

Heterologous encapsidation in mixed infections among four isolates of barley yellow dwarf virus

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We used immunohybridization and ELISA to investigate heterologous encapsidation (transcapsidation and phenotypic mixing) between paired isolates of barley yellow dwarf virus (BYDV) in doubly infected oat plants, *Avena sativa* L. cv. Clintland 64. Virions in samples extracted from plants doubly infected with two viruses were trapped with an antibody specific to one virus, and the nucleic acids of the trapped virions were identified with a cDNA probe specific to the other. Heterologous encapsidation was found in mixed infections between isolates NY-RPV and NY-MAV-PS1, NY-RPV and P-PAV, NY-RMV and NY-MAV-PS1, P-PAV and NY-MAV-PS1, and NY-RPV and NY-RMV. Heterologous encapsidation between NY-RPV and P-PAV, and between NY-RPV and NY-MAV-PS1, occurred in one direction, while the heterologous encapsidation between P-PAV and NY-MAV-PS1

occurred in both directions. Further analysis by heterologous ELISA and immunohybridization assays with immunoprecipitated samples demonstrated that transcapsidation was the predominant type of heterologous encapsidation in mixed infections of NY-RPV and P-PAV, NY-RPV and NY-MAV-PS1, and NY-RMV and NY-MAV-PS1; phenotypic mixing was the predominant type of heterologous encapsidation in mixed infections of P-PAV and NY-MAV-PS1. Phenotypic mixing was also detected in mixed infections of NY-RPV and NY-RMV. These results suggest that among BYDV isolates transcapsidation is more common between distantly related isolates than between more closely related isolates, and phenotypic mixing is more common between more closely related isolates than distantly related isolates.

Introduction

Barley yellow dwarf virus (BYDV) is a general name for a group of luteoviruses which infect small-grain cereals and other grasses. Five isolates described by Rochow (1970*a*) represent five serotypical categories of BYDV from New York (NY), namely NY-PAV, NY-MAV, NY-SGV, NY-RPV and NY-RMV. These isolates are more or less specifically transmitted by, and were named after, various vector aphids, and separation by vector specificity approximately corresponds to separation on the basis of serotype differences (Rochow, 1970*a*). Isolates can also be grouped by serological relationship into group 1, which includes the first three mentioned, and group 2, which includes the last two (Rochow, 1970*a*; Waterhouse *et al.*, 1988). A further feature is that the vector specificities of different isolates can be altered if two isolates co-infect and replicate simultaneously in one plant. For example, *Rhopalosiphum padi* transmits NY-RPV but not NY-MAV from single infections, but can transmit the NY-MAV isolate from a plant which is infected with both NY-RPV and NY-MAV (Rochow, 1970*b*). This altered transmission is referred to as

dependent transmission, in which aphids transmit one virus (the dependent virus) only in the presence of a second virus (the helper virus). It also occurs among other luteoviruses (summarized by Waterhouse *et al.*, 1988). Dependent transmission may affect the epidemiology of luteovirus diseases by altering the vector specificity of the dependent virus, so that the virus can be transmitted by a non-vector aphid (i.e. the helper virus vector). It may also increase the incidence of mixed infections in the field, thus influencing synergistic effects on disease severity and yield loss in the doubly infected plants (Halstead & Gill, 1971; Gill & Comeau, 1977).

Vector specificity in BYDV is probably determined by interactions between the viral capsid protein and the membrane surfaces of the aphid's accessory salivary gland (Gildow & Rochow, 1980). It has been suggested that in BYDV altered vector specificity in dependent transmission results from the formation of atypical virions formed by encapsidation of viral RNA of one virus by the coat protein of the other during simultaneous replication of the two viruses in mixed infections, a phenomenon referred to as heterologous encapsidation (Rochow, 1977). As a result, the protein capsid of

heterologously encapsidated virions could contain protein subunits either from one virus or from both viruses thus forming an antigenically mixed or chimeric protein capsid (Rochow, 1977; Hu *et al.*, 1988). The former type of heterologous encapsidation is transcapsidation or genomic masking, and the latter is phenotypic mixing.

