

キュウリモザイクウイルスY系統(CMV-Y)RNA1の全塩基配列ならびにCMV全ゲノムRNAの比較

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Complete Nucleotide Sequence of RNA1 of Cucumber Mosaic Virus Y Strain and Evolutionary Relationships among Genome RNAs of the Virus Strains

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Abstract

cDNA clones covering full-length of cucumber mosaic virus Y strain (CMV-Y) RNA1 were synthesized and the complete nucleotide sequence of the RNA was determined. It consisted of 3,361 nucleotides and contained a long single open reading frame of 2,979 nucleotides. The putative "nucleotide binding site" and the other regions conserved in proteins involved in nucleic acid replication were found in the protein encoded by the RNA. There was almost complete sequence homology between CMV-Y and CMV-Fny RNAs (96.4% sequence homology at the nucleotide level) and lower degree of homology between CMV-Y and CMV-Q RNAs (76.1% sequence homology at the nucleotide level). The 5' terminal sequence of CMV-Y RNA1 showed substantial homology with that of CMV-Y RNA2, but virtually no homology with that of CMV-Y RNA3. By contrast, the 3' terminal sequences were highly conserved among all genome RNAs of CMV-Y and other CMV strains, Fny, O and D, and the variations were mainly found in one stem and loop structure.

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Key words: cucumber mosaic virus, RNA1, cDNA cloning, nucleotide sequence.

INTRODUCTION

Cucumber mosaic virus (CMV) is a positive-sense RNA plant virus with a tripartite genome consisting of single-stranded RNAs, designated RNA1, 2 and 3 in decreasing order of molecular weight (M_r)^{13,18}. In the course of our study on molecular aspects of CMV, we have already made cDNA clones and reported the complete nucleotide sequences of RNA3¹⁶) and RNA2¹²) of Japanese Y strain of CMV (CMV-Y)²⁵). In this paper, we described the synthesis of cDNA clones covering full-length of RNA1 of CMV-Y and its complete nucleotide sequence. Since RNAs1 and 2 of CMV were suggested to encode proteins associated with the replication of the virus genome¹⁷), it is of interest to know the nucleotide sequence and amino acid sequence encoded by the RNA and to compare them with those of other proteins involved in nucleic acid replication.

A nucleotide sequence of CMV RNA1 was first reported on CMV-Q strain²¹) followed by Fny strain²³). The computer analysis of these sequences revealed that there is a significant homology between the two RNAs²³) and sequences in the central region of the RNAs are highly conserved among other tripartite plant viruses²¹), alfalfa mosaic virus³) and brome mosaic virus²). Computer-assisted comparison of the sequences of RNAs of these CMV strains and evolutionary relationships among all genome RNAs of CMV are also described.

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MATERIALS AND METHODS

RNA purification, cDNA cloning and sequencing. All procedures were almost the same as fully described in the accompanying paper¹²⁾ for determination of nucleotide sequence of CMV-Y RNA2, except following points. Since the 5' terminal nucleotide sequence of the RNA was already determined by Hidaka *et al.* (personal communication), a specific primer was synthesized according to the sequence and primed in the second strand synthesis to create the complete 5' terminal cDNA of the RNA. *Pst*I site and *Not*I site were introduced at the upstream of the 5' end and at the downstream of the 3' end, respectively.

Computer analysis. The Microgenie (Beckman) and the DNASIS (Hitachi) sequence analysis programs were used in the analysis of CMV genome RNAs.

RESULTS AND DISCUSSION

The cloning and sequencing strategies for CMV-Y RNA1 are shown in Fig. 1. Three overlapping cDNA clones, designated pBCY11, pBCY12 and pCY1N4, were used for determination of the nucleotide sequence of CMV-Y RNA1. Since the 3' terminus, 5'-ACCA_{OH}, were lost in pBCY11, probably excised on the same reason described in our previous paper¹²⁾, pCY1C15 was constructed to cover the complete 3' terminal region of the RNA. As a result, the full-length CMV-Y RNA1 was cloned in the three overlapping cDNAs which contained two additional restriction sites at the both terminal regions as described in Materials and Methods, and these clones would facilitate any future reengineering of the inserts.

