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A domain of the readthrough protein of barley yellow dwarf virus (NY-RPV isolate) is essential for aphid transmission

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Abstract

Luteoviruses are obligately transmitted by aphids and contain two capsid proteins, the coat protein (CP) coded for by open reading frame (ORF) 3, and the readthrough protein (RTP), produced by readthrough of the amber termination codon of ORF 3 into the contiguous ORF 5. Previous studies have suggested that it is the RTP that determines transmissibility and vector specificity. To investigate which capsid protein or protein part contains determinants for the transmission of the NY-RPV isolate of barley yellow dwarf virus (BYDV) by its vector *Rhopalosiphum padi*, we produced three fusion proteins by expressing NY-RPV cDNA in *E. coli*. These respectively represented the CP alone (P3), a region of the RTP immediately following the amber termination codon (P5a), and the remainder of the RTP (P5b). Polyclonal antisera raised against the P3, P5a and P5b proteins each gave distinctive reactions against purified NY-RPV on Western blots. Also, in ELISA tests, antisera raised against all three fusion proteins detected purified intact virions. When mixed with purified virions and fed to *R. padi* through Parafilm membranes, immunoglobulins (Igs) from antisera raised against P3 and P5b had no effect on transmission, whereas Ig from antiserum against P5a interfered with transmission. P5a antiserum Ig had no effect on the transmission of the P-PAV isolate of BYDV by *R. padi*. The results demonstrate that while neither the CP itself nor the terminal region of the RTP are key determinants for transmission, a specific domain in the central part of the RTP is an important determinant in the transmission of NY-RPV by *R. padi*, though apparently not of P-PAV.

Introduction

Barley yellow dwarf viruses (BYDV) typify the luteovirus group (Waterhouse et al., 1988; Martin and D'Arcy, 1995), infect many species of gramineous plants and cause significant yield losses in grain crops worldwide (Conti et al., 1990). The luteoviruses are phloem-limited and obligately transmitted by aphids in the circulative/persistent manner. Virions pass through the cellular linings of the hindgut and accessory salivary glands by what appears to be a receptor-mediated endocytosis-exocytosis mechanism (Gildow, 1987, 1993). The specificity of this process depends on the viral capsid (Rochow, 1970), and is presumed to involve the interaction of some domain(s) of the capsid proteins with the relevant aphid membranes.

BYDV particles are isometric, about 26nm in diameter, and contain a single, positive-sense RNA genome. The organization of the genome differs somewhat between the three subgroups of luteoviruses (Martin et al., 1990), i.e. Subgroup 1, containing BYDV -PAV, -MAV and -SGV, Subgroup 2, containing BYDV -RPV and -RMV as well as beet western yellows virus (BWYV) and potato leafroll virus (PLRV), and Subgroup 3, containing soybean dwarf virus (SDV) (Rathjen et al., 1994). Following the open reading frame (ORF) numbering system of Martin et al. (1990), ORFs in the 5′ part of the genome include those encoding putative replicase proteins. ORFs 3 and 4, in the central part, encode a 22–23 kD coat protein (CP) and a smaller (17–19 kD) protein respectively, and ORF 6, found only in Subgroup 1 members, encodes a small

protein of unknown function. In all cases, a further sequence, ORF 5, following ORF 3, is expressed by readthrough of the ORF 3 amber termination codon, producing a readthrough protein (RTP) (for reviews see Domier, 1995; Martin et al., 1990). Although the RTP is present in virus particles as a fusion between the products of ORF 3 and ORF 5 (Bahner et al., 1990; Brault et al., 1995, Veidt et al., 1988; Vincent et al., 1991), it is not necessary for viral replication or assembly in protoplasts (Filichkin et al., 1994; Reutenauer et al., 1993). It has been suggested, however, that the RTPs of luteoviruses may play a part in virus transmission (e.g. Bahner et al., 1990; Tacke et al., 1990; Wang et al., 1995) as with the RTPs of other viruses such as pea enation mosaic virus (PEMV) (Adam et al., 1979) and the fungus-transmitted beet necrotic yellow vein virus (BNYVV) (Tamada and Kusume, 1991). Recent work has addressed testing this notion by sequence studies of PLRV (Jolly and Mayo, 1994) and mutagenic analysis of BWYV (Brault et al, 1995). Here we report a serological approach to mapping the capsid domains important in transmission of the NY-RPV isolate of BYDV. Polyclonal antisera were raised against three fusion proteins produced in *Escherichia coli*, using genomic segments of NY-RPV, i.e. to the CP alone and to each of the two halves of the RTP product encoded by ORF 5, respectively. We then investigated their effects on the transmission of NY-RPV when incubated with purified virions prior to membrane feeding to aphids.

