PSEUDO-RECOMBINATION OF RNA SPECIES OF CUCUMOVIRUSES

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ABSTRACT

Among the viruses isolated in Japan, cucumber mosaic virus (CMV), soybean stunt virus (a soybean stunt strain of DMV), peanut stunt virus and chrysanthemum mild mottle virus belong to the cucumovirus group. All of these viruses contain four genomic RNA species (RNA1, RNA2, RNA3 and RNA4), and the three largest are necessary for infection. Pseudorecombinants (PRs) could be constructed either between these viruses or isolates of the same virus by exchange of RNA3. By comparing the properties among PRs and parents used, the determinant(s) of properties were located in certain RNA species. Serological specificity and particle mobility were found to be determined by RNA3 of the cucumoviruses, while the symptoms on two plant species were determined by RNA1+2. When fractionated RNA1 and RNA2 were exchanged between CMV isolates, symptoms on asparagus bean depended on RNA2.

Introduction

Cucumber mosaic virus (CMV) is a representative member of the cucumovirus group. CMV has been shown to contain four genomic RNA species (RNA's), RNA1 to RNA4 from the largest to the smallest. The three largest RNA's (RNA1, RNA2 and RNA3) are necessary for infection (Peden and Symons, 1973). Thus CMV has a tripartite genome like the bromoviruses or alfalfa mosaic virus (van Vloten-Doting *et al.*, 1981). In Japan numerous isolates of CMV have been recovered, and other viruses such as soybean stunt virus (SSV, Takahashi *et al.*, 1980), peanut stunt virus (PSV, Tsuchizaki *et al.*, 1981) and chrysanthemum mild mottle virus (CMMV, Tochihara 1970) were reported to belong to the cucumovirus group. As these viruses show considerable variations in their biological or biochemical properties, some of them are extremely useful as genetic markers. This is the reason why we used cucumoviruses for genetic analysis. Cucumovirus isolates used in this work are listed in Table 1. Tripartite genome and analysis using pseudorecombinant of cucumoviruses will be reviewed in this article.

Table 1 Virus isolates used

Virus isolate	Original host	Supplied by
CMV-Y	Tobacco	Dr. K. Kiriyama
CMV-P	Japanese butterbur	
CMV-L	Pea	Dr. M. Iwaki
CMV-E	Pea	Dr. T. Inoue
SSV-A	Soybean	Dr. K. Takahashi
PSV-J	Bean	Dr. T. Tsuchizaki
CMMV	Chrysanthemum	

See Hanada and Tochihara (1980) for reference of each isolate.

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Tripartite genome of cucumoviruses

Polyacrylamide gel electrophoresis of all the tested isolates of CMV, SSV, PSV and CMMV showed the presence of four RNA's. Typical patterns are shown in Fig. 1. The role played by RNA's in infectivity was tested by inoculating RNA's singly or in combination to *Chenopodium quinoa* plants, since *C. quinoa* is a suitable local lesion host of all cucumoviruses (Table 2). Inoculum containing RNA1+2 (mixture of RNA1 and RNA2) alone obtained from each virus except CMMV was infectious to some extent, while RNA3 alone was scarcely infectious in all the trials. Inoculum containing RNA1+2 and RNA3 showed the highest infectivity in all instances. Low infectivity of RNA1+2 alone was considered to be due to the contamination with RNA3 in the cases of CMV, SSV and PSV. Further addition of RNA4 to the inoculum containing RNA1-3 did not affect the infectivity. For CMMV, inoculum containing RNA1+2 alone was highly infectious and the addition of CMMV-RNA3 or CMV-L-RNA3 did not enhance the infectivity of CMMV-RNA1+2. However, the addition of CMV-RNA3 increased appreciably the infectivity of CMV-L-RNA1+2. When RNA's were fractionated under denaturing conditions by electrophoresis instead of non denaturing conditions, CMMV-RNA1+2 alone became non infectious and the addition of CMMV-RNA3 induced a high level of infectivity (unpublished results), suggesting that under non denaturing conditions CMMV-RNA1+2 was heavily contaminated with RNA3. Therefore, all of the cucumoviruses used have a tripartite genome.

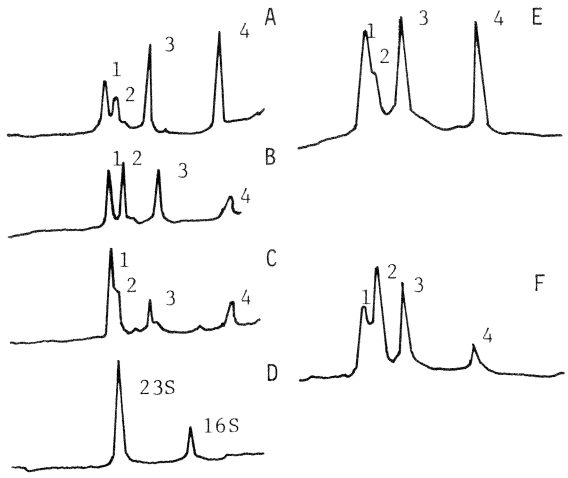

Fig. 1 Typical electrophoretic patterns of RNA's of cucumoviruses.
Purified virus samples were subjected to electrophoresis on 2.5% polyacrylamide disc gels after disruption with sodium dodecyl sulfate. After electrophoresis, gels were scanned at 260 nm.
Migration is from left to right. 1, 2, 3 and 4 indicate RNA1, RNA2, RNA3 and RNA4, respectively. (A) CMV-E, (B) PSV-J, (C) CMMV, (D) *Escherichia coli* ribosomal RNA 23S + 16S, (E) SSV-A and (F) PSV-J. From (A) to (D) from Hanada and Tochihara (1980).

Table 2 Infectivity of fractionated RNA's of CMV, SSV, PSV and CMMV (Hanada and Tochihara, 1980 and unpublished results)

Experiment number	Virus isolate	Infectivity of RNA's[a]			
		1+2	3	1+2+3	1+2+3+4
1	CMV-P	14	0	311	312
	CMV-L	13	0	244	166
2	SSV-A	6	2	231	NT[b]
3	PSV-J	31	0	373	337
4	CMV-L	50	1	425	NT
	CMMV	643	0	596	NT

a) Total number of local lesions in four leaves (Expts. 1 and 4) or six leaves (Expts. 2 and 3) of *C. quinoa*.
b) Not tested.

Exchange of RNA3 among cucumoviruses

RNA1+2 preparations from CMV-Y or CMV-P were mixed together with RNA3 from CMV-P or CMV-Y, respectively, and they were subsequently inoculated to *C. quinoa* plants. For the control experiments, homologous mixtures of RNA1+2 and RNA3 from either isolate were inoculated separately. After single lesion isolation, the serotype of each single lesion isolate (SLI) was determined by gel diffusion tests using partially purified virus samples from each SLI as antigen and antisera prepared to CMV-Y and CMV-P. Almost all the progeny SLIs had the serotype of the isolate supplying RNA3 to the original inoculum (data not shown). A few exceptional SLIs with the serotype of RNA1+2 donor probably originated from contamination of RNA3 in the original RNA1+2 preparations. Similar results were obtained by RNA3 exchange trials either between CMV-L and CMV-P or between CMV-e and SSV-A. Hence, serotypes of CMV and SSV were determined by RNA3. SLIs with the serotype of RNA3 donor were considered to be pseudo-recombinants (PRs). Gel diffusion tests of PRs constructed by RNA3 exchange between CMV-Y and CMV-P are shown in Fig. 2. Symptoms on cucumber and asparagus bean induced by

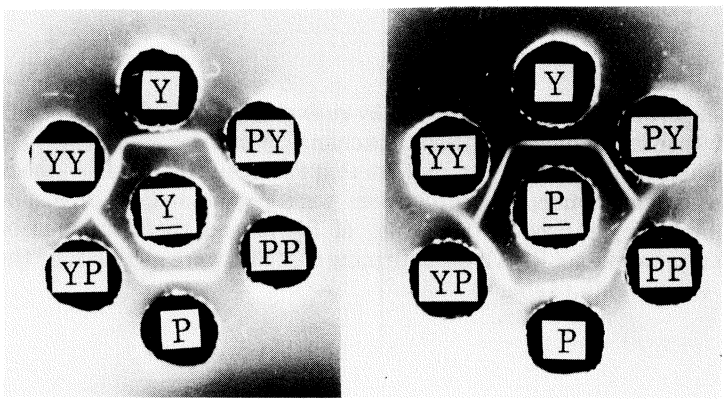

Fig. 2 Immunodiffusion tests with pseudo-recombinants (PRs) constructed by RNA3 exchange between CMV-Y and CMV-P. Outer wells contain purified viruses of (Y) parental CMV-Y and (P) CMV-P; the others contain PRs and controls, the first letter represents the parent contributing RNA1+2 and the second letter the parent supplying RNA3, respectively. Central wells contain antisera to *(Y)* CMV-Y and *(P)* CMV-P. From Hanada and Tochihara (1980).

PRs were compared with those induced by the parent viruses, and it was found that symptoms on these plants were determined by RNA1+2 (Table 3). RNA3 was similarly exchanged between CMMV and CMV-L, and it was shown that the serotype was determined by RNA3 and sympton appearance on cucumber or asparagus bean was determined by RNA1+2 in the case of CMMV also.

Table 3 Symptoms produced by pseudo-recombinants constructed by RNA3 exchange between CMV isolates or between CMV and CMMV (Hanada and Tochihara, 1980)

RNA's in inoculum		Symptoms[a] on	
RNA1+2	RNA3	Cucumber	Asparagus bean
Between CMV-Y(Y) and CMV-P(P)			
Y	P	ChS[b], YM[c]	NL, -
P	Y	- , -	NL, -
Y	Y	ChS, YM	NL, -
P	P	- , -	NL, -
Between CMV-L(L) and CMV-P(P)			
L	P	- , YM	ChS, M
P	L	- , -	NL, -
L	L	- , YM	ChS, M
P	P	- , -	NL, -
Between CMV-L(L) and CMMV(M)			
L	M	- , YM	ChS, M
M	L	- , -	NL, -
L	L	- , YM	ChS, M
M	M	- , -	NL, -

a) Symptoms produced on plants: ChS = chlorotic spots, YM = yellow mottling, M = mosaic, NL = necrotic lesions and - = no symptoms appeared.
b) Symptoms on inoculated leaves.
c) Systemic symptoms.

Backcross experiments were carried out by re-exchanging RNA3 between the two types of PR, which had been constructed by RNA3 exchange either between CMV-Y and CMV-P or between CMV-E and SSV-A, in order to confirm that PRs resulted only from the re-assortment of RNA's from the parent viruses. Parent viruses were regenerated in almost all instances as evidenced by the serotype used as a marker of RNA3 and the symptoms on cucumber or asparagus bean as a marker of RNA1+2, confirming that PRs were produced by the re-assortment of RNA's from the parents (Table 4).

Table 4 Regeneration of parent isolates by RNA3 exchange between two types of pseudo-recombinants (Hanada and Tochihara, 1980)

Donor of RNA's		Number of isolates with the serotype of		Symptoms[a] on
RNA1+2	RNA3	Y type	P type	Cucumber
Y P[b]	P Y[c]	3	0	ChS, YM
P Y	Y P	0	2	-, -
Y P	Y P	0	2	ChS, YM
P Y	P Y	2	0	-, -

a) See Table 3.
b) One of pseudo-recombinants constructed by RNA3 exchange between CMV-Y(Y) and CMV-P(P). The first and the second letter represent the origin of RNA1+2 and RNA3, respectively.
c) Another type of pseudo-recombinant constructed by RNA3 exchange between Y and P.

Exchange of RNA1 and RNA2 between CMV isolates

To determine which one of RNA1 or RNA2 in RNA1+2 preparations controls the symptoms, RNA1 and RNA2 were exchanged between CMV-Y and either CMV-E or CMV-L. These isolates produced distinct symptoms on asparagus bean (Table 3), thereafter symptoms on asparagus bean induced by each progeny SLI obtained by the exchange of RNA1 or RNA2 were compared (Table 5). Almost all the tested SLIs caused symptoms of the isolate contributing RNA2. Similar results were obtained by RNA1 and RNA2 exchange between CMV-L and CMV-P, indicating that the symptoms on asparagus bean are determined by RNA2 of CMV.

Table 5 Symptoms on asparagus bean caused by single lesion isolates produced by exchanging RNA1 and RNA2 between CMV isolates (Hanada and Tochihara, 1980)

Donor of RNA's			Total number of isolates tested	Number of isolates which caused symptoms of
RNA1	RNA2	RNA3		
Between CMV-Y(Y) and CMV-L(L)				
L	Y	L	12	All Y
Y	L	Y	12	All L
L	Y	Y	6	All Y
L	L	L	8	All L
Y	Y	Y	8	All Y
Between CMV-Y(Y) and CMV-E(E)				
E	Y	E	9	All Y
Y	E	Y	5	4 E, 1 Y
Y	E	E	7	All E
E	Y	Y	7	All Y
E	E	E	6	All E
Y	Y	Y	6	All Y

Discussion

Lot *et al.*, (1974) confirmed that CMV contained a tripartite genome, then Habili and Francki (1974) suggested that tomato aspermy virus (TAV) had also a tripartite genome. TAV is a member of the cucumovirus group and is serologically related to CMMV but not to CMV. We demonstrated that CMMV, SSV and PSV also have a tripartite genome. SSV is now considered to be a soybean strain of CMV (Hanada and Tochihara, 1982).

Pseudo-recombinant analysis of CMV performed by several workers (Habili and Francki, 1974; Mossop and Francki, 1977; Hanada and Tochihara, 1980 and unpublished results; Rao and Francki, 1982) consistently showed that virus properties related to viral coat protein (i.e. serotype, aphid transmissibility and particle mobility) are determined by RNA3, and that RNA2 usually plays an important role in determining symptoms on plant. The location of the coat protein gene in RNA3 was confirmed by the determination of the complete nucleotide sequence of RNA3 (Gould and Symons, 1982). Recently the sequences of RNA1 and RNA2 were completed (Rezaian *et al.*, 1974 and 1975), which indicate that RNA2 encodes one kind of protein with a molecular weight of 94K. Although this protein would be considered to play an important role in symptom determination, the mechanism of the determination remains unknown. An attenuated tobacco mosaic virus is presently used in Japan for the control of tomato mosaic. Similar attenuated CMV may be useful for the control of CMV in the field. Since attenuation is a phenomenon closely related to symptom expression, it may become possible to produce an attenuated CMV by inducing artificial mutation(s) in RNA2.

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Discussion

Reddy, D.V.R. (ICRISAT): Can you shorten the procedure by cloning individual RNA species and then use them for such studies by recombination?

Answer: The cloning technique should be very useful for further analysis of the cucumovirus genome. Presently we are trying to obtain some cDNA of cucumovirus RNA species.