Serological Relationships and In Vitro Translation of an Antigenically Distinct Strain of Papaya Ringspot Virus

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ABSTRACT

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The serological relationships between a potyvirus isolated from squash in Guadeloupe (isolate Q10), a watermelon mosaic virus-1 isolate of papaya ringspot virus (PRSV-W), a papaya isolate of papaya ringspot virus, and a virus isolated from cucurbits in Morocco (WMV-M) were analyzed by sodium dodecyl sulfate immunodiffusion tests with inclusion body protein and coat protein antisera. The products of in vitro translation of purified Q10-RNA were also analyzed by immunoprecipitation. A 114k product obtained in the rabbit reticulocyte lysate (RRL) system and a 51k product obtained in both the RRL and the wheat germ systems were immunoprecipitated by PRSV-W amorphous inclusion protein antiserum. A 70k product and a 36k product obtained in the lysate system were precipitated by PRSV-W cylindrical inclusion protein antiserum and by isolate Q10 coat protein antiserum, respectively. The SDS-immunodiffusion tests showed that the amorphous inclusions of isolate Q10 and PRSV-W were serologically closely related, that their cylindrical inclusions had at least one common antigenic determinant and one specific determinant, and that isolate Q10 coat protein had at least three antigenic determinants: one specific to isolate Q10; one that was common to isolate Q10 and PRSV-W; and a third one that was common to isolate Q10, PRSV-W, and WMV-M. Since most of its properties were similar to those of PRSV-W, isolate Q10 is considered to be a serologically distinct strain of papaya ringspot virus.

In 1982, a virus was isolated from naturally infected squash on the island of Guadeloupe in the Caribbean Sea. A preliminary study showed that this isolate belonged to the potyvirus group and that it had biological properties very similar to those described for the papaya ringspot virus type W (PRSV-W ex watermelon mosaic virus 1 [16]). However, noticeable serological differences were reported (18).

We present herein studies of relationships of this isolate to PRSV-W, PRSV-P (a PRSV isolate from papaya), and a virus isolated from cucurbits in Morocco (WMV-M) (1,2,8).

MATERIALS AND METHODS

Virus source and propagation. The isolate (Q10) used in this experiment was collected in 1982 from a naturally infected zucchini squash (*Cucurbita pepo* L. 'Diamant F1') in Guadeloupe (18).

The virus was kept in leaves dried over calcium chloride at 4 C. Zucchini squash (*C. pepo* L. 'Chefini') was used for further propagations.

Virus purification, RNA isolation, and in vitro translation. Virus was purified according to the method described for PRSV-W (16).

RNA was extracted from freshly purified virus as described by Dougherty and Hiebert (6) except that the buffer used was 200 mM tris, pH 9.0, instead of 200 mM ammonium carbonate, pH 9.0.

Rabbit reticulocyte lysate (RRL) preparation and the in vitro translation conditions were as described previously (6). The wheat germ system was prepared according to the method described by Marco and Dudock (13), and the reactions were carried out as described by Salerno-Rife et al (20).

Immunoprecipitation and analysis by polyacrylamide gel electrophoresis (PAGE) were done as described by Hiebert and Purcifull (10). Antisera to the following antigens were used: untreated, purified PRSV-W (17); PRSV-W cylindrical inclusion protein (11); PRSV-W amorphous inclusion protein (4); tobacco etch virus (TEV) 49k and 54k nuclear inclusion protein (7,11) and untreated, purified isolate Q10.

Purification and analysis of cylindrical and amorphous inclusion proteins. Partial purification of cylindrical inclusions and purification of cylindrical inclusion protein by sodium dodecyl sulfate (SDS)-PAGE were done as previously described (11).

Amorphous inclusion protein was purified and then analyzed by Western blot procedures as described by de Mejia et al (4). The antisera used were pepper mottle virus (PeMV) and PRSV-W amorphous inclusion protein antisera (4) and tobacco vein mottling virus (TVMV) helper component protein antiserum provided by D. W. Thornbury (12,21).

Antisera production and serological tests. Antisera were prepared in rabbits by injecting immunogens emulsified in Freund's complete adjuvant (first injection) or in incomplete adjuvant (subsequent injections) as described by Purcifull and Batchelor (15). Each time injections were made into thigh muscles and toe pads.

For the virus coat protein antiserum, rabbit 1062 was injected initially with 2 mg of virus followed 3 wk later by an injection of 2 mg.

For the cylindrical inclusion protein antiserum, rabbit 1078 was injected with 0.6 mg of cylindrical inclusion protein initially and 30 days later with 0.4 mg of protein. Blood was collected weekly starting 1 wk after the second injection.