Materials and methods

Aphid and virus cultures

The NY-RPV BYDV isolate was that described previously (Rochow, 1969, 1970; Vincent et al., 1991) and was maintained at Purdue University by mass transfer of viruliferous *Rhopalosiphum padi* (L.) to oat plants (*Avena sativa* L., cultivar Clintland-64). It was cultured and purified as described for the P-PAV isolate of BYDV (Hammond et al., 1983). Purified P-PAV was also used in one experiment.

Aviruliferous *R. padi* were maintained on healthy barley (*Hordeum vulgare* L., cultivar Moore or Dickson) and regular checks of plant extracts were made by ELISA to ensure that the aphids remained virus-free and that the NY-RPV culture did not become contaminated with other BYDV serotypes (Webby et al., 1993).

Fusion protein and antiserum production

To produce fusion proteins, three segments of the NY-RPV genome (Vincent et al., 1991) were each separately subcloned into pATH vectors for fusion with the amino terminus of the *E. coli* anthranilate synthetase (*trp* E) gene (Koerner et al., 1991). All inserts and vectors were incubated with the Klenow fragment of DNA polymerase and dNTPs to generate blunt ends. The inserts were gel purified and clones identified by hybridization with labelled inserts. Orientation was determined by restriction analysis. All techniques were based on standard procedures (Sambrook et al., 1989).

Figure 1 indicates the restriction sites and nucleotide regions (after Vincent et al., 1991) used to create the three fusion proteins. Fusion protein P3 was made using nucleotides 3649–4303 of the *Acc*I fragment corresponding to the CP gene (ORF 3) and subcloned into pATH2 at the *Xba*I site. P3 included 13 extra amino acids translated from the NY-RPV sequence 5′ to the CP start codon, with a predicted MW of 57 kD (33 kD from Trp and 24 kD from NY-RPV). Fusion protein P5a was made to the 5′ part of ORF 5 (nucleotides 4302–4972) using the *Acc*I and *Eco*RI sites, and subcloned into pATH3 at the *Bam*HI site. The stop codon from the vector was utilized, and hence P5a contains 21 amino acids from the vector (2.6 kD) plus 33 kD from Trp and 24 kD from NY-RPV to give a predicted fusion protein of 59.6 kD. P5b was made from the 3′ end of ORF 5 (nucleotides 4961 to the end of the NY-RPV clone 6.4 which includes the ORF 5 stop codon at nucleotide 5495 (Vincent et al., 1991)) and subcloned into pATH2 at the *Xba*I site. The predicted MW of the resulting fusion protein is 33 kD from Trp plus 19 kD from NY-RPV, giving a total of 52 kD. Fusion proteins were expressed in bacterial cells by induction with indoleacrylic acid (Koerner et al., 1991). The fusion proteins were purified following electrophoresis in SDS-polyacrylamide gels (Laemmli, 1970). The gels were incubated in cold 0.25 M KCl to visualize the protein bands. After excision of the bands, the proteins were recovered by electroelution into dialysis membranes.

Polyclonal antisera to the NY-RPV and P-PAV isolates and to the three fusion proteins (anti-P3, anti-P5a and anti-P5b) were produced in New Zealand White rabbits by intramuscular injections of samples emulsified with Freund's adjuvants using standard procedures (Lister et al., 1982; Dunbar and Schoehel, 1990). Immunoglobulins (Igs) from the virus antisera