Evidence of heterologous encapsidation among BYDV isolates has mostly been indirect and based on studies of altered vector specificity and serological behaviour, e.g. by infectivity neutralization (Rochow, 1970*b*). Since some pairs of BYDV isolates (such as NY-PAV and NY-RPV, and NY-PAV and NY-MAV) share certain vectors, it has not been feasible to investigate fully their heterologous encapsidation and dependent transmission by examining aphid transmission specificity. More recently however, direct evidence of heterologous encapsidation has been obtained from studies using a novel analytical method referred to as immunohybridization (Creamer & Falk, 1990). In an immunohybridization assay, virions in the samples are trapped with antibodies, and the RNAs of the trapped virions are probed with labelled cDNAs. Heterologous encapsidation can be easily detected by this assay with heterologous antibodies and cDNA probes, i.e. virions are trapped with antibodies specific to one virus, and the RNAs of the trapped virions are detected with cDNA probes specific to the other virus in the mixed infection. In this study, we used immunohybridization and heterologous ELISA to investigate heterologous encapsidation (transcapsidation and phenotypic mixing) among the NY-RPV, NY-RMV, NY-MAV-PS1 and P-PAV isolates of BYDV.

Methods

Plants, virus isolates and inoculations. Plants were maintained in growth chambers at 20 ± 1 °C with a 14 h photoperiod (200 $\mu\text{E}/\text{m}^2/\text{s}$). The BYDV isolates used were NY-MAV-PS1, a Purdue subculture of the NY-MAV isolate (Rochow, 1970*a*) which differs slightly from the latter in serological reactions (Lister & Sward, 1988), P-PAV ('Purdue PAV'), a PAV-like isolate from wheat in Indiana (Hammond *et al.*, 1983), NY-RMV and NY-RPV (Rochow, 1970*a*). Each virus was cultured in oat seedlings, *Avena sativa* L. cv. Clintland 64, which were inoculated by mass infestation with appropriate viruliferous aphids, i.e. *R. padi* L. for P-PAV and NY-RPV, *Sitobion avenae* Fabr. for NY-MAV-PS1 and *R. maidis* Fitch for NY-RMV. Apterous aphids from cultures established on these plants for 1 to 2 weeks were used for virus inoculation.

Uniform 5-to-7-day-old oat seedlings grown singly in 8 cm diameter polystyrene cups were simultaneously doubly inoculated with paired viruses. Plants inoculated singly with each virus were used as controls. After inoculation, plants were incubated in a growth chamber for 10 to 15 days, and then harvested for sample processing.

Sample processing. Shoots, i.e. all parts above ground level, of the plants singly and doubly infected with paired viruses were harvested, ground in liquid nitrogen with a mortar and pestle and then extracted at a ratio of 1:10 (w/v) in 0.1 M-potassium phosphate buffer pH 7.0. The

extracts were used in ELISA and immunohybridization assays. In experiments designed to detect phenotypic mixing, extracts were subdivided into two subsamples: one was processed directly for immunohybridization tests, and the other was absorbed with an appropriate monoclonal antibody (MAb) before use in immunohybridization tests. Such absorption was with an MAb reacting with an epitope not detected by the trapping antibody. For example, to detect phenotypic mixing in the heterologous encapsidation of NY-MAV-PS1 RNA, we absorbed the subsamples with an MAb specific to NY-MAV-PS1 to remove virions bearing epitopes specific to NY-MAV-PS1, and then unreacted virus from the absorbed subsample was trapped on ELISA plates with P-PAV-specific MAb and probed with NY-MAV-PS1-specific cDNA probes. If only transcapsidation were involved, this procedure would not affect the hybridization signal with the NY-MAV-PS1 probe. However, if phenotypic mixing were involved, the hybridization signal would be reduced or eliminated. Absorption was carried out as follows. One ml of each subsample was mixed with 50 to 100 μl (5 to 8.5 mg) of appropriate mouse anti-virus MAb (ascites). The mixture was incubated at 37 °C for 2 h, incubated further at 4 °C overnight, then centrifuged at 7000 *g* for 10 min, and the supernatant liquid was collected for immunohybridization assay.