Other antisera used were isolate Q10 virus antiserum 83-1C (18), PRSV-W virus antisera 852 and 862 (17) and 643 (D. E. Purcifull, *unpublished*), watermelon mosaic virus-2 (WMV-2) antiserum 868 collected 5 and 10 mo after immunization (17), watermelon mosaic virus-Morocco (WMV-M) virus and cylindrical inclusion protein antisera 955 and 928, respectively (1,2), zucchini yellow mosaic virus (ZYMV) antiserum 1028 (14), and PRSV-W amorphous inclusion protein antiserum 1039 (4). Antiserum to a French isolate of PRSV-W was obtained from H. Lecoq; PRSV-P (PRSV-HA)

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virus and cylindrical inclusion protein antisera 86 and 106, respectively, from D. Gonsalves (9,22); and clover yellow vein virus and bean yellow mosaic virus antisera from F. W. Zettler (23).

The antigens used were a Florida isolate of PRSV-W obtained from W. C. Adlerz (17), a Florida isolate of WMV-2 (17), PRSV-P (isolate HA) from Hawaii (9), a WMV-M isolate from Morocco (8), and a ZYMV isolate from Florida (14).

SDS-immunodiffusion tests were conducted as described by Purcifull and Batchelor (15).

Alkaline phosphatase-immunoglobulin G conjugates were prepared with IgG purified from antiserum 1062 to purified Q10 collected 7 wk after immunization, and with IgG from antiserum 852 to PRSV-W. Double-antibody sandwich, enzyme-linked immunosorbent assays (ELISA) were carried out as described by Clark and Adams (3) by using crude plant extracts as test antigens.

RESULTS

Serological relationships of the capsid protein. The results of SDS-immunodiffusion tests performed with eight antisera and four viruses are shown in the drawings of Fig. 1. Fig. 2 illustrates the spur formations (reactions of partial identity) in reciprocal tests with isolate Q10 and PRSV-W. Intragel absorption test results are summarized in Table 1. None of the antisera gave perceptible reactions with sap from uninoculated plants in these tests.

Three antisera were found to be very specific and they reacted only with the homologous antigen: isolate Q10 antiserum 83-1C, PRSV-W antiserum 643, and WMV-M antiserum 955. The five other antisera showed some cross-reactivity (Fig. 1).

Antiserum 1062 had a titer of 1/8 in SDS-immunodiffusion tests. The precipitin line developed by isolate Q10 formed a spur over the precipitin lines developed by PRSV-W, PRSV-P, or WMV-M, and the precipitin line developed by PRSV-W formed a spur over the precipitin line developed by WMV-M. This evidence, along with the intragel cross-absorption tests, indicated that isolate Q10 had at least three classes of antigenic determinants: one common for isolate Q10, PRSV-W, and WMV-M; a second type common for isolate Q10 and PRSV only, and the third specific for isolate Q10.

Antiserum 862 reacted with PRSV-W, PRSV-P, isolate Q10, and WMV-M. The precipitin line developed by PRSV-W formed a spur over the precipitin line developed by isolate Q10 and WMV-M and the precipitin line developed by isolate Q10 formed a spur over the line developed by WMV-M. So, PRSV-W antiserum 862 also has three antibody classes. Intragel cross-absorption tests also showed that one antibody class reacted with PRSV-W (isolate Q10 and WMV-M), one with PRSV and isolate Q10 (but not with WMV-M), and one with PRSV-W only. Antiserum against PRSV-W from France, antiserum 852, and antiserum 86 did not react with WMV-M antigen. In SDS-immunodiffusion tests, the patterns observed when tested against isolate Q10 were similar to those observed in the case of antiserum 862, and they showed that antiserum 852 contained two classes of antibodies, one specific to PRSV-W and another common to PRSV-W and isolate Q10. Intragel absorptions performed with antiserum 852 also showed the presence of two classes of antibodies. It was observed that in SDS-immunodiffusion tests with antiserum to isolate Q10, the spur formed by the PRSV precipitin line over the WMV-M precipitin