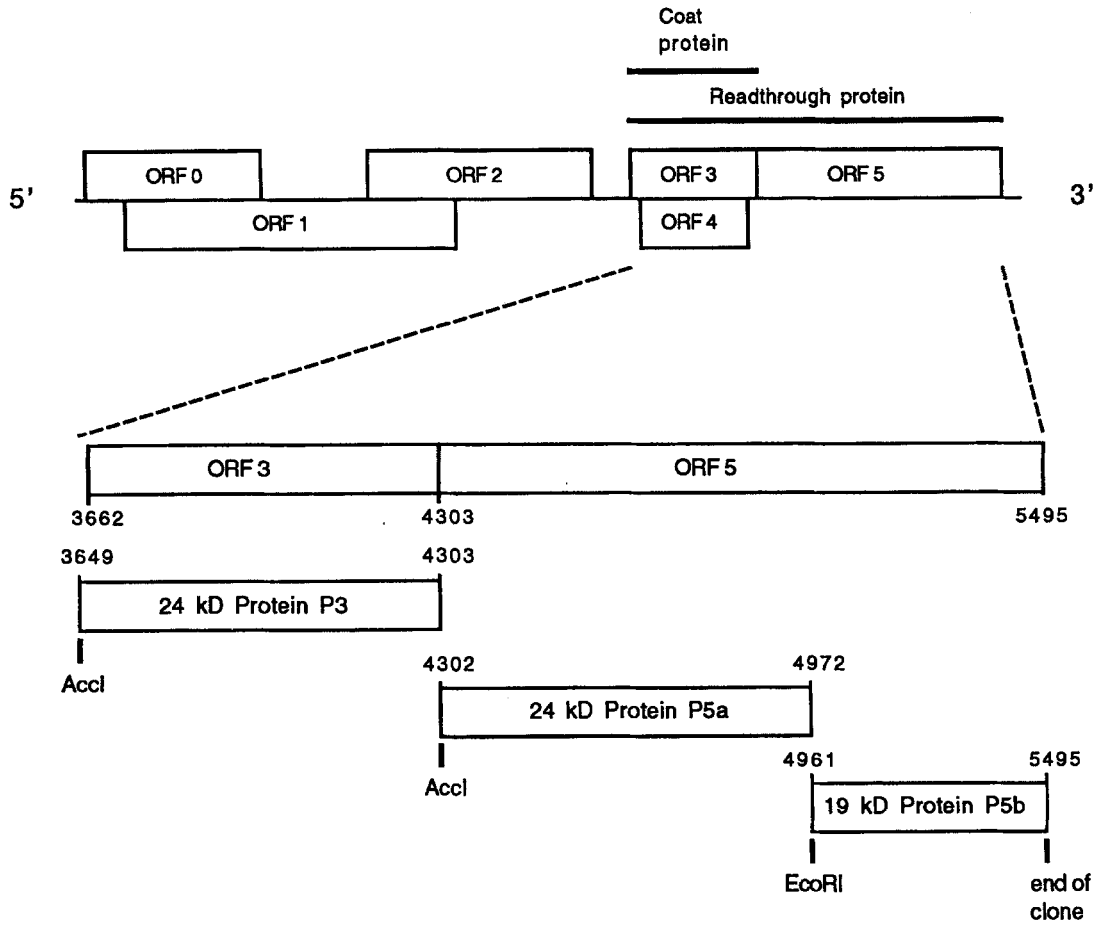

Figure 1. Schematic diagram of the genomic map of NY-RPV (after Vincent et al., 1991; Martin et al., 1990) showing the details of the restriction enzyme sites used to make fusion proteins P3, from ORF 3, and P5a and P5b from the N- and C-termini of ORF 5 respectively.

were prepared by ammonium sulphate precipitation, and from the three fusion protein antisera and pre-immune serum from the rabbit inoculated with P5a (pre-immune P5a) by using an E Z Sep kit (Pharmacia).

Electrophoresis and western blotting

Total protein lysates of the *E. coli* expressing the three fusion proteins and purified NY-RPV virions were run on 10% acrylamide gels and transferred to nitrocellulose. Acrylamide gels of total *E. coli* protein lysates were also stained with Coomassie brilliant blue. Western blotting of the *E. coli* lysates and of purified virions was done using a Bio-Rad Mini Protean II system according to the manufacturer's protocol. Detection of the fusion proteins was with NY-RPV antiserum Ig followed by goat anti-rabbit alkaline phosphatase conjugate (Sigma). Antigenic bands from purified virions were detected with the NY-RPV Ig (1 mg ml^{-1}) at 1:1000 dilution and with the three fusion protein Igs (10–20 mg ml^{-1}) at 1:1000 dilutions in milk diluent (Kirkegaard and Perry, Gaithersburg, MA, 1:20) after previously blocking for 30 min in milk diluent (1:10). Detection was with goat anti-rabbit alkaline phosphatase conjugate (Sigma) at 1:2000 (also in 1:20 milk diluent) and the substrate was BCIP plus NBT (Sigma Fast, Sigma).