Antibodies. Trapping and second antibodies were either mouse anti-virus MAbs or rat anti-virus MAbs. The mouse anti-virus MAbs (and their specificities among the isolates used) included MAV-1 (NY-MAV-PS1-specific), MAV-2 (P-PAV-specific), MAV-3 (P-PAV-specific), MAV-4 (NY-MAV-PS1-specific), RPV-1 (NY-RPV-specific) (Hsu *et al.*, 1984) and RMV-1 (NY-RMV-specific) (G. N. Webby & R. M. Lister, unpublished results). Similarly, the rat anti-virus MAbs (Torrance *et al.*, 1986) included MAC 91 (P-PAV-specific) and MAFF-2 (NY-MAV-PS1-specific). Antibodies for conjugates were the appropriate rabbit anti-virus polyclonal antibodies, or anti-rat antibodies raised in goats (BRL).

cDNA clones. In most experiments the cDNA clones used, in pUC18 (Vieira & Messing, 1982), were as described previously (Fattouh *et al.*, 1990) and were defined by prefixes derived from the initial letters of Purdue and their virus acronym. They were pPP26, pMP7+ and pRP23, detecting the viral RNAs of P-PAV, NY-MAV-PS1 and NY-RPV, respectively. Because pPP26 and pMP7+ cross-react weakly with their heterologous viral RNAs, subclones pPP26S4 (specific for P-PAV) and pMP7+S5 (specific for NY-MAV-PS1) were kindly prepared for us by Dr P. P. Ueng from pPP26 and pMP7+, respectively (Wen, 1990), and these subclones were used to distinguish P-PAV and NY-MAV-PS1 in plants doubly infected with these two viruses. ³²P-labelled cDNA probes pPP26, pMP7+ and pRP23 were prepared by nick translation, and the cDNA probes pPP26S4 and pMP7+S5 were labelled by a DNA multiprime labelling technique (Amersham).

Immunohybridization assay procedures. Immunohybridization procedures were essentially those of Creamer & Falk (1990) with slight modifications. MAbs were diluted in coating buffer (Clark *et al.*, 1986) at 1:1000. The diluted MAbs (200 μl) were added to each well of MicroELISA Immulon 2 plates (Dynatech Laboratories). The plates were incubated at 37 °C for 4 h in plastic bags containing moist paper towels, and then washed four times by filling the wells with PBS-Tween buffer (Clark *et al.*, 1986) with a squeeze-bottle. Plant extract (200 μl) (either pre-absorbed with MAb or not pre-absorbed) was added to each well, and the plates were incubated and washed as before. Each well was then filled with 200 μl of denaturing buffer (0.02 M-sodium phosphate buffer containing 0.02 M-EDTA pH 6.5). Plates were incubated at 95 °C on a water bath for 10 min, then individually chilled on ice.

The denatured virus samples were spotted onto nitrocellulose prewetted with 20 \times SSC (0.45 μm , S&S NC, Schleicher & Schuell)

through a HybriDot manifold (BRL), and washed once with 100 µl 10 × SSC per well. The nitrocellulose membrane was baked at 80 °C under vacuum for 2 h. Prehybridization and hybridization procedures were as described previously (Barbara *et al.*, 1987; Wen, 1990).

Homologous and heterologous ELISAs. 'Homologous ELISA', the standard ELISA (Clark *et al.*, 1986), is so called in this paper to distinguish it from heterologous ELISA (also called two-site ELISA if only MAbs were used). In heterologous ELISA, the trapping (coating) antibodies and detecting antibodies (or conjugates) detect different epitopes which are normally located on virions of different viruses (Creamer & Falk, 1990; Hu *et al.*, 1988). Thus, in direct heterologous ELISA, virions in extracts from a mixed infection were trapped with a MAb specific to one virus, and then detected with a conjugate (anti-virus polyclonal antibodies conjugated with alkaline phosphatase) specific to the other virus. In indirect heterologous ELISA, virions were trapped with an MAb (raised in a mouse) specific to an epitope on one virus, and then a second MAb (rat) specific to an epitope on the other virus was added to react with the trapped virions. The second MAb was detected by goat anti-rat antibodies conjugated with alkaline phosphatase (mouse-absorbed) (BRL). The general procedures for direct and indirect ELISAs were as described by Clark *et al.* (1986). The reaction for a sample was considered as positive if the ELISA value (A_{405}) for the sample was at least twice the ELISA value for healthy plant extracts.

Results

Antigenic mixing

In heterologous ELISA, extracts from plants doubly infected with P-PAV and NY-MAV-PS1 showed positive reactions, but extracts from plants singly infected with P-PAV or NY-MAV-PS1 showed negative reactions (Table 1). The results were consistent in two trials in which either MAV-specific or PAV-specific MAbs were used as trapping antibodies. They indicated that some of the virions in doubly infected plants were antigenically mixed virions.