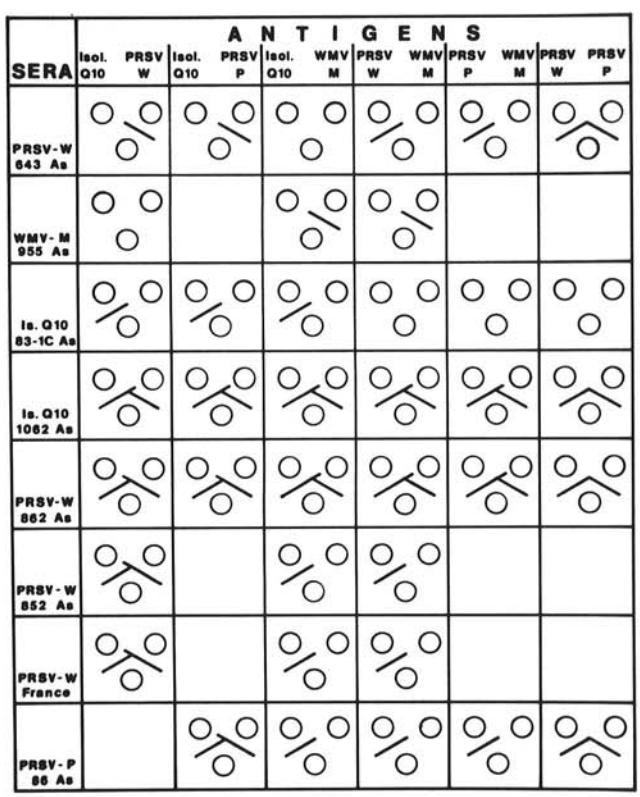

Fig. 1. Schematization of precipitin lines obtained in sodium dodecyl sulfate immunodiffusion tests with different antisera to coat protein of isolate Q10, papaya ringspot virus-W and P (PRSV-W and PRSV-P), and watermelon mosaic virus from Morocco (WMV-M). In each square, the upper circles represent wells containing antigens that are indicated above every lane of circles, and the lower circle represents the well containing antiserum which is indicated on the left. Blank squares indicate combinations that were not tested.

TABLE 1. Comparison of results in SDS-immunodiffusion tests when antisera are nonabsorbed or absorbed with different viruses[a]

Sera[b]	Antigens	Sera nonabsorbed	Sera absorbed with: Isol.Q10	PRSV-W	PRSV-P	WMV-M
Isol.Q10	Isol.Q10	++++	0	+++	+++	+++
(No. 1062)	PRSV-W	++	0	0	0	+
	PRSV-P	++		0	0	+
	WMV-M	+	0	0	0	0
PRSV-W	Isol.Q10	++	0	0		
(No. 852)	PRSV-W	++++	+++	0		
	WMV-M	0	0	0		
PRSV-W	Isol. Q10	++				+
(No. 862)	PRSV-W	++++				+++
	PRSV-P	++++				+++
	WMV-M	+				0

[a] Relative intensity of immunoprecipitin lines is indicated (++++ = strongest reactions, 0 = no reaction). Antisera were absorbed by using the intragel method.

[b] Antiserum number in parentheses.

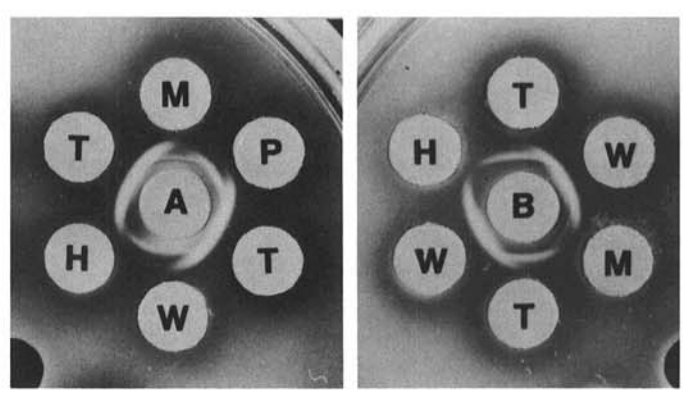

Fig. 2. Serological relationships between isolate Q10 and papaya ringspot virus type W (PRSV-W). Contents of center wells were: A = isolate Q10 virus antiserum, B = PRSV type W virus antiserum. Peripheral wells contained sodium dodecyl sulfate-treated antigens from squash leaves: healthy (H) or infected with isolate Q10 (T), PRSV-W (W), watermelon mosaic virus from Morocco (M), and a papaya isolate of PRSV (P). Antigens tested against isolate Q10 antiserum were previously boiled; antigens tested against PRSV antiserum were not boiled.

line was often more distinct when WMV-M antigen preparations were heated for 4 min in a boiling water bath.

No cross-reaction was observed between isolate Q10 and clover yellow vein virus, bean yellow mosaic virus, or ZYMV. Antiserum 868 against WMV-2 collected 5 mo after immunization did not react with isolate Q10, but a fraction collected from the same rabbit 10 mo after immunization did react. This fraction gave similar results with several other potyviruses (D. E. Purcifull, *unpublished*). Antiserum 1062 to isolate Q10 did not react with WMV-2.

ELISA tests performed using conjugates prepared with antisera 1062 and 852 showed a closer specificity than SDS-immunodiffusion tests (Table 2). The conjugates reacted strongly with their homologous antigens and slightly with the heterologous ones. Furthermore, PRSV-P antigen reacted more strongly with 852 conjugate than with 1062 conjugate.

Serological relationships between cylindrical inclusion proteins. Three antisera were assayed against extracts from squash infected singly by isolate Q10, PRSV-W, PRSV-P, or WMV-M (Fig. 3). The homologous titer of antiserum 1078 (made against isolate Q10 cylindrical inclusion protein) was 1/8 in SDS-immunodiffusion tests and it reacted with sap from leaves infected with either isolate Q10 or PRSV-W (Fig. 4A). This antiserum sporadically gave a weak reaction with WMV-M. The precipitin line developed by isolate Q10 cylindrical inclusions formed a spur over the precipitin lines developed by PRSV-W or WMV-M cylindrical inclusions. No spur was observed between PRSV-P and PRSV-W precipitin lines. When absorbed with sap of plants infected by PRSV-W, antiserum 1078 no longer reacted with PRSV-W and PRSV-P cylindrical inclusion protein, but it still reacted with isolate Q10 cylindrical inclusion protein.