DAS-ELISA tests

Standard double antibody sandwich (DAS)-ELISA tests (Clark et al., 1986; Webby et al., 1993) were used to determine the ability of the three antisera raised against the fusion proteins to detect intact virions. Plates (Corning 25801, Corning Inc., Corning, NY) were coated for 3 h with polyclonal anti-NY-RPV Ig at 1 $\mu g\ ml^{-1}$, or with one of the three fusion protein Igs at 100–200 $\mu g\ ml^{-1}$. After washing, the

plates were then incubated for 3 h with either purified NY-RPV virions or phosphate buffer as a negative control, followed by a further wash and then incubation overnight at 4 °C with NY-RPV-Ig conjugated to alkaline phosphatase. The plates were then developed with 1 mg ml^{-1} p-nitrophenyl phosphate substrate in 10% diethanolamine buffer, pH 9.8, and read after overnight incubation at 4 °C. All tests were carried out in duplicate wells and on replicate plates.

Aphid transmissions

Purified NY-RPV (50 μl) at a concentration of approximately 26 μg ml^{-1} was mixed with 10 μl of Ig (anti-NY-RPV at 1 mg ml^{-1}, or anti-P3, anti-P5a or anti-P5b at 10 to 20 mg ml^{-1}) or with 10 μl of 7.5% (Experiment 1) or 15% (Experiments 2 and 3, Table 3) bovine serum albumen (BSA). Each mixture was made up to a final volume of 75 μl with 0.1M phosphate, pH 7.0, and incubated for 30 min at 37 °C, then overnight at 4 °C (Rochow, 1970). Pre-immune P5a was also used as a control in Experiments 2 and 3 (Table 3). The mixtures were then centrifuged for 5 min at 5000 rpm and 70 μl of the supernatants were mixed with an equal volume of 40% sucrose in 0.1 M phosphate buffer, pH 7.0. Aviruliferous *R. padi* were allowed a 24 h acquisition access period on the mixtures through a stretched Parafilm M membrane (American National Can, Greenwich, CT), before being transferred in groups of 20–25 to individual Clintland-64 oat seedlings. Aphid survival was excellent, and after a 3-day inoculation access period, the plants were sprayed with Malathion insecticide and maintained in an aphid-free greenhouse for 14 days. Negative controls, with no aphid inoculation, were used to check for contamination; none of these plants were ever found to be infected. The number of infections was determined by DAS-ELISA with the NY-RPV antiserum, essentially as described (Webby et al., 1993). The experiment was also repeated using similar concentrations of P-PAV mixed with NY-RPV Ig, the three fusion protein Igs, pre-immune P5a, or P-PAV Ig. In this case, the number of plants becoming infected was determined using P-PAV Ig in DAS-ELISA. Also, to check the availability of virus for acquisition by the aphids from the membrane feeding solution, a comparable set of NY-RPV and P-PAV samples were subjected to the Ig treatments and then tested by DAS-ELISA (Webby et al., 1993) using their respective Igs. Dilutions of virus solutions in the ELISA wells were equivalent to one fourth and one twelfth of that present in the feeding solutions (i.e. approximately 2.2 μg ml^{-1} and 0.7 μg ml^{-1} respectively, assuming no precipitation of the virus caused by the Igs).

Results

Identification of fusion proteins in E. coli *extracts*

Total protein extracts of *E. coli* transformed to produce fusion proteins were run on a 10% acrylamide gel in comparisons with extracts of *E. coli* transformed with the pATH2 vector with no insert. When stained with Coomassie brilliant blue, two induced (i.e. specific) proteins were detected for the P3-producing *E. coli*, one was detected for the P5a-producing bacteria, and two were detected for the P5b-producing bacteria (Figure 2A). The P5a band had a larger apparent MW than expected, presumably because of anomalous migration. After transfer to nitrocellulose and probing with NY-RPV Ig, the P3-transformed culture extract produced a double band plus a band of smaller size that is probably a breakdown product. The P5a-transformed culture extract gave a single band of the predicted MW, but the P5b-transformed culture extract gave no reaction (Figure 2B).