The complete nucleotide sequence and amino acid sequence of putative translation product of CMV-Y RNA1 are shown in Fig. 2. It consists of 3,361 nucleotides and contains a long ORF of 2,979 nucleotides encoding a 111,462 Mr protein (993 amino acids). Nucleotide sequence alignment of RNA1s of CMV-Y, -Q and -Fny is shown in Fig. 3, and variations found among them are summarized in Table 1 in nucleotides and amino acids. There are almost complete homologies between the sequences of RNA1s of Y and Fny strains in both the nucleotide and the amino acid levels (Table 1). By contrast, many variations are found between the two RNA1s of CMV-Y and Q strains (Table 1). It is known that CMV strains fall into two Sub-

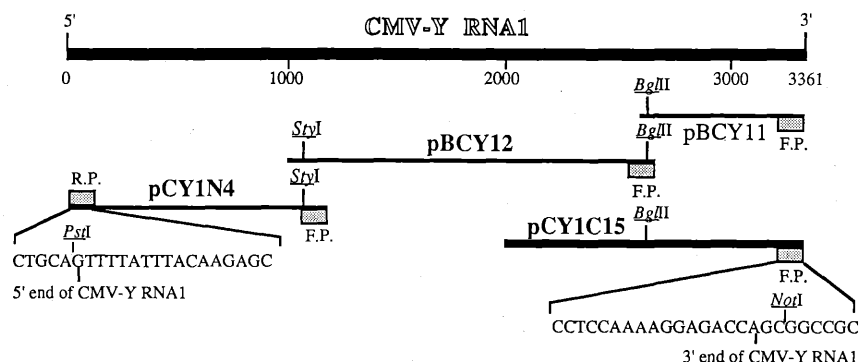


Fig. 1. Schematic representation of the cloning and sequencing strategies for CMV-Y RNA1. CMV-Y RNA1, the first set of overlapping cDNA clones used for sequencing and the newly constructed 3' terminal cDNA clone containing a specific *Not*I site are indicated by broad, thin and middle lines, respectively. Specific primers used for the synthesis of first strand cDNA (F.P.) and second strand cDNA (R.P.) are represented by dotted area. The nucleotide sequences of 5' and 3' terminal primers are shown.

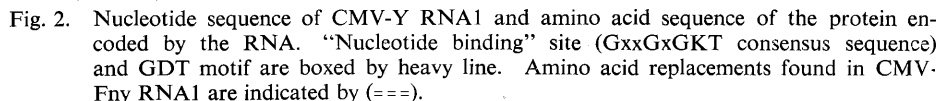


Fig. 3. Alignment of the nucleotide sequences of three CMV RNA1s, CMV-Q RNA1 (upper line), CMV-Y RNA1 (middle line) and CMV-Fny RNA1 (lower line). Nucleotides identical to CMV-Y RNA1 are indicated by (—). Deletions are indicated by (*).

Table 1. Differences found in the RNAs of CMV-Fny and CMV-Q compared to that of CMV-Y

Region	5' non-coding		ORF		3' non-coding		Total	
Length ^{a)}	97		2979		285		3361	
CMV strain	Fny	Q	Fny	Q	Fny	Q	Fny	Q
Length ^{b)}	94	97	2979	2973	284	319	3357	3389
Insertion	0	1	3	12	0	35	3	48
Deletion	3	1	3	18	1	1	7	20
Base Change	4	12	97	666	8	85	109	763
A.A. Rep. ^{c)}	—	—	21	164	—	—	21	164

a) Number of nucleotides in CMV-Y RNA1.

b) Number of nucleotides in RNAs of CMV-Fny (Fny) and -Q (Q) strains.

c) Amino acid replacement.