TABLE 2. Serological cross-reactivity of isolate Q10 and papaya ringspot virus type W in double antibody sandwich ELISA tests

Antigens	Absorbance ($A_{405\ nm}$)[a]	
	Isolate Q10 antiserum (No. 1062)	PRSV-W antiserum (No. 852)
Isolate Q10	1.56 ± 0.04	0.30 ± 0.02
PRSV-W	0.07 ± 0.04	1.03 ± 0.04
PRSV-P	0.24 ± 0.01	1.89 ± 0.03
WMV-M	0.03 ± 0.01	0.01 ± 0.01
Healthy plant	0.02 ± 0.01	0.02 ± 0.01

[a] Absorbance values and confidence intervals ($P = 0.05$) were obtained at 405 nm. All values were obtained in the same test by measuring crude extracts. Concentration of No. 1062 and No. 852 coating antibody was 1 μg/ml. Conjugates were used at dilutions of 1/500 (No. 1062) and 1/1,000 (No. 852).

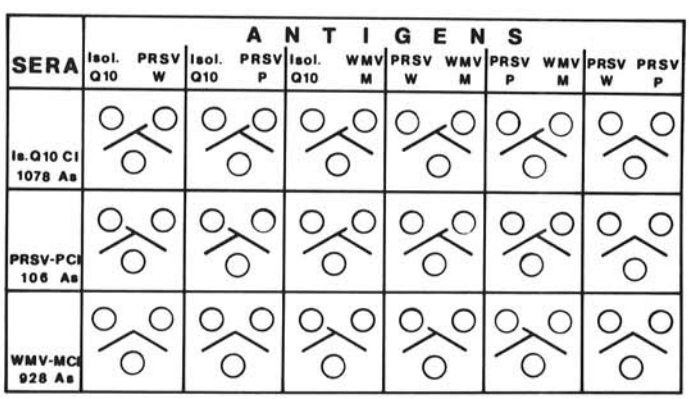

Fig. 3. Schematization of precipitin lines obtained in sodium dodecyl sulfate-immunodiffusion tests with antisera to cylindrical inclusion proteins of isolate Q10 (ISQ10 CI), papaya ringspot virus-P (PRSV-PCI), and watermelon mosaic virus from Morocco (WMV-MCI). In each square, upper circles represent wells containing antigens that are indicated above every lane of circles, and the lower circle represents the well containing antiserum which is indicated on the left.

PRSV-P cylindrical inclusion antiserum reacted with PRSV, with isolate Q10, and sometimes with WMV-M cylindrical inclusions (Fig. 4B). The precipitin line developed by PRSV-W cylindrical inclusions formed a spur with the precipitin lines developed by isolate Q10 and WMV-M cylindrical inclusions. The spur of the isolate Q10 cylindrical inclusion precipitin line over the WMV-M cylindrical inclusion precipitin line was not always very distinct.

Antiserum to WMV-M cylindrical inclusions reacted with WMV-M, isolate Q10, PRSV-W, and PRSV-P cylindrical inclusion proteins. The precipitin line developed by WMV-M cylindrical inclusions formed a spur over the precipitin lines developed by isolate Q10 and PRSV-W cylindrical inclusions (Fig. 4C). No spur was observed between isolate Q10 and PRSV-P precipitin lines or between PRSV-P and PRSV-W precipitin lines.

Serological relationships between amorphous inclusion proteins. Antiserum to PRSV-W amorphous inclusion protein reacted against sap from leaves infected with PRSV-W or isolate Q10 but not against sap from leaves infected with WMV-M. PRSV-W and isolate Q10 precipitin lines fused without spur formation.

Semi-purified, SDS-degraded amorphous inclusion protein of isolate Q10 was submitted to SDS-PAGE and its serological relatedness to PRSV-W amorphous inclusion protein was studied by Western blot analysis. The major protein component of semipurified isolate Q10 amorphous inclusions comigrated with a similar component of PRSV-W amorphous inclusions. It reacted efficiently with PRSV-W amorphous inclusion antiserum and less efficiently with antisera to PeMV amorphous inclusions and to TVMV helper factor. No reaction was detected with normal serum tested against the amorphous inclusion protein of either isolate Q10 or PRSV-W.

Immunological analysis of in vitro translation products. The in vitro translation products of isolate Q10 were analyzed by SDS-PAGE and compared to WMV-2, PRSV-W, and ZYMV products. Translation of the viral genome in the lysate system resulted in unique products under identical conditions (Fig. 5). The major product of isolate Q10 RNA (Fig. 5, lane 4) translation had an estimated size of 114k and was larger than the major translation products of WMV-2 (lane 1), PRSV-W (lane 2), and ZYMV (lane 3) RNAs.

