NUCLEOTIDE SEQUENCE NEAR THE 5′-TERMINAL OF CUCUMBER MOSAIC VIRUS RNA No. 5 SEGMENT

Soh HIDAKA, Kunitada SHIMOTOHNO, Kin-ichiro MIURA, Yoichi TAKANAMI* and Susumu KUBO*

National Institute of Genetics, Mishima 411 and **Central Research Institute, the Japan Tobacco and Salt Public Corporation, Umegaoka, Yokohama 227, Japan*

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1. Introduction

A definite piece of gene containing single cistron is a valuable material for study on the mechanism of gene expression. Cucumber mosaic virus (CMV) gene is known to consist of 4 or 5 (different according to the strain) single-stranded RNA segments, which are separated by gel electrophoresis by difference of size [1,2]. The smallest segment (segment no. 5) is estimated as about 300 nucleotides long [1,3]. As the protein synthesis starts at the initiation codon and proceeds in the direction 5′→3′ of the template RNA, we analyzed here the nucleotide sequence near the 5′-terminal of no. 5 segment of CMV RNA. The 5′-terminal carries the cap structure, $m^7G^{5'}ppp^{5'}G$, as reported [4]. The initiation codon AUG appears at the 11th position in no. 5 segment, although it does not appear until 30th in no. 4 segment, the second smallest RNA segment. The sequence between the 5′-terminus and the initiation codon in no. 5 RNA is quite simple as $m^7G^{5'}ppp^{5'}$G–U–U–U–U–G–U–U–U–G–A–U–G.

2. Materials and methods

CMV yellow strain (CMV-Y) [5] was propagated in tobacco plants (*Nicotiana tabacum* cv. Ky 57), and viral RNA was purified as in [6,7]. Segments no. 4 and 5 were separated from other segments and from each other in a sucrose density gradient by ultracentrifugation.

The nucleotide sequence analysis was carried out by gel electrophoresis of partial digests of RNA [8–10]. RNA was oxidized by periodate and β-eliminated by anilin and treated with phosphomonoesterase to remove the 5′-blocked cap structure, before [^{32}P]phosphate was introduced to the 5′-terminus of the pretreated RNA with [γ^{32}P]ATP and T4-infected polynucleotide kinase [8,11]. The labeled RNA was electrophoresed on a gel containing urea [12] and was eluted electrophoretically. The ^{32}P-labeled RNA was partially digested with alkali, RNase T_1 and U_2, respectively. These partial digests were separated on 20% gel electrophoresis. The sequence of adenine, guanine and pyrimidine was discriminated. The sequence of cytosine and uracil was deduced by mobility shift in the two-dimensional gel electrophoresis method in [10]. The alkaline partial digest of RNA was run in a 10% gel adjusted to pH 3.5 by citric acid. Then the gel column was put in the second dimension gel (20%, pH 8.3) and electrophoresed. The gels were autoradiographed by Kodak X-ray film XR-1 with the use of intensifying screen.

The first nucleotide at the 5′-terminus of RNA was analyzed by nuclease P_1 digestion, followed by column chromatography using anion exchanger resin Bio Rad AG 1 [11]. The neighbouring nucleotide was identified by digestion with pancreatic RNase A, followed by column chromatographies with DEAE–Sephadex A-25 in 7 M urea and with AG 1 resin [11].

Address correspondence to: Dr K. Miura, National Institute of Genetics, Mishima 411, Japan

3. Results and discussion

Since the 5′-terminus of CMV RNA was a m^7G-blocked cap structure, it was required to remove this residue prior to the 5′-terminal labeling. The 5′-terminal first nucleotide was determined as G by the AG 1 column chromatography of the nuclease P_1 digest of the 5′-terminal ^{32}P-labeled RNA. This is not methylated at the 2′-position like other plant viral RNAs so far analyzed [13–19]. Thus, the 5′-terminal structure of CMV RNA no. 5 is $m^7G^{5'}ppp^{5'}G$ as mentioned [4]. The second nucleotide was determined as pU, because $^{32}pGpUp$ was identified for the terminal oligonucleotide obtained from pancreatic RNase A digestion by the use of column chromatography as mentioned in section 2 (data are not shown here).

