the two HMW proteins separated and treated as described in Materials and Methods. The autoradiography of the tryptic peptides is presented in figure 1. The tryptic-initiator

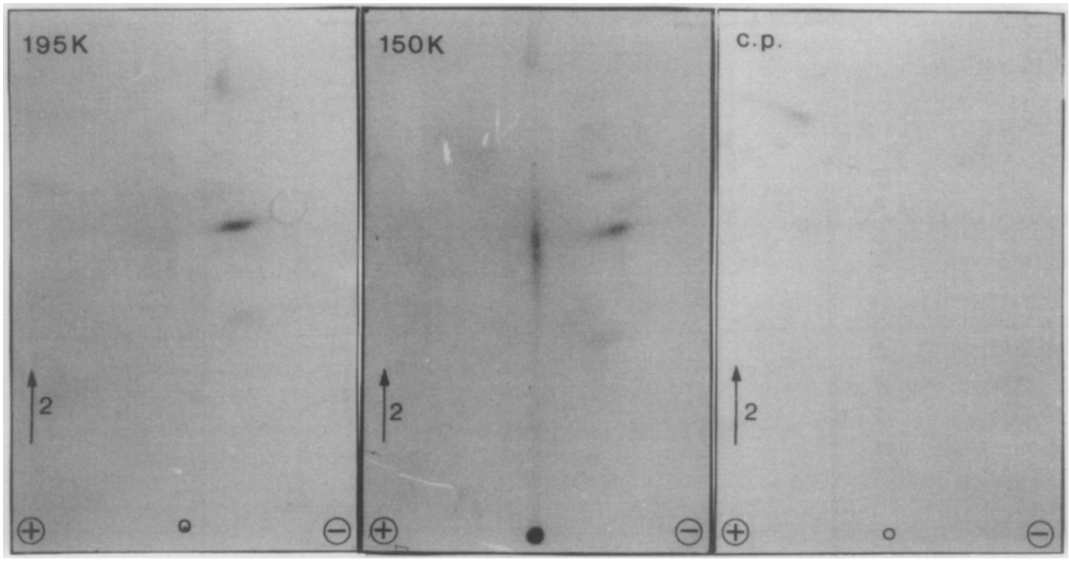

Figure 1 - Autoradiography of the initiator peptides of HMW proteins and coat protein synthesized in vitro in response to TYMV RNA in the presence of f-^{35}S-Met-tRNA$_f^{Met}$. 195 K, 150 K, and C.P. correspond respectively to 195 000; 150 000 and coat proteins eluted from a polyacrylamide gel and treated as described in Materials and Methods.

peptides from the two HMW proteins are identical and different from that of the coat protein. This implies that the two HMW proteins are initiated at the same site on the mRNA and that genomic TYMV RNA possesses only one initiation site accessible for the ribosomes. Thus total TYMV RNA constitutes a mixture of two mRNA species which can be used to study interactions between mRNA and ribosomes.

2. Translation of total TYMV RNA in eucaryotic cell-free systems

Total protein synthesis obtained in response to the addition of various amounts of total TYMV RNA to an mRNA-dependent reticulocyte cell-free system is represented in figure 2. Maximal incorporation is reached with very low RNA concentrations (25 µg/ml); incorporation then decreases linearly with further

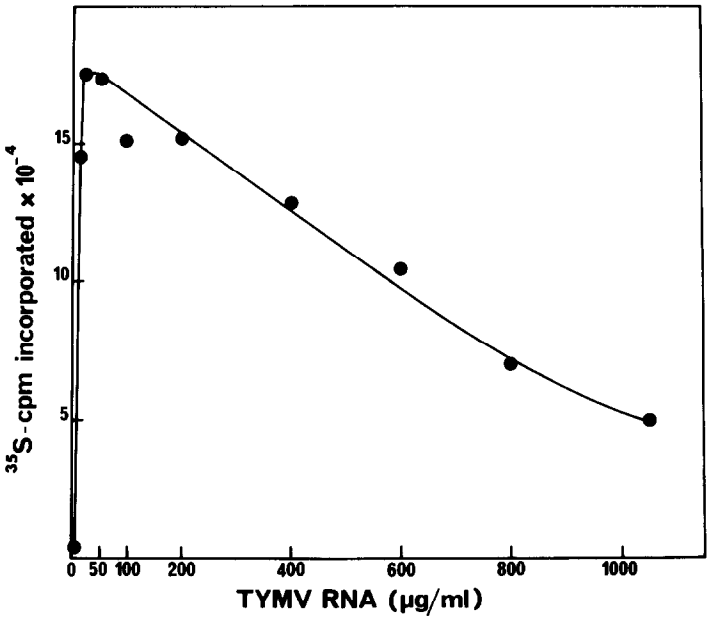

Figure 2 - Effect of total TYMV RNA concentration on protein synthesis in a reticulocyte lysate. Aliquots (2 µl) were spotted onto Whatman 3MM disks and treated as previously described (13) prior to counting in a toluene-based scintillator.

increase in RNA concentration. This decrease of amino acid incorporation may be due to the interaction of Mg^{++} with the viral RNA which lowers the concentration of free Mg^{++} in the incubation mixture.

To analyze the products synthesized at various RNA concentrations, equal amounts of radioactivity from the in vitro synthesized products were layered into the wells of a 15 % polyacrylamide-0.1 % SDS slab gel. The corresponding autoradiography is shown in figure 3. As the RNA concentration is raised, the proportion of coat protein synthesized increases with respect to the HMW proteins. Consequently the coat protein mRNA is more efficiently translated than genomic RNA at high RNA concentrations.

In order to define whether this phenomenon is due to the reticulocyte ribosomal machinery or to the structural features

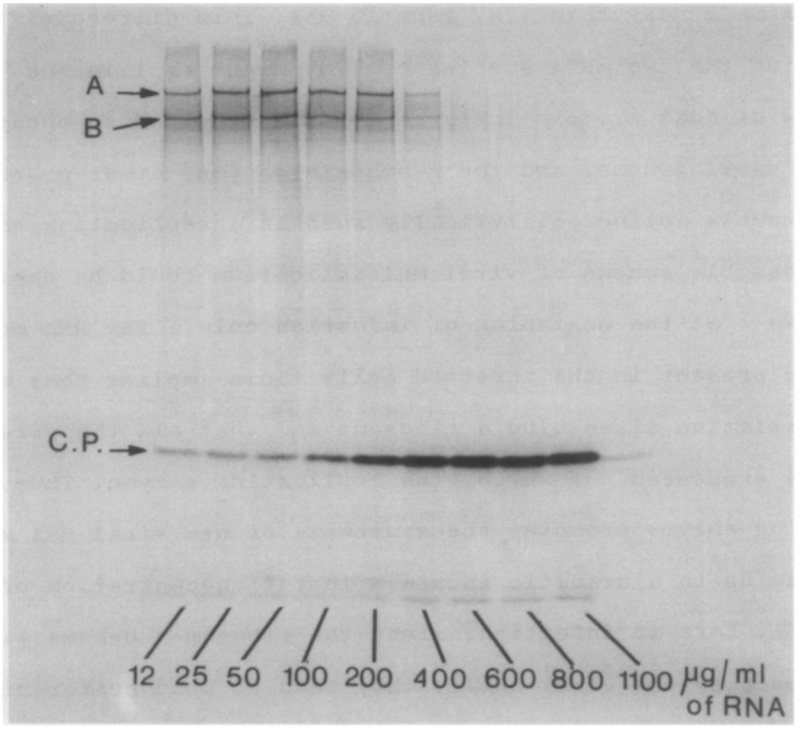

Figure 3 - Effect of total TYMV RNA concentration on differential translation of genomic and coat protein mRNAs of TYMV in a reticulocyte lysate. Equal amounts of radioactive products synthesized in response to different concentrations of total TYMV RNA were layered into the wells of a 15 % polyacrylamide-0.1 % SDS gel. After electrophoresis the gel was fixed (methanol-acetic acid-water, 300; 75; 625), dried and autoradiographed.

of the RNA, analogous experiments were performed in a wheat germ cell-free system (13). The same phenomenon is observed : as the concentration of TYMV RNA increases, the proportion of coat protein molecules synthesized increases relatively to the total *in vitro* synthesized products (results not shown). This implies that differential translation is a consequence of the relative affinity of the two mRNA species for the ribosomal machinery.

DISCUSSION

The results presented here show clearly that the coat protein mRNA of TYMV possesses a much higher affinity for the

ribosomal machinery than TYMV genomic RNA. This differential property of the two mRNA species of TYMV might explain the synthesis of coat protein in large amounts required to encapsidate the viral genome, and the synthesis of (an) other protein(s) in low amounts acting catalytically such as a replicating enzyme.

A possible scheme of viral multiplication could be described as follows : at the beginning of infection only a few RNA molecules are present in the infected cells. This implies that most viral initiation sites bind a ribosome and that all the viral genes are expressed, of which, the replicating enzyme. Thus, this replicating enzyme promotes the synthesis of new viral RNA molecules leading to a dramatic increase in the concentration of viral mRNA. Late in infection, since the ribosomes become limiting with respect to the viral mRNAs, they tend to bind preferentially to the mRNA for which they have a greater affinity, and the coat protein gene is virtually the only one expressed. New viral particles are then formed by the assembly of coat proteins with the viral RNA molecules. Indications for such a differential recognition have been presented for both viral (19,20) and cellular mRNAs (20,21) and were explained in terms of differences in the secondary structure of the initiation site or of lack of selective factors required for the initiation process.

Whilst this manuscript was being written, similar experiments were reported by Zagorski using Brome Mosaic Virus RNA and a wheat germ cell-free system (22); the results lead to analogous conclusions concerning the infectious cycle of the virus.

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