The in vitro translation products of isolate Q10 in the RRL system were analyzed serologically by immunoprecipitation with various antisera prepared against potyvirus-specified proteins. When translation products were treated with PRSV-W amorphous inclusion antiserum, the 114k major product was immunoprecipitated (Fig. 6, lane 2). One minor product of 51k was also immunoprecipitated. The other products visible between 51k and 114k may be due to partial processing or due to premature terminations during the in vitro translation. Antiserum to PRSV-W cylindrical inclusions precipitated a 70k product (cylindrical

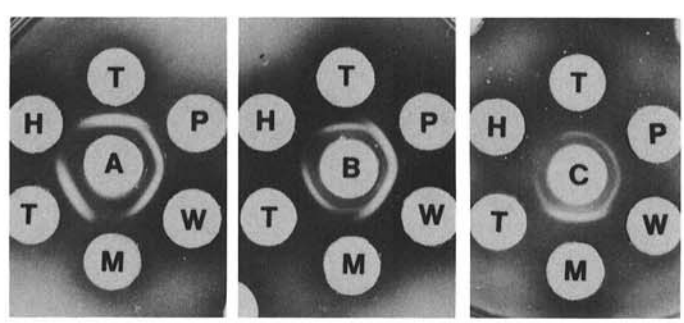

Fig. 4. Serological relationships between isolate Q10 and PRSV cylindrical inclusions. Contents of center wells: A = isolate Q10 cylindrical inclusion protein antiserum, B = PRSV-P HA cylindrical inclusion protein antiserum, C = WMV-M cylindrical inclusion protein antiserum. Peripheral wells contained sodium dodecyl sulfate-treated antigens from squash leaves: healthy (H) or infected with isolate Q10 (T), PRSV-W (W), PRSV-P (P), or WMV-M (M).

inclusion protein monomer) and a larger product with a molecular weight close to 114k (Fig. 6, lane 3). Antiserum to isolate Q10 capsid protein precipitated the 36k product (capsid protein) and larger products which are also immunoprecipitated with the TEV 54k nuclear inclusion protein antiserum (Fig. 6, lanes 4 and 5). Antiserum to TEV 49k nuclear inclusion protein only precipitated a 114k product (Fig. 6, lane 6). Immunoprecipitation with normal serum was negative (Fig. 6, lane 7).

Translation of isolate Q10 RNA in the wheat germ (WG) cell-free system produced a major polypeptide with estimated molecular weight of 63k and a minor product of 51k (Fig. 7, lane 3). The 51k product was immunoprecipitated with PRSV-W amorphous inclusion antiserum (Fig. 7, lane 4). No product other than 63k (which was presumed to be precipitated nonspecifically) was immunoprecipitated with coat protein antiserum, cylindrical inclusion antiserum, and TEV 54k and 49k nuclear inclusion antisera (Fig. 7, lanes 5–8). Immunoprecipitation with normal serum was negative (Fig. 7, lane 9).

DISCUSSION

Preliminary studies had shown that isolate Q10 was a potyvirus and that it had biological and physical properties similar to those of PRSV-W (18). No hosts of Q10 were found outside the family Cucurbitaceae, and genotypes that are resistant to PRSV-W are also resistant to isolate Q10. Isolate Q10 does not infect papaya nor does it induce lesions in *Chenopodium quinoa* Willd. or *C. amaranticolor* Coste et Reyn. like some PRSV-W isolates (19). Isolate Q10 is also degraded by *n*-butanol.

Studies at the genome level reported in this paper confirm the close relationships between isolate Q10 and PRSV. The major product obtained by in vitro RNA translation in the lysate system is slightly larger compared to those of PRSV: 114k instead of 110k and 112k for PRSV-W and PRSV-P, respectively. This molecular weight is in agreement with the sum of molecular weights of the

