TOBACCO MOSAIC VIRUS RNA CARRIES 5′-TERMINAL TRIPHOSPHORYLATED GUANOSINE BLOCKED BY 5′-LINKED 7-METHYLGUANOSINE

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1. Introduction

An unusual structural feature has been found during the past year at the 5′-end of all mRNAs of animal cells that were studied in this regard, namely that the terminal nucleotide(s) carried ribose methyl groups and a 5′-triphosphate which was blocked by 7-methylguanosine linked at the 5′-position (7-mG (5′)ppp(5′) ($N^mp)_1$ or $_2$Np-) [1–8].

The same type of inverted ending was found for viral mRNAs produced as the result of infection, and more recently for certain intraviral RNAs, including the RNA of Rous sarcoma virus [9]. A plant viral RNA, that of tobacco mosaic virus (TMV), was historically the first nucleic acid to be obtained in pure state and to be shown to carry genetic information, and to represent a mRNA in that it was directly translated [10–13]. Thus it was imperative to search for the new type of 5′-terminus in this RNA, which had been reported as lacking terminal phosphates [14], but carrying adenosine at the 5′-end [15]. Unphosphorylated adenosine was also reported as the 5′-terminus of several other plant, bacterial, and even tumor virus RNAs [16–19].

2. Materials and methods

Four plants (*Nicotiana tabaccum*) inoculated 3 days earlier with TMV (0.1 mg/ml), were cut off and allowed to imbibe 5 mCi of carrier-free ^{32}P, then floated in water in baking dishes with 16 hr illumination for 1 week. TMV and TMV RNA were isolated by standard procedures. The RNA, usually at least 80% 30S, was at times further purified by sucrose gradient centrifugation after heating for 45 sec at 100°C.

Digestion of the RNA with pancreatic, T1, and T2 ribonucleases, followed by alkaline phosphatase, electrophoretic isolation and redigestion of the remaining labeled oligonucleotides by the same enzymes, and subsequent digestion of the twice-nuclease-resistant fragments by venom diesterases were done as recently described [9]. Subsequent identification of 7-methylguanylic acid was by chromatography in ethanol-1 M(pH 7.5) ammonium acetate (75:30, v/v) (Solvent B) [9], and 5′-guanylic acid was differentiated from ribose-methylated guanylic acid by chromatography in isopropanol-concentrated ammonia – 0.1 M boric acid (70:10:20, v/v) (Solvent C) [9].

3. Results and discussion

Gradient-purified ^{32}P-labeled TMV RNA (8–20 × 10^6 cpm, about 50 000 cpm/μg) was digested with a mixture of three ribonucleases (pancreatic, T1 and T2) followed by bacterial alkaline phosphatase, and the digest electrophoresed at pH 3.5. The relatively small amounts of radioactivity behind the large amount of inorganic phosphate at the anodic end of the paper showed two main peaks located between the adenylate and uridylate markers. These two, as well as other radioactive areas, were eluted and again digested with the above four enzymes. This generally caused complete dephosphorylation of all but two sharp peaks located slightly behind

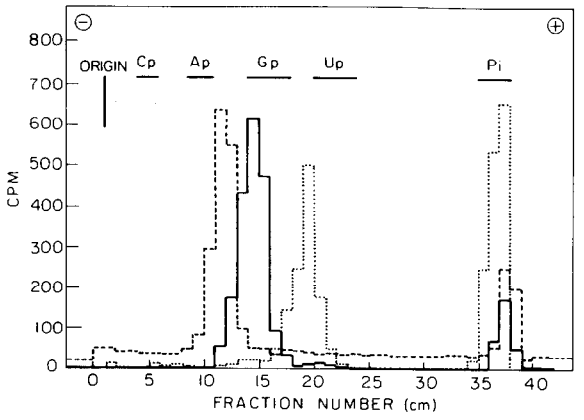

Fig.1. Electrophoresis in pH 3.5 pyridine acetate of resistant fragments of viral RNAs twice treated with nuclease and phosphatase [9]. The solid line represents the terminal fragment of TMV RNA and the small amount of inorganic phosphate released upon repeated enzyme treatment of the corresponding area of the first electropherogram. The dotted line represents the partially resistant guanylic oligonucleotide found in TMV RNA digests and the inorganic phosphate released from it upon retreatment with enzymes. The dashed line represents the resistant fragment from Rous sarcoma RNA [9], included on the figure only for purposes of comparison.

Gp (14 cm) and between Gp and Up (19 cm) respectively, which remained to 90% and 40% enzyme resistant (see fig.1). They represented about 3000 cpm and 1800 cpm in a typical experiment starting with 9.5×10^6 cpm of gradient-purified TMV RNA.

The nuclease-resistant fragments were then treated with a high level of snake-venom phosphodiesterase (37°C, 150 μg, 4 hr), and again electrophoresed as above. The main peak yielded three areas of similar radioactivity, located in the inorganic phosphate, guanylic acid, and origin areas (624, 466 and 380 cpm, respectively). The identity of the latter two as 5′-guanylic and 5′-(7-methyl)guanylic acid (pG and p7mG) was established by chromatography in Solvents C and B, which also confirmed their purity. These results differ from those recently reported for RSV RNA in that in that case the only nuclease-resistant fragment moved 3 cm less far (see fig.1), and proved to contain four stoichiometric components, namely, inorganic phosphate, guanylate (which upon chromatography proved to be ribose-methylated), cytidylate, and 7-methylguanylate [9]. Thus RSV RNA had the terminal sequence 7-mG(5′)ppp(5′)G^{m}C, while TMV RNA has the simpler terminal structure 7-mG(5′)-ppp(5′)G which is split by diesterase at the indicated arrows to yield the three products found. The same terminus recently been reported from another plant-virus source, in that part of the small component (RNA 4) of brome mosaic virus (BMV) terminates in this manner [20].

In quantitative respects the data on TMV RNA are similar to those obtained with RSV RNA, with the inorganic phosphate being a little higher than the other products. As previously discussed [9] this may be due to a trace of phosphatase or 5′-nucleotidase activity in the snake-venom diesterase. The low radioactivity detected in the Up area on the other hand may be due to some residual diphosphate. Concerning the total amount of nuclease-resistant fragment, it appears to be only about two-thirds of that calculated for a triphosphate terminal component of TMV RNA containing 6400 nucleotides. Whether this signifies that part of the RNA molecules terminate otherwise, or that the resistance of this particular fragment to the enzymes used is not absolute, remains to be established. Concerning the nature of the lesser peak centered at fraction 19 (fig.1), it yielded only one product, electrophoresing and chromatographing in Solvent B like guanylic acid, both upon venom- and spleen-diesterase treatment. The absence of 7-mpG and inorganic phosphate suggests that this product is not part of the terminal structure. Its structure and origin are under further study. TMV RNA not gradient-purified also yielded a peak preceding Ap on the electropherogram, which proved to be degraded to adenylic acid by both diesterases.

The question remains why we and/or others, using various methods, have previously found unphosphorylated terminal adenosine in TMV, as well as all BMV components, a strain of MS2 phage, RSV RNA etc. [17–19]. We believe that this may be due to the possibility of contamination of any RNA with traces of fragments. Since the most labile bond and most likely break in RNA was shown to be –CpA– → –Cp, A– [21], 5′-terminal adenosine would predominate in such fragments. It would appear imperative to re-investigate other RNA 5′-termini in

the light of these findings. It may well be that all eukaryotic cellular and viral mRNAs show endings of the type indicated, with terminal ribose-methylation differentiating the animal from the plant viruses.

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