Similarly, antigenic mixing was also found in plants

doubly infected with NY-RPV and NY-RMV. The NY-RMV conjugate (antibodies conjugated with alkaline phosphatase) used did not react with virions trapped by NY-RPV-specific antibody from plants inoculated with NY-RPV alone, but it reacted with virions trapped by NY-RPV-specific antibody from extracts of doubly infected plants (Table 2). The reciprocal experiment was not done, as NY-RPV conjugate cross-reacts slightly with NY-RMV.

Antigenic mixing was not detected in mixed infections with NY-RPV and P-PAV (Table 3), nor in mixed infections with NY-RPV and NY-MAV-PS1, NY-RMV and NY-MAV-PS1, and NY-RMV and P-PAV (data not shown).

Heterologous encapsidation of viruses in mixed infections

(i) Heterologous encapsidation between isolates in group 1 and isolates in group 2

In immunohybridization assays, NY-MAV-PS1 RNA was readily detected in virions trapped by NY-RPV-specific MAb (RPV-1) from mixed infections with the two viruses, but in contrast, RNA of NY-RPV could not be detected in virions trapped by NY-MAV-PS1-specific MAb (MAV-1) (Fig. 1). These results demonstrate that in addition to the encapsidation of RNAs of NY-RPV and NY-MAV-PS1 in their homologous capsid proteins, heterologous encapsidation also occurred in the mixed infections in one direction, i.e. RNA of NY-MAV-PS1 could be encapsidated in the capsid of NY-RPV, but NY-RPV RNA was not encapsidated in the protein of NY-MAV-PS1.

One-way heterologous encapsidation was also found between NY-RPV and P-PAV in mixed infections. The RNAs of P-PAV were detected in virions trapped by NY-RPV-specific MAb RRV-1, but the RNAs of NY-RPV

Table 1. Detection of antigenically mixed virions by indirect ELISA in extracts from oat plants doubly or singly infected with P-PAV and NY-MAV-PS1 isolates of BYDV

MAb*		Mean ELISA values (\pm S.D.) for extracts from plants infected with viruses indicated†				
Coating	Detecting	PAV	MAV	PAV/MAV	D	None (control)
Heterologous ELISA						
MAV-4 (MAV)	MAC91 (PAV)	0.042 \pm 0.006	0.038 \pm 0.003	0.040 \pm 0.008	0.192 \pm 0.003	0.032 \pm 0.002
MAV-1 (MAV)	MAC91 (PAV)	0.032 \pm 0.002	0.032 \pm 0.004	0.035 \pm 0.005	0.090 \pm 0.009	0.032 \pm 0.002
MAV-2 (PAV)	MAFF-2 (MAV)	0.042 \pm 0.007	0.040 \pm 0.003	0.039 \pm 0.008	0.285 \pm 0.081	0.034 \pm 0.002
MAV-3 (PAV)	MAFF-2 (MAV)	0.041 \pm 0.006	0.040 \pm 0.006	0.046 \pm 0.007	0.375 \pm 0.086	0.033 \pm 0.003
Homologous ELISA						
MAV-1 (MAV)	MAFF-2 (MAV)	0.046 \pm 0.004	1.087 \pm 0.135	0.981 \pm 0.122	0.884 \pm 0.147	0.040 \pm 0.003
MAV-3 (PAV)	MAC91 (PAV)	1.150 \pm 0.112	0.041 \pm 0.004	1.015 \pm 0.139	1.096 \pm 0.195	0.036 \pm 0.003

* Coating antibodies were mouse anti-virus, detecting antibodies were rat anti-virus and the third antibodies (conjugates) were goat anti-rat IgG conjugated with alkaline phosphatase (mouse-absorbed) which reacted with the detecting antibodies. The virus specifically detected by each MAb is listed in parentheses after the antibody.

† MAV, NY-MAV-PS1; PAV, P-PAV; PAV/MAV, mixture of extracts from plants singly infected with P-PAV or NY-MAV-PS1; D, extracts from plants infected with both P-PAV and NY-MAV-PS1.

Table 2. Detection of antigenically mixed virions by direct ELISA in extracts from oat plants doubly or singly infected with NY-RPV and NY-RMV isolates of BYDV

Specificity of antibodies*		Mean ELISA values (\pm s.d.) for extracts from plants infected with viruses indicated†				
Coating	Detecting	RPV	RMV	RMV/RPV	D	None (control)
RPV	RMV	0.033 \pm 0.005	0.035 \pm 0.005	0.027 \pm 0.004	0.115 \pm 0.016	0.033 \pm 0.005
RMV	RMV	0.051 \pm 0.010	0.638 \pm 0.061	0.431 \pm 0.065	0.568 \pm 0.095	0.047 \pm 0.004
RPV	RPV	1.580 \pm 0.062	0.038 \pm 0.004	1.409 \pm 0.104	1.525 \pm 0.123	0.039 \pm 0.007

* MAbs RPV-1 and RMV-1 were used to trap NY-RPV and NY-RMV, respectively. Detecting antibodies were rabbit anti-virus polyclonal antibodies conjugated with alkaline phosphatase.