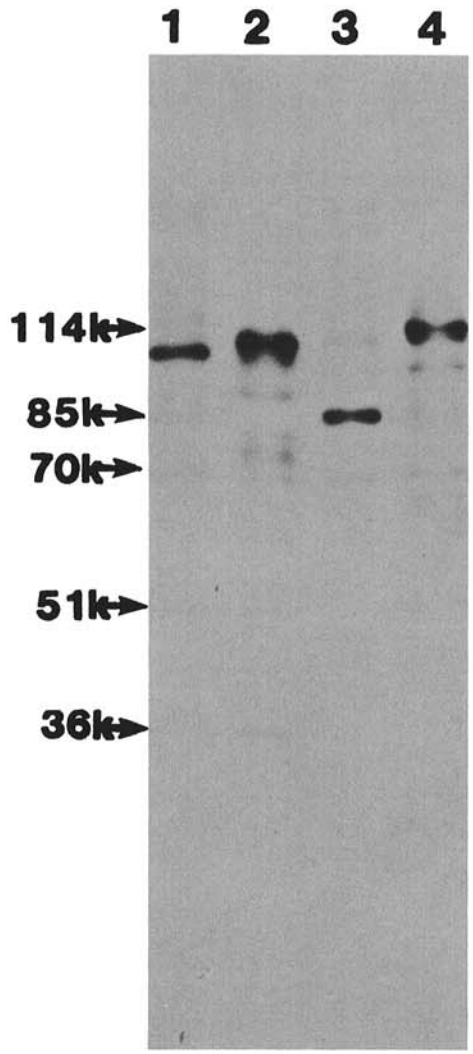

Fig. 5. Electrophoretic fractionation of the in vitro translation products of isolate Q10 in comparison with those of watermelon mosaic virus-2 (WMV-2), papaya ringspot virus-W (PRSV-W), and zucchini yellow mosaic virus (ZYMV). The products were fractionated on a sodium dodecyl sulfate-polyacrylamide slab gel (7.5–15%) gradient (PAGE) and detected by fluorography. Translations were carried out in the rabbit reticulocyte lysate system with [^{35}S]-methionine as the labeled amino acid and conditions were identical for all viral RNAs. Molecular weight markers used in all electrophoretic studies were: myosin, 200,000 (200k), phosphorylase b (93k), bovine serum albumin (67k), ovalbumin (43k), carbonic anhydrase (29k), and tobacco mosaic virus capsid monomer (17.5k). Total products were from RNA translation of WMV-2 (lane 1), PRSV-W (lane 2), ZYMV (lane 3), and isolate Q10 (lane 4). Molecular weights of some of the major translation products are given on the left side of the figure.

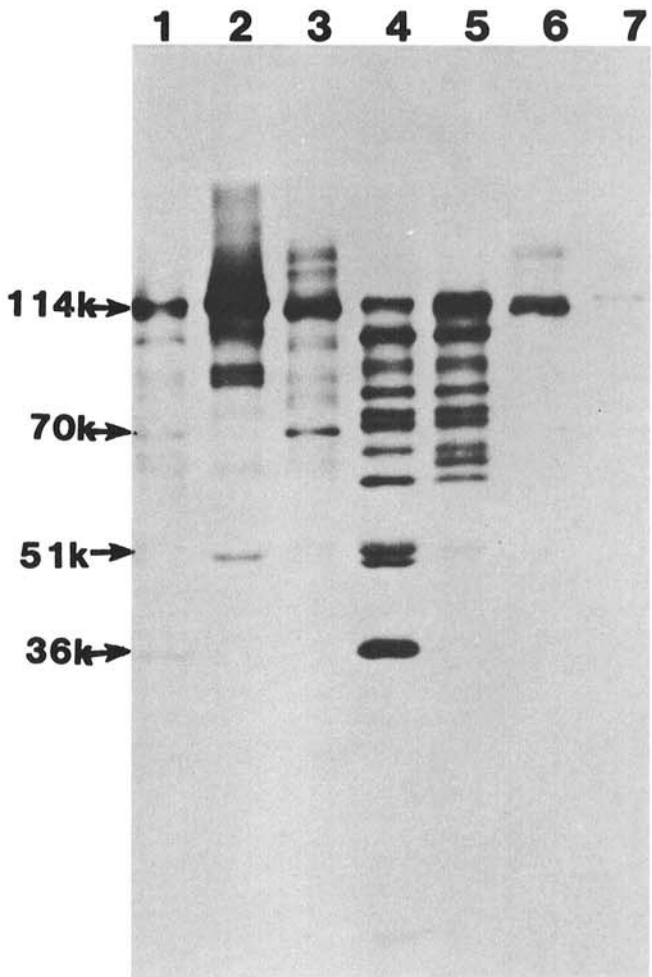

Fig. 6. Analysis of the products of isolate Q10 RNA translated in the rabbit reticulocyte lysate system. The figure illustrates [^{35}S]-methionine-labeled products separated by polyacrylamide gel electrophoresis and detected by fluorography. Lane 1, total products of isolate Q10 RNA translation; lane 2, products immunoprecipitated with antiserum to papaya ringspot virus-W (PRSV-W) amorphous inclusion protein; lane 3, products immunoprecipitated with antiserum to PRSV-W cylindrical inclusion protein; lane 4, products immunoprecipitated with antiserum to isolate Q10 coat protein; lane 5, products immunoprecipitated with antiserum to tobacco etch virus (TEV) 54k nuclear inclusion protein; lane 6, products immunoprecipitated with antiserum to TEV 49k nuclear inclusion protein; and lane 7, products immunoprecipitated with normal serum. Molecular weights of some of the major translation products are given on the left side of the figure.

major product obtained in the wheat germ system (63k) and of the amorphous inclusion protein (51k). The amorphous inclusion proteins of isolate Q10 and PRSV-W have the same molecular weight (5) and they are closely related serologically as shown by immunoprecipitation of the in vitro RNA translation products, Western blot analysis, and SDS-immunodiffusion.