The nucleotide sequence following after the 5′-terminus was derived from gel electrophoresis of the partial digests of RNA. The partial digestion by alkali and RNase T_1 and U_2 gave the sequence of G, A and pyrimidine nucleotide (fig.1). The sequences of C and U were judged from the mobility shift in the two-dimensional gel electrophoresis as shown in fig.2. The nucleotide sequence from the 5′-terminus to the 30th position of CMV RNA no. 5 segment is summarized in fig.3.

The initiation codon AUG is found at the 11th position. The sequence between 1 and 10 non-coding region, is very simple and U-rich. In brome mosaic virus (BMV) no. 4 RNA, AUG appears at the 10th position and the sequence between 1 and 9 is U-rich [20]. Some other plant viral RNAs contain U-rich sequence near the 5′-terminus [21–23]. However, the long common sequence was not detected in the non-coding region near the 5′-terminal. The sequences of these regions so far analyzed, including mRNAs of

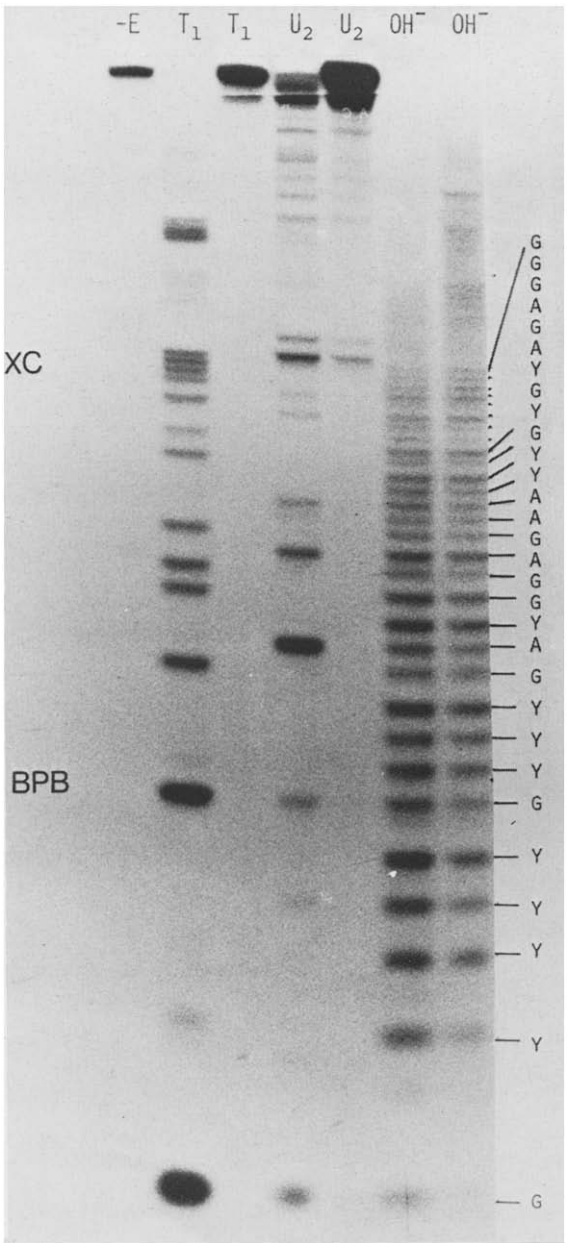

Fig.1. Autoradiogram of single-directional electrophoresis of partial digests of CMV [5′-^{32}P]RNA 5. Electrophoresis was carried out on 20% polyacrylamide gel slab (0.15 × 20 × 40 cm) in the presence of 7 M urea. Running buffer was 50 mM Tris–borate (pH 8.3) containing 1 mM EDTA. Patrial digestion with RNase T_1 and U_2 were carried out in 20 mM Na-citrate (pH 5.0), 1 mM EDTA, 7 M urea, 0.025% xylene cyanol and 0.025% bromphenol blue at 50°C for 15 min. Limited alkaline hydrolysis was carried out in 50 mM $NaHCO_3/Na_2CO_3$ buffer (pH 9.0) and 1 mM EDTA at 90°C for 15 min or 25 min. A 10 μl solution of 10 M urea, 0.05% xylene cyanol and 0.05% bromophenol blue was added to the 10 μl hydrolysate after digestion [8]. Tracks: E, undigested [5′-^{32}P]RNA; T_1, partial digestion with RNase T_1 at two different units (0.05 and 0.005 unit); U_2, partial digestion with RNase U_2 at two different units (2.5 and 0.5 unit); OH^-, partial digestion with alkali; BPB, Location of bromophenol blue dye; XC, location of xylene cyanol dye.

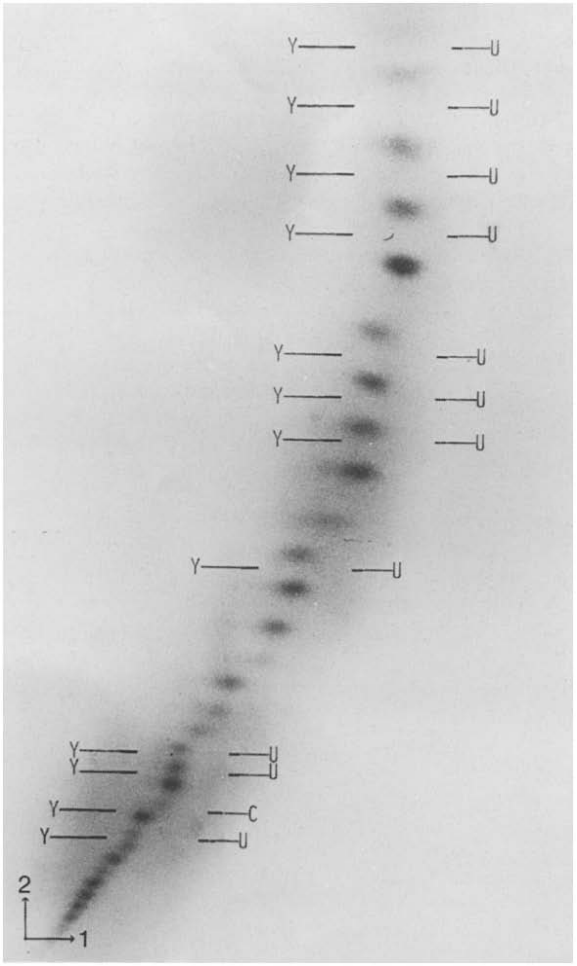

Fig.2. Autoradiogram of two-dimensional electrophoresis of a partial alkali digest of CMV [5′-^{32}P]RNA 5. First dimension was electrophoresed on 10% polyacrylamide gel slab (0.15 × 20 × 40 cm) at pH 3.5 until the bromophenol blue tracking dye had migrated 12 cm at 200 V. A 20 cm long strip from it was used for the second dimension electrophoresis on 20% polyacrylamide gel (0.15 × 25 × 40) at pH 8.3. Running buffer was 25 mM citric acid and 4 mM EDTA adjusted to pH 3.5 with sodium hydroxide for the first dimension and 90 mM Tris–borate, pH 8.3 containing 4 mM EDTA for the second dimension. The letter Y between two radioactive spots indicates that the two oligonucleotides differ by a pyrimidine residue (judged from the data in fig.1). C or U was identified by the mobility shifts in this system [10].

$m^7G^{5'}ppp^{5'}$GUUUUGUUUGAUGGAGAAUUGCGUAGAGGG

10 20 30

Fig.3. Nucleotide sequence from the 5′-terminus to the 30th nucleotide of CMV RNA no. 5 segment.

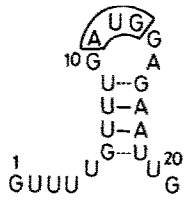

Fig.4. A possible secondary structure around the initiation codon.

animal cells and viruses were compared [24] and it was claimed that AUG is the only recognisable signal sequence in the 5′ non-coding regions of eukaryotic mRNA.

As shown in phage RNA at first [25–27], the initiation codon seems to be located in the looped-out part in a hair-pin type secondary structure in some cases. If a hair-pin structure is constructed around the initiation codon sequence in CMV RNA no. 5, a possible structure is as written in fig.4. Even if it is the case, this region does not keep the rigid structure, as only two A–U pairs and two G–U pairs are contained. Thus the initiation codon AUG and the neighbouring region in CMV RNA no. 5 would be almost in a single-stranded state and in a quite reactive state.

Preliminary analysis of no. 4 RNA of CMV showed that the intiation codon does not appear until 30th position from the 5′-terminal and this non-coding region does not show a distinct similarity to that of no. 5 RNA, except the first 3 nucleotide sequence G–U–U. The nucleotide mapping [28] and the competition hybridization experiments [29] also indicate that the CMV RNA no. 5 is less homologous to RNA no. 1–4.

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