Motif	1	2	3	4
Q:	-707-ISQVDGVAGCGKTTAISKMFN-49-RVLVDEVVLLHFGQLCAV-5-AVRALCFGDSEQIAFSS-23-FRS-			
				
Y:	-710-ISQVDGVAGCGKTMPIKSIFE-49-RVLEDEVVLLHFGQLCAV-5-AVRAICFGDAEQIAFSS-23-FRS-			
				
F:	-709-ISQVDGVAGCGKTTAISKIFE-49-RVLVDEVVLLHFGQLCAV-5-AVRAICFGDSEQIAFSS-23-FRS-			
				
Motif	5	6		
Q:	-81-DRIKTVHESQGISDHVTLVR-15-LVAVTRHKVTF-20			
				
Y:	-82-ERIKTVHESQGISDHVTLVR-15-LVALTRHKVTF-19			
				
F:	-82-ERIKTVHESQGISDHVTLVR-15-LVALTRHKVTF-20			

Fig. 4. Alignment of highly conserved motifs from proteins of RNAs of three CMV strains.

Bold numbers are the motif numbers presented by Gorbalenya *et al.*⁶⁾

groups I and II^{4,5,19)}, and we have reported that CMV-Y would belong to Subgroup I based on the result of sequence analysis of CMV-Y RNA2¹²⁾. This proposal seems to be also supported by the sequence comparison of RNAs of Y, Fny and Q strains.

By computer analysis of accumulated sequence data, existence of a series of conserved motifs has been revealed in proteins involved in nucleic acid replication from various sources including several plant viruses¹⁰⁾, and the conserved regions are also found in bacterial helicases^{7,11)}. These conserved sequence motifs are also found in the putative protein encoded by CMV-Y RNA1, and are well conserved among CMV strains as shown in Fig. 4 which was modeled after Gorbalenya's sequence alignment⁶⁾. Motif 1 in Fig. 4 is well known as a conserved sequence found in ATP and GTP binding proteins²⁰⁾ and motif 3 was noted to resemble a conserved region from viral DNA polymerase and plant virus proteins involved in RNA replication¹¹⁾. Considering the facts that the translation product of CMV RNA2 contains the GDD motif which is known as the polymerase site¹⁴⁾ and that inoculation only with RNAs1 and 2 was sufficient to induce viral RNA replicase activity¹⁷⁾, non-structural proteins encoded by the both RNAs are suggested to be the main constituents of CMV replicase.

The sequences of the 5' non-coding region of CMV-Y RNAs 1, 2¹²⁾ and 3¹⁶⁾ were compared each other (Fig. 5). There is substantial homology between RNA1 and RNA2 (69.7% homology), but virtually no homology between RNA1 and RNA3 (35.0%). It was shown that synthesis of RNA3 in CMV-infected tobacco protoplasts was predominant compared to those of RNAs1 and 2²⁴⁾. Though little is known about the role(s) of the 5' non-coding region of CMV genome RNA, this finding may reflect the difference in replication efficiency between RNAs1, 2 and RNA3 in the infected cells.

In contrast with the 5' non-coding region, high levels of homology are conserved among 3' terminal regions of all genome RNAs of CMV strains so far sequenced. A secondary structure model of the 3' non-coding region of CMV-Y RNA1 is shown in Fig. 6, mimicking to that of

1
 RNA2: m7GpppG-UUUUUUUACAAGAGCGUACGGUUCUAAUCCCU-GCCUCCCCUGUAAAACUCCUAGACU

 RNA1: m7GpppGUUUUUUUACAAGAGCGUACGGUUCUAAUCCGU-GCCUCCCCUGUAAAACUACC--CUUU

 RNA3: m7GpppGUAAUCUAACCACCUGUGUGUGUGUGUGUGUUAUCGAGUCGUGUUGCCGCAC--AUUU

 RNA2: UGAAACUUCUUCUUUCUAGUAUCUUUUUAUG

 RNA1: GAAACUUCUCUCUCUUAUUCUUUUUUUUUUUUAAUUCCUAUG

 RNA3: GAGUCGUGUGUCCGCACAUUAUAUUUUUUUUCGUUGUACAGUGUGUAGAUUUCCCGAGGCAUG

Fig. 5. Nucleotide sequence alignment of the 5' non-coding regions of CMV-Y genome RNAs. Nucleotides identical to RNA1 are indicated by (*). AUG shows a start position for the translation of respective RNA.