† RPV, NY-RPV; RMV, NY-RMV; RMV/RPV, mixture of extracts from plants singly infected with NY-RPV or NY-RMV; D, extracts from plants infected with both NY-RPV and NY-RMV.

Table 3. Detection of antigenically mixed virions by direct ELISA in extracts from oat plants doubly or singly infected with NY-RPV and P-PAV isolates of BYDV

Specificity of antibodies*		Mean ELISA values (\pm s.d.) for extracts from plants infected with viruses indicated†				
Trapping	Detecting	PAV	RPV	PAV/RPV	D	None (control)
RPV	PAV	0.030 \pm 0.003	0.023 \pm 0.004	0.033 \pm 0.007	0.022 \pm 0.004	0.024 \pm 0.005
RPV	RPV	0.037 \pm 0.003	0.702 \pm 0.027	0.672 \pm 0.129	0.605 \pm 0.108	0.030 \pm 0.004
PAV	RPV	0.045 \pm 0.004	0.046 \pm 0.007	0.045 \pm 0.010	0.041 \pm 0.004	0.041 \pm 0.006
PAV	PAV	0.803 \pm 0.157	0.044 \pm 0.006	0.795 \pm 0.117	0.708 \pm 0.164	0.039 \pm 0.004

* MAbs RPV-1 and MAV-3 were used to trap NY-RPV and P-PAV, respectively. Detecting antibodies were rabbit anti-virus polyclonal antibodies conjugated with alkaline phosphatase.

† PAV, P-PAV; RPV, NY-RPV; PAV/RPV, mixture of extracts from plants singly infected with P-PAV or NY-RPV; D, extracts from plants infected with both P-PAV and NY-RPV.

were not detected in virions trapped by the P-PAV-specific antibody (MAV-3) (Fig. 2). Similar one-way heterologous encapsidations were also obtained in mixed infections of NY-RPV with P-PAV or NY-MAV-PS1 when there was a 9-day interval between inoculations with the paired viruses, regardless of the order of inoculation.

Since a cDNA probe was not available for NY-RMV, heterologous encapsidation between NY-RMV and various BYDV isolates was tested only in one direction, i.e. the MAb RMV-1 was used to trap virions, and a cDNA probe specific for the other isolate was used to detect RNA. Heterologous encapsidation occurred between NY-RMV and NY-MAV-PS1 (Fig. 3). However, heterologous encapsidation was not detected between NY-RMV and P-PAV when the NY-RMV-specific MAb (RMV-1) was used to trap the virions from mixed infections, and either pPP26 or pPP26S4 was used as the cDNA probe (data not shown).

(ii) Heterologous encapsidation between isolates within group 1 or 2

In three trials, two-way (i.e. mutual) heterologous encapsidation was found between P-PAV and NY-

MAV-PS1, i.e. the RNAs of P-PAV and NY-MAV-PS1 could be encapsidated in virions trapped with MAbs MAV-1 (NY-MAV-PS1-specific) and MAV-3 (P-PAV-specific), respectively (Fig. 4). NY-MAV-PS1 RNAs were readily detected in the virions trapped with MAb MAV-3 in all three trials. P-PAV RNA was detected in two of the three trials if the virions had been trapped with MAb MAV-1, and in all three trials if the virions had been trapped with MAb MAV-4 (NY-MAV-PS1-specific).

Other experiments also showed that phenotypically mixed particles predominated among heterologously encapsidated particles in mixed infections of P-PAV and NY-MAV-PS1. When samples absorbed with MAb MAV-4 (NY-MAV-PS1-specific) were trapped with MAb MAV-3 (P-PAV-specific) and probed with cDNA probe pMP7 + S5 (NY-MAV-PS1-specific), the samples gave no hybridization signals (Fig. 5). Corresponding non-absorbed samples or the samples absorbed with MAb RPV-1 (NY-RPV-specific) showed positive reactions. Similar results were also obtained when the samples were absorbed with MAb MAV-3, followed by trapping with MAb MAV-4 and probing with labelled cDNA pPP26S4 (PAV-specific) (data not shown).