Serological identification of virion proteins

Several bands were detected in western blots of purified NY-RPV virions probed with NY-RPV Ig and the three Igs produced against the fusion proteins (Figure 3). As expected, NY-RPV Ig detected the CP (23 kD) and the RTP, or part thereof (estimated size: 66kD), together with a weaker band slightly smaller in size than the putative RTP (62kD, Figure 3A) which is probably a breakdown product of the 66 kD protein (Vincent et al., 1991). Indeed, the 66kD band may be, as is typical of luteoviruses, a truncated form of the full length RTP with anomalous migration. The anti-P3 Ig also detected the CP plus several bands similar in size to the RTP as detected by the NY-RPV Ig, the strongest of these bands being equivalent to the 62kD band detected by the NY-RPV Ig (Figure 3B). The anti-P5a Ig detected the 66 kD and 62 kD bands with equal intensity, together with a smaller protein of 17 kD (Figure 3C). The anti-P5b Ig also detected the 66 kD and 62 kD products, as well as a medium sized protein of about 36 kD (Figure 3D). Neither anti-P5a or anti-P5b detected the CP, demonstrating specificity of these two Igs for the product(s) of ORF 5.

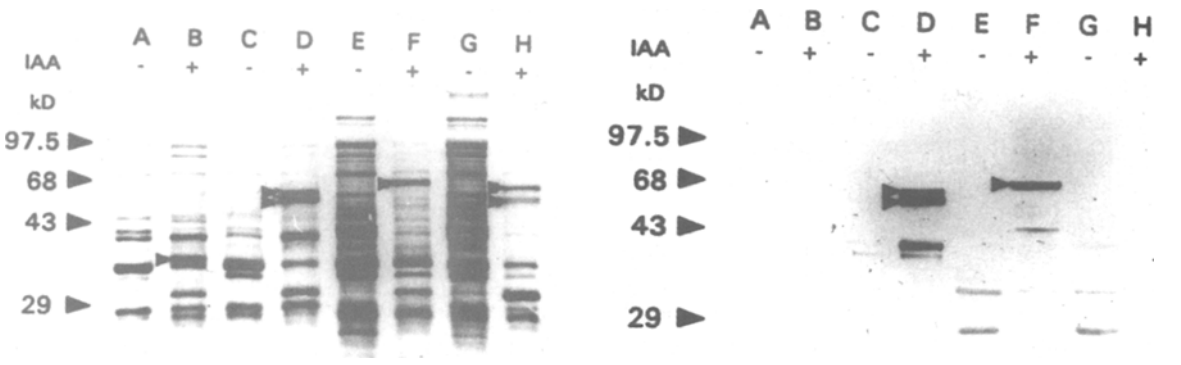

Figure 2. (A) Coomassie blue stained proteins extracted from *E. coli* separated on a 10% acrylamide gel and (B) Western blot of gel with bands detected with NY-RPV Ig followed by goat anti-rabbit alkaline phosphatase conjugate and developed with NBT/BCIP. Lanes A and B, *E. coli* containing vector (pATH) alone. The 33kD band is part of the trpE protein which was fused to all the NY-RPV proteins. Other lanes correspond to *E. coli* transformed with various regions of NY-RPV cDNA. Lanes C and D, transformed with ORF3 (CP region, P3); Lanes E and F, transformed with 5′ part of ORF5 (P5a); Lanes G and H, transformed with 3′ part of ORF5 (P5b). + or − refers to whether the bacterial cells were induced with indoleacrylic acid (IAA) or not. Small arrows indicate induced bands.

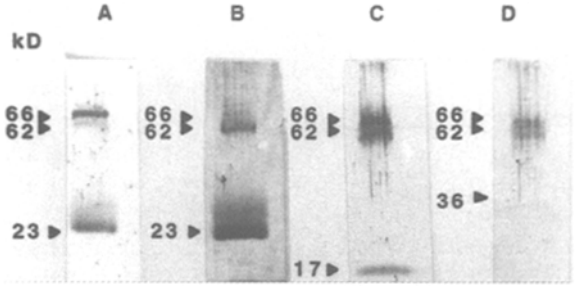

Figure 3. Western blots of purified NY-RPV detected with Igs against: (A) intact virus, (B) P3, (C) P5a and (D) P5b (see text for details), followed by goat anti-rabbit alkaline phosphatase conjugate and NBT/BCIP substrate. Values on the left hand side of the blots correspond to the MW (kD) of the major bands.

DAS-ELISA of intact virions

DAS-ELISA tests using NY-RPV Ig and the three fusion protein Igs demonstrated that all were able to trap intact virions. The experiment was repeated three times with consistent results. Typical absorbance values obtained from one of these replications are shown in Table 1. As expected, tests with NY-RPV Ig detected the purified virions strongly. Anti-P5a Ig also had a strong affinity for the NY-RPV virions, while the reactions of anti-P3 Ig and anti-P5b were relatively weak (Table 1).