The translation of isolate Q10 RNA in the wheat germ system resulted in a product profile distinct from that observed in the lysate system. The products observed with the wheat germ system have been mapped to the 5′ end of the viral genome (5). The template activity of Q10 RNA in the wheat germ system appears to be confined to the 5′ end, whereas in the lysate system the entire genome appears to be expressed. This disparity in template activity between the two translation systems has been observed with a number of different potyviral RNAs (E. Hiebert and W. Dougherty, *unpublished*).

Cylindrical inclusion proteins obtained by in vitro RNA translation have the same size of 70k for isolate Q10 and PRSV-W. The SDS-immunodiffusion tests, however, showed that cylindrical inclusion proteins of isolate Q10 and PRSV-W have at least one antigenic site in common and one specific antigenic site.

Coat proteins obtained by in vitro translation of isolate Q10 and PRSV-W RNA have a similar size (36k) but have at least two antigenic sites in common and one specific antigenic site as shown by SDS-immunodiffusion tests. PRSV-W and PRSV-P have some different biological and physical properties but have serologically indistinguishable coat protein (16). Isolate Q10 has biological and physical properties similar to those of PRSV-W, yet its coat protein is distinct from, albeit related to, the PRSV-W coat protein. This fact provides evidence that mutations can occur on the coat protein gene and even on the gene for cylindrical inclusion protein without apparent effects on the biological and physical properties of the virus.

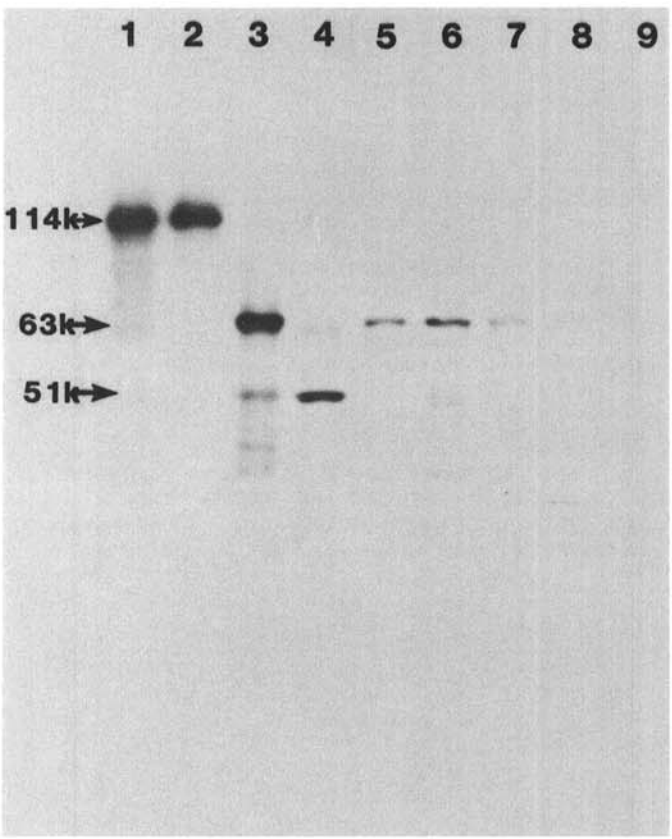

Fig. 7. Analysis of the products of isolate Q10 RNA translated in the wheat germ system (lanes 3–9). The figure illustrates [^{35}S]-methionine-labeled products separated by polyacrylamide gel electrophoresis and detected by fluorography. Lane 1, total products of isolate Q10 RNA translation in rabbit reticulocyte lysate system; lane 2, products of RNA translation in the lysate system immunoprecipitated with antiserum to papaya ringspot virus-W (PRSV-W) amorphous inclusion protein; lane 3, total products of isolate Q10 RNA translation in the wheat germ system; lane 4, products of RNA translation in wheat germ system immunoprecipitated with antiserum to PRSV-W amorphous inclusion protein. Lanes 5–9 are products of the wheat germ system immunoprecipitated with (lane 5) antiserum to PRSV-W cylindrical inclusion protein; (lane 6) antiserum to isolate Q10 coat protein; (lane 7) antiserum to tobacco etch virus 54k nuclear inclusion protein; (lane 8) antiserum to TEV 49k nuclear inclusion protein; and (lane 9) normal serum. Molecular weights of some of the major translation products are given on the left side of the figure.

Serological studies reported here have also shown that isolate Q10 is distantly related to WMV-M. The WMV-M host range presents slight differences such as reactions on *C. amaranticolor* or *Lavatera trimestris* L. (8,18). Isolate Q10 coat protein and cylindrical inclusion protein antisera each has one of the three antibody classes in common with WMV-M. These classes also are common with PRSV.

We consider that the properties of isolate Q10 indicate that it is a distinct type of PRSV. With respect to the "tiger"-like stripe symptoms it induces in squash (18), this isolate is designated as papaya ringspot virus type T.