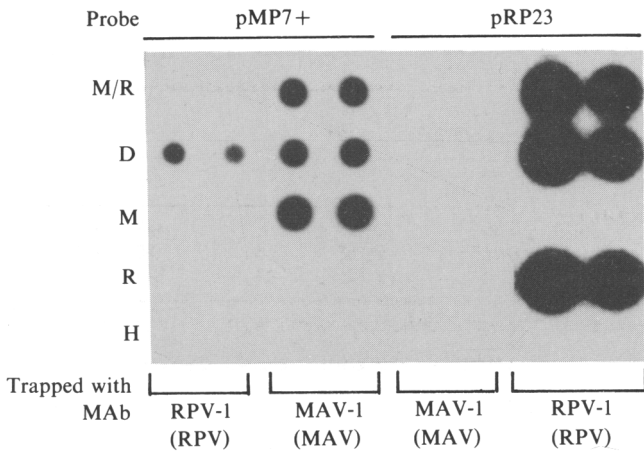


Fig. 1. Detection of viral RNAs of NY-RPV and NY-MAV-PS1 in virions trapped with MAbs RVPV-1 and MAV-1 from the extracts of plants doubly or singly infected with NY-RPV and/or NY-MAV-PS1. M/R, mixed extracts from plants singly infected with NY-MAV-PS1 or NY-RPV; D, extracts of plants infected with both NY-MAV-PS1 and NY-RPV; H, extracts of non-infected healthy plants. Viruses specifically detected by each MAb are indicated in parentheses under each MAb.

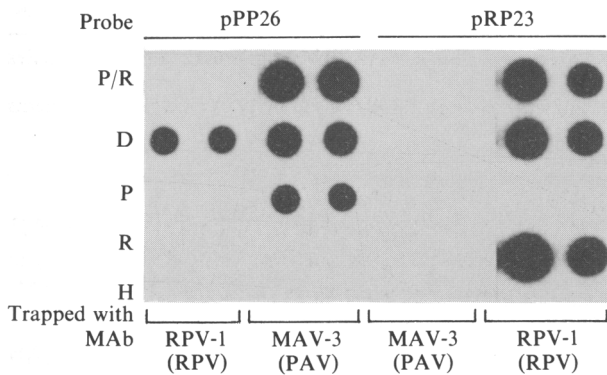


Fig. 2. Detection of viral RNAs of NY-RPV and P-PAV in virions trapped with MAbs RVPV-1 and MAV-3 from the extracts of plants doubly or singly infected with NY-RPV and/or P-PAV. P, extracts of plants infected with P-PAV; R, extracts of plants infected with NY-RPV; P/R, mixed extracts from plants singly infected with P-PAV or NY-RPV; D, extracts of plants infected with both P-PAV and NY-RPV; H, extracts of non-infected healthy plants. Viruses specifically detected by each MAb are indicated in parentheses under each MAb.

Evidence of heterologous encapsidation involving some phenotypic mixing was also found in mixed infections of NY-RPV and NY-RMV (Fig. 6). NY-RPV RNA was detected in virions trapped with the MAb RMV-1 (NY-RMV-specific) from samples doubly infected with NY-RPV and NY-RMV by probing with cDNA pRP23. When the samples were absorbed with NY-RPV-specific antibody RVPV-1, the hybridization signal was greatly reduced, but did not disappear even in samples treated with a high concentration of antibody

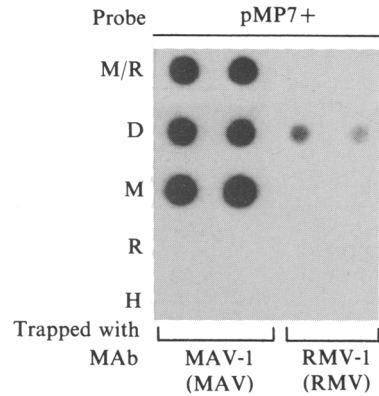


Fig. 3. Detection of NY-MAV-PS1 RNA in virions trapped with MAbs MAV-1 and RMV-1 from extracts of plants doubly or singly infected with NY-RMV and/or NY-MAV-PS1. M/R, mixed extracts from plants singly infected with NY-MAV-PS1 or NY-RMV; D, extracts of plants infected with both NY-RMV and NY-MAV-PS1; M, extracts of plants infected with NY-MAV-PS1; R, extracts of plants infected with NY-RMV; H, non-infected plants. Viruses specifically detected by each MAb are indicated in parentheses under each MAb.