Table 1. Absorbance values in DAS-ELISA of tests using NY-RPV Ig and fusion protein antisera Igs to trap intact purified NY-RPVvirions. Values are the means of overnight readings of duplicate wells on each of two plates

Trapping antibody	Absorbance reading	
	NY-RPV virions	Phosphate buffer
NY-RPV Ig	>2.0	0.043
Anti-P3 Ig	0.182	0.050
Anti-P5a Ig	>2.0	0.065
Anti-P5b Ig	0.110	0.037

Aphid transmissions

Preliminary experiments to determine the efficiency of transmission of NY-RPV by *R. padi* through membranes used 20 μl of purified NY-RPV at a concentration of about 26 μg ml^{-1} mixed with 200 μl 0.1M phosphate buffer, pH 7.0, containing 20% sucrose. Three attempts, using 10–25 aphids per plant, failed to transmit the virus to any of a total of 32 test plants. However, when in further similar experiments, the virus concentration was increased (50 μl purified NY-RPV plus 75 μl sucrose solution), one plant of eight became infected. With 20 aphids per plant, the addition of BSA to such mixtures, as suggested for PLRV transmission (van den Heuvel et al., 1991) increased the rate

of transmission over controls without BSA from none of ten plants to five of 13 plants (38.5%). So as to give a suitable baseline level of transmission it was therefore decided to use a combination of virus and BSA as the control in the experiments investigating the effects of added antisera on transmission efficiencies (Tables 2 and 3).

DAS-ELISA tests showed that incubation of purified virus with NY-RPV Ig or P-PAV Ig followed by centrifugation specifically removed the homologous virus from solution (Table 2). However, incubation of virus with the three fusion protein Igs or with pre-immune P5a or BSA, followed by centrifugation, did not do so (Table 2). Also, as expected, NY-RPV Ig did not remove P-PAV from the feeding solution. It is assumed, therefore, that the interactions that occurred between fusion protein Igs and intact virions (Table 1) did not precipitate them from the feeding solutions used in our experiments.

Results from three experiments showed that aphid transmission was not prevented after feeding on mixtures of purified NY-RPV virions incubated with BSA or pre-immune P5a serum. Nor did either anti-P3 or anti-P5b Igs inhibit the transmission of NY-RPV by *R. padi*. All these four treatments gave rates of transmission (25.8 to 44.8%) similar to that obtained in the preliminary experiments with NY-RPV preparations incubated with BSA (38.5%), as described above. If, however, the virus was pre-treated with NY-RPV Ig or anti-P5a Ig, then transmission was either abolished or greatly reduced, to zero or 3.2 % respectively (Table 3). In the case of the NY-RPV Ig, this is presumably because virions were precipitated from the feeding solution or insolubilized (Table 2). In contrast, purified P-PAV was transmitted efficiently after pre-treatment with NY-RPV Ig or any of the three fusion protein Igs, as well as the pre-immune P5a control. The number of plants infected and the number tested for P-PAV incubated with NY-RPV Ig, anti-P3, anti-P5a, anti-P5b Igs and pre-immune P5a were 8/9, 6/6, 4/4, 6/6 and 8/8 respectively. Only when pre-treated with P-PAV Ig was transmission prevented, with 0/9 plants becoming infected, again presumably due to the absence of virus particles in the feeding solution (Table 2).

Discussion

The genomes of all luteoviruses that have been sequenced to date contain the arrangement whereby ORF 5 is expressed by readthrough of the ORF 3 amber termination codon. It has been proposed that the RTP expressed by this mechanism is involved in virus transmission by specific aphid vectors (Bahner et al., 1990; Tacke et al., 1990). Supporting this idea is that the RTP is present as a minor component in the viral capsid along with the CP (reviewed by Domier, 1995; Martin et al., 1990), but it is not required for particle assembly (Filichkin et al., 1994; Reutenauer et al., 1993). As well as in particle assembly and capsid integrity, capsid proteins have been shown to be important in the transmission of several plant viruses by vectors (Briddon et al., 1990; Chen and Francki, 1990, Harrison and Robinson, 1988; Rochow, 1970), and studies of other virus groups that contain RTPs in their capsids, such as PEMV and BNYVV, demonstrate that the RTP is necessary for transmission (Adam et al., 1979; Tamada and Kusume, 1991). Among the luteoviruses, comparisons of the sequences of the capsid proteins and specific aphid vectors of the different viruses (Vincent et al., 1990) showed that the determinants for transmission were most likely to be in the non-CP region of the RTP, as this region shows most sequence diversity. However, there is conflicting evidence as to which of the capsid proteins are involved in transmission by *M. persicae*. Thus, by comparing the sequences of efficiently and poorly aphid-transmitted isolates (Jolly and Mayo, 1994) a determinant for transmission was mapped to two possible residues towards the 3′ region of the RTP. Also, while the present work was in progress, Brault et al. (1995) found by mutational analysis that a region in the C-terminal part of the RTP seems necessary for the aphid transmission of BWYV. In contrast, using membrane feeding experiments similar to those described here, van den Heuvel et al. (1994) were able to inhibit transmission of PLRV after incubation with a monoclonal antibody that reacts with the CP but not with any other part of the RTP, hence concluding that it was the CP itself that governed transmission.

Ig produced against NY-RPV detects several bands in western blots of purified virus preparations (Vincent et al., 1990, Figure 3A). Each of the three Igs raised against the fusion proteins described herein, representing various parts of the NY-RPV capsid proteins, were able to detect some but not all of these bands and each produced a different pattern of bands (Figure 3B, C and D). Each of the three Igs was, therefore, specific to a particular part of the products of ORF 3 and ORF 5, as expected. However, anti-P5a and anti-P5b Igs also detected bands that were not detected by the NY-RPV Ig. The reason for this is unclear,

Table 2. Absorbance values in DAS-ELISA tests of virus remaining in solution in control samples and samples incubated with various immunoglobulins (Igs) followed by centrifugation at 5000 rpm for 5 min

Treatments	Dilutions[2]	NY-RPV[1]		P-PAV[1]	
		2.2 μg ml^{-1}	0.7 μg ml^{-1}	2.2 μg ml^{-1}	0.7 μg ml^{-1}
NY-RPV Ig		0.043[3]	0.055	1.910	0.913
anti-P3 Ig		1.701	1.045	1.496	0.821
anti-P5a Ig		1.415	0.893	1.228	0.690
anti-P5b Ig		1.557	1.013	1.328	0.718
Pre-immune P5a		1.628	0.992	1.228	0.660
0.55% BSA		1.869	1.150	–	–
P-PAV Ig		–	–	0.029	0.018
Buffer control		0.048	0.048	0.013	0.013

[1] NY-RPV and P-PAV Igs were used to detect NY-RPV and P-PAV, respectively.
[2] Virus concentrations equivalent to 1/4 and 1/12 of the solutions used in the membrane feed experiments.
[3] Readings were taken after 2h.

Table 3. Results of transmission experiments with *R. padi* given an acquisition access feed of 24 h through Parafilm membrane on purified NY-RPV pre-treated with (Igs) raised against purified virus or to various parts of the viral capsid proteins (see text for details)

Treatment	Expt 1	Expt 2	Expt 3	Totals	Percent
NY RPV Ig	0/11[1]	0/12	0/9	0/32	0
anti-P3 Ig	5/10	2/10	6/9	13/29	44.8
anti-P5a Ig	1/10	0/12	0/9	1/31	3.2
anti-P5b Ig	6/9	1/10	5/9	12/28	42.8
Pre-immune P5a	–	3/8	3/7	6/15	40.0
BSA[2]	2/10	2/12	4/9	8/31	25.8
Negative controls[3]	0/6	0/6	0/8	0/20	0

[1] Numerator equals number of plants becoming infected, denominator is number of plants tested. 20–25 aphids were transferred to each test plant.
[2] Final concentration of BSA was 0.28% in Expt. 1 and 0.55% in Expts. 2 and 3.
[3] No aphids were placed on these plants.

but it is likely that the bands represent breakdown products of the RTP, which is unstable and can be degraded by repeated freezing and thawing (Vincent et al., 1990). The detection of bands from purified NY-RPV by anti-P5b Ig is also somewhat unexpected, as in other luteoviruses examined to date the C-terminal part of the RTP is not present in the viral capsid (Bahner et al., 1990; Filichkin et al., 1994; Brault et al., 1995). Absence of the C-terminus from the virion could explain why anti-NY-RPV Ig did not detect the P5b fusion protein in western blots of expressed proteins from P5b-transformed *E. coli* (Figure 2B), and also why anti-P5b Ig only weakly detected intact virions (Table 1), much depending on the degree of overlap between the region of ORF 5 used to create P5b and the site of truncation of the RTP in the virion. However, since NY-RPV proteins were detected by anti-P5b Ig in western blots (Figure 3D) it may be that the C-terminus is either folded within the tertiary structure of the virion, or for some other reason is only weakly immunogenic in intact particles.