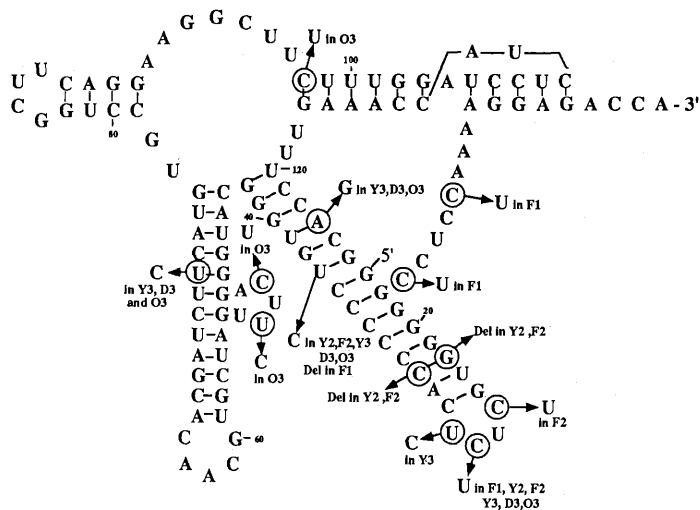


Fig. 6. Secondary structure model of the 3' terminus of CMV-Y RNA1 in the presence of magnesium ions. Nucleotide numbers are from the 3' end. Nucleotide substitutions and deletions that occur in other CMV RNAs relative to CMV-Y RNA1 are marked by circles, accompanying with the substitutive nucleotides, genome RNA number and CMV strain. D, F, O and Y represent CMV-D, -F, -O and -Y, respectively, and numbers affixed to the large capitals indicate genome RNA number. Del shows nucleotide deletion in the specified CMV RNAs.

CMV-Fny RNA2²²). Nucleotide differences that occur in CMV genome RNAs^{8,9,12,16,22,23} relative to CMV-Y RNA1 are mapped on the figure at corresponding positions. Most of the variations are conserved in the same genome RNAs beyond CMV strains and are mapped at the first stem and loop region from the 3' end, which was designated "B" region in CMV-Fny RNA2²²). Nucleotide differences in this region between Y and Q strains are too many to be mapped in this figure, while the 3' region of Q strain^{8,20,21}) has basically the same structure as those of Y and Fny strains. Although function of the 3' non-coding region of the RNAs of CMV is unclear, it may play some essential role in the initiation of RNA replication as demonstrated by *in vitro* experiments with brome mosaic virus¹) and turnip yellow mosaic virus¹⁵).

We are now constructing a full-length clone using the inserts of pCY1N4, pBCY12 and pCY1C15, in order to produce biologically-active RNAs *in vitro*. This construct will be useful for understanding the function(s) of CMV RNA1 and study on the mechanism of CMV replication.

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和 文 摘 要

片岡二郎・増田 税・高浪洋一：キュウリモザイクウイルス Y 系統 (CMV-Y) RNA1 の全塩基配列ならびに CMV 全ゲノム RNA の比較

CMV RNA1 の完全鎖長に相補的な cDNA を作成しその全塩基配列を決定した。RNA1 は 3,361 塩基よりなり分子量 111,462 のタンパク質をコードしていた。このタンパク質には GxxGxGKT で示される nucleotide binding site と GDT motif に似た配列とが認められた。RNA1 間の塩基配列の比較を行った結果、Y 系統は Fny 系統と高い相同性 (96.4%) を示し、Q 系統とは低い相同性 (76.1%) を示した。Y 系統内の 5' 非翻訳領域を比較すると、RNA1 は RNA2 と部分的に相同性があったが RNA3 とは相同性が認められなかった。3' 末端非翻訳領域を Fny, O および D 系統と比較したところ各 RNA について 95% 以上の相同性があり、認められる塩基置換も一つのステムループに集中する傾向があった。