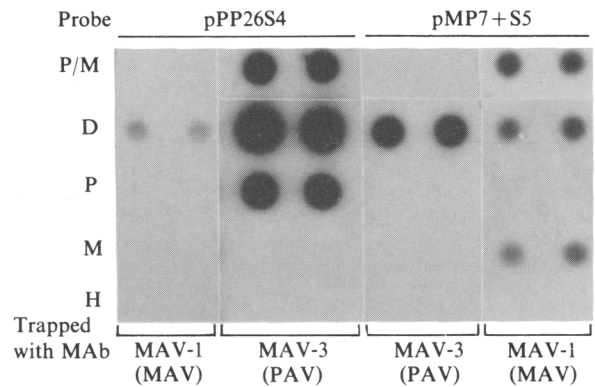


Fig. 4. Detection of viral RNAs of P-PAV and NY-MAV-PS1 in virions trapped with MAbs MAV-1 and MAV-3 from the extracts of plants doubly or singly infected with P-PAV and/or NY-MAV-PS1. P/M, mixed extracts from plants singly infected with P-PAV and NY-MAV-PS1; D, extracts of plants infected with both P-PAV and NY-MAV-PS1; P, extracts from P-PAV-infected plants; M, extracts from NY-MAV-PS1-infected plants; H, extracts of non-infected healthy plants. Extracts for each treatment were pooled from shoot extracts of five plants. Viruses specifically detected by MAb are indicated in parentheses under each MAb.

(100 µl of 85 mg/ml MAb was added in 1 ml of sample) (Fig. 6).

Discussion

This study provides direct evidence of one-way heterologous encapsidation in mixed infections with the NY-RPV and P-PAV isolates or with the NY-RPV and NY-MAV-PS1 isolates. Some of the viral RNA of either P-PAV or NY-MAV-PS1 was encapsidated in the protein

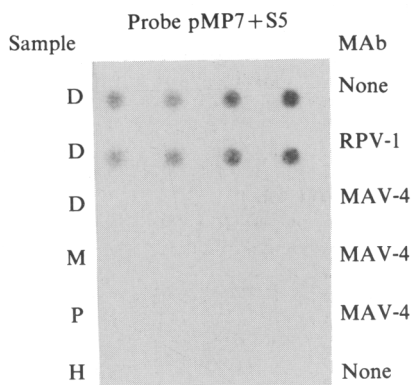


Fig. 5. Detection of phenotypic mixing between P-PAV and NY-MAV-PS1 by cross-absorbing the samples with various MABs, followed by trapping with MAB MAV-3 and probing with cDNA probe pMP7+S5. MABs listed in the figure were used to cross-absorb the various samples. MAB MAV-4 is MAV-specific, MAB MAV-3 is PAV-specific. Non-absorbed samples and MAB RPV-1 (RPV-specific)-absorbed samples were used as controls. D, extracts of plants infected with both P-PAV and NY-MAV-PS1; P and M, extracts of plants singly infected with P-PAV and NY-MAV-PS1, respectively; H, healthy plants.

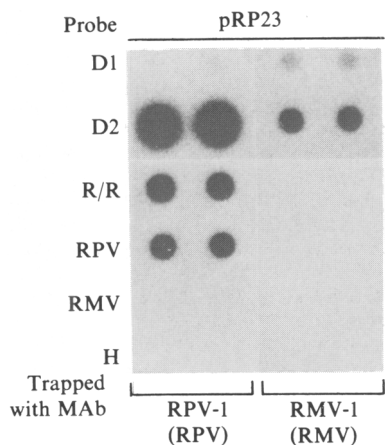


Fig. 6. Detection of heterologous encapsidation between NY-RPV and NY-RMV in mixed infections. D1 and D2, extracts of plants infected with both NY-RPV and NY-RMV; R/R, mixed extracts from plants singly infected with NY-RPV and NY-RMV; RPV, extracts of plants singly infected with NY-RPV; RMV, extracts of plants singly infected with NY-RMV; H, non-infected plants. The D1 sample had been cross-absorbed with MAb RPV-1 (8.5 mg/ml) before being trapped with MABs RPV-1 and RMV-1. Viruses specifically detected by each MAB are indicated in parentheses under each MAB.

capsid of NY-RPV if P-PAV or NY-MAV-PS1 replicated simultaneously with NY-RPV in doubly infected plants. However, as in the recent work of Creamer & Falk (1990), there was no evidence of heterologous encapsidation of NY-RPV RNA in the protein capsids of P-PAV or NY-MAV-PS1. Our study also provides the first direct evidence of two-way (mutual) heterologous encapsidation between P-PAV and NY-MAV-PS1. It also provides direct evidence of heterologous encapsida-

tion between NY-RPV and NY-RMV and between NY-MAV-PS1 and NY-RMV.