Preliminary experiments on transmission of NY-RPV through Parafilm membranes indicated that the addition of a protein such as BSA was necessary to obtain reasonable rates of transmission, possibly by inhibiting virus agglutination (van den Heuvel et al., 1991). Even so, the rates of transmission obtained, 25.8 to 44.8% with 20–25 aphids per plant (Table 3), are low, and this seemed to be a peculiarity of NY-RPV in our hands. In contrast, transmissible P-PAV was efficiently acquired through membranes by *R. padi* (see above) and in other tests an MAV serotype isolate (MAV-PS1 (Lister and Sward, 1988)) was also acquired efficiently through membranes by *Sitobion avenae* (P. McGrath, unpublished results). But, given the baseline rate of transmission provided by the BSA and pre-immune P5a serum controls (25.8% and 40% respectively), the evidence is convincing that both anti-P3 Ig (raised against the CP) and anti-P5b Ig (raised against the terminus of the ORF 5 product) consistently had no effect on the transmission of NY-RPV by *R. padi*, although both reacted serologically with NY-RPV virions, albeit relatively weakly. In contrast, anti-P5a Ig, containing antibodies to the central portion of the RTP, reacted strongly with intact virions and greatly reduced transmission. It is reasonable to conclude therefore that a determinant for transmission lies between nucleotides 4302 and

4972, the section of the NY-RPV cDNA used to make the P5a fusion protein. Interestingly, this region overlaps somewhat with the region of the RTP of BWYV thought to be involved in aphid transmission, downstream of nucleotide 4822 (Brault et al., 1995).

Anti-P3 and anti-P5b Igs react strongly with dissociated virus in western blots (Figure 3B, 3D) but only weakly with virions in DAS-ELISA (Table 1). These reactions are typical of fusion protein antisera which primarily detect linear or sequence-specific epitopes and therefore we cannot preclude the possible importance in transmission of other domains on the virion surface produced by tertiary or quaternary structures of the capsid proteins. However, the weak DAS-ELISA reactions suggest that there may be some linear epitopes corresponding to these two regions on the virion surface. The lack of interference in transmission by these two Igs (Table 3) suggests that these linear epitopes must play little or no role in aphid transmission. Indeed, it is probable that much of the N-terminal region of the CP is located within the interior of the virus particle (Rossman and Johnson, 1989) and this would explain the difference between reaction strengths of the dissociated viral proteins on the western blots and the intact particles in the ELISA tests, at least for anti-P3 Ig, and possibly for anti-P5b (see above). The reduction of transmission efficiency by anti-P5a Ig could conceivably be due to steric hindrance rather than direct interaction with viral domains, but the fact that no similar effect is observed with anti-P3 Ig or anti-P5b Ig suggests an important role in the region of the RTP analogous to P5a. For more precise mapping of the domain involved by the techniques described here, a range of overlapping fusion proteins and their respective antisera would be needed. Alternatively, an infectious, full length clone would need to be made and manipulated to investigate the effect on transmissiblilty of induced deletions and mutations in the region of interest, as recently done for BWYV (Brault et al., 1995). However, such results would still be difficult to interpret due to the possibility of alterations in protein folding induced by genomic changes.

Virus transmission by insect vectors seems to be dependent on two factors, efficiency and specificity, and it is possible that different domains of the viral capsid proteins are responsible for each process. This could explain differences in the results described above regarding transmission determinants. For example, Jolly and Mayo (1994) may have studied a determinant governing efficiency, whereas van den Heuvel et al. (1994) may have found a monoclonal antibody that blocks a site of the CP responsible for recognition of PLRV by *M. persicae*. It would be interesting if this same monoclonal antibody were not able to prevent the transmission of PLRV by another vector, for example *Macrosiphum euphorbiae*. In a comparable experiment described above, anti-P5a Ig did not interfere with the transmission of P-PAV by *R. padi*. This indicates that determinants for the transmission of RPV and PAV in the virions are different, and that there are at least two different receptor sites within *R. padi*, each utilised by a different isolate of BYDV. It seems likely therefore, that different domains in virions and different receptors in vectors account for transmission specificity among BYDVs.

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