LITERATURE CITED

1. Baum, R. H. 1980. Purification, partial characterization, and serology of the capsid and cylindrical inclusion proteins of four isolates of watermelon mosaic virus. Ph.D. dissertation, University of Florida, Gainesville. 95 pp.
2. Baum, R. H., Purcifull, D. E., and Hiebert, E. 1979. Purification and serology of a Moroccan isolate of watermelon mosaic virus (WMV). (Abstr.) Phytopathology 69:1021-1022.
3. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
4. de Mejia, M. V. G., Hiebert, E., and Purcifull, D. E. 1985. Isolation and partial characterization of the amorphous cytoplasmic inclusions associated with infections caused by two potyviruses. Virology 142:24-33.
5. de Mejia, M. V. G., Hiebert, E., Purcifull, D. E., Thornbury, D. W., and Pirone, T. P. 1985. Identification of potyviral amorphous inclusion protein as a nonstructural, virus-specific protein related to helper component. Virology 142:34-43.
6. Dougherty, W. G., and Hiebert, E. 1980. Translation of potyvirus RNA in a rabbit reticulocyte lysate: Reaction conditions and identification of capsid protein as one of the products of *in vitro* translation of tobacco etch and pepper mottle viral RNAs. Virology 101:466-474.
7. Dougherty, W. G., and Hiebert, E. 1980. Translation of potyvirus RNA in a rabbit reticulocyte lysate: Identification of nuclear inclusion proteins as products of tobacco etch virus RNA translation and cylindrical inclusion protein as a product of the potyvirus genome. Virology 104:174-182.
8. Fischer, H. U., and Lockhart, B. E. L. 1974. Serious losses in cucurbits caused by watermelon mosaic virus in Morocco. Plant Dis. Rep. 58:143-146.
9. Gonsalves, D., and Ishii, M. 1980. Purification and serology of papaya ringspot virus. Phytopathology 70:1028-1032.
10. Hiebert, E., and Purcifull, D. E. 1981. Mapping of the two coat protein genes on the middle RNA component of squash mosaic virus (comovirus group). Virology 113:630-636.
11. Hiebert, E., Purcifull, D. E., and Christie, R. G. 1984. Purification and immunological analyses of plant viral inclusion bodies. Pages 225-280 in: Methods in Virology. Vol. VIII. K. Maramorosch and H. Koprowski, eds. Academic Press, Inc., Orlando, FL. 396 pp.
12. Hiebert, E., Thornbury, D. W., and Pirone, T. P. 1984. Immunoprecipitation analysis of potyviral *in vitro* translation products using antisera to helper component of tobacco vein mottling virus and potato virus Y. Virology 135:1-9.
13. Marco, K., and Dudock, B. 1974. Characterization of a highly efficient protein synthesizing system derived from commercial wheat germ. Nucleic Acid Res. 1:1385-1397.
14. Purcifull, D. E., Adlerz, W. C., Simone, G. W., Hiebert, E., and Christie, S. R. 1984. Serological relationships and partial characterization of zucchini yellow mosaic virus isolated from squash in Florida. Plant Dis. 68:230-233.
15. Purcifull, D. E., and Batchelor, D. L. 1977. Immunodiffusion tests with sodium dodecyl sulfate (SDS)-treated plant viruses and plant viral inclusions. Fla. Agric. Exp. Stn. Tech. Bull. 788. 39 pp.

16. Purcifull, D. E., Edwardson, J., Hiebert, E., and Gonsalves, D. 1984. Papaya ringspot virus (revised). No. 292 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England.
17. Purcifull, D. E., and Hiebert, E. 1979. Serological distinction of watermelon mosaic virus isolates. Phytopathology 69:112-116.
18. Quiot-Douine, L., Quiot, J. B., and Labonne, G. 1986. Description preliminaire d'un potyvirus isole de courgette (*Cucurbita pepo* L.) en Guadeloupe. Agronomie (In press).
19. Russo, M., Martelli, G. P., Vovlas, C., and Ragozzino, A. 1979. Comparative studies on Mediterranean isolates of watermelon mosaic virus. Phytopathol. Mediterr. 18:94-101.
20. Salerno-Rife, T., Rutgers, T., and Kaesberg, P. 1980. Translation of southern bean mosaic virus RNA in wheat embryo and rabbit reticulocyte extracts. J. Virol. 34:54-58.
21. Thornbury, D. W., and Pirone, T. P. 1983. Helper component of two potyviruses are serologically distinct. Virology 125:487-490.
22. Yeh, S.-D., and Gonsalves, D. 1984. Purification and immunological analyses of cylindrical inclusion protein induced by papaya ringspot virus and watermelon mosaic virus 1. Phytopathology 74:1273-1278.
23. Zettler, F. W., and Abo El-Nil, M. M. 1977. Bean yellow mosaic virus infections of gladiolus in Florida. Plant Dis. Rep. 61:243-247.