It has been suggested that heterologous encapsidation in mixed infections of viruses is the basis of dependent transmission of different BYDV isolates (Rochow, 1977). Dependent transmission among BYDV isolates was intensively studied by Rochow using aphid transmission and serological analysis, and was shown to occur in six of the eight combinations of paired viruses tested (Rochow, 1982). Occasionally, only one of the viruses in a mixed infection is able to serve as a helper virus, while the other behaves as a dependent virus, suggesting that in some combinations heterologous encapsidation may be possible in one direction only. Such one-way heterologous encapsidation was confirmed in the present study between NY-RPV and P-PAV and between NY-RPV and NY-MAV-PS1. Although recent aphid transmission work indicates that two-way heterologous encapsidation may also occur between RPV and PAV isolates, this was not confirmed by the immunohybridization assay (Creamer & Falk, 1990). Apart from the two-way heterologous encapsidation between P-PAV and NY-MAV-PS1 demonstrated in our study, the aphid transmission studies by Rochow suggest that two-way heterologous encapsidation also occurs between NY-RPV and NY-RMV isolates of BYDV (Rochow, 1982). In both systems, the two BYDV isolates involved in mutual heterologous encapsidation are serologically related and otherwise interrelated to the extent that they are grouped together: P-PAV and NY-MAV-PS1 in group 1, and NY-RPV and NY-RMV in group 2 (Rochow, 1970a; Waterhouse *et al.*, 1988). Taken together, these results therefore suggest that one-way heterologous encapsidation may be more common between distantly related BYDV isolates, whereas two-way heterologous encapsidation occurs more frequently between more closely related isolates. However, this generalization should be tested further among various interactions of paired isolates representing the different BYDV serotypes.

Heterologous encapsidation can involve either encapsidation of the RNA of one virus in the homogeneous protein capsid of the other virus (transcapsidation), or encapsidation of the RNA of one virus in a chimeric protein capsid containing protein subunits from both viruses, thus resulting in antigenically mixed virions (phenotypic mixing). Chimeric protein capsids were not detected as a result of interactions between NY-RPV and P-PAV, NY-RPV and NY-MAV-PS1, or NY-RMV and NY-MAV-PS1 in mixed infections. The predominant type of heterologous encapsidation in mixed infections of these pairs of viruses was apparently transcapsidation. By contrast, extensive phenotypic mixing occurred during virion assembly in mixed

infections of P-PAV and NY-MAV-PS1, and NY-RPV and NY-RMV (Tables 1 and 2), as shown by analysis using immunoprecipitation and immunohybridization. Transcapsidation may also be involved, especially in mixed infections of NY-RPV and NY-RMV (Fig. 6). It was also noticed that, although both MAbs MAV-1 and MAV-4 could be used as trapping antibodies in heterologous ELISA and immunohybridization, MAV-4 trapped relatively more antigenically mixed virions than did MAV-1, indicating that the phenotypically mixed virions contained more MAV-4-specific epitopes than MAV-1-specific epitopes, or MAV-4 had higher avidity than MAV-1 in reacting with its specific epitopes.

The work described here reveals interesting parallels between the ability of strains of BYDV to cross-protect against each other (Wen *et al.*, 1991) and their capacity for different types of heterologous encapsidation. No heterologous encapsidation or only transcapsidation was observed in mixed infections of distantly related isolates which had previously shown no cross-protection against each other. On the other hand, phenotypic mixing occurred between closely related isolates, which also cross-protect against each other.

In at least some cases, cross-protection may be due to the prevention of RNA uncoating (Sherwood, 1987), a process that may involve a 'cotranslational disassembly' (Shaw *et al.*, 1986; Wilson & Shaw, 1985; Roenhorst *et al.*, 1989), i.e. the sequential stripping of coat protein subunits in synchrony with RNA translation. Exogenous viral coat protein inhibits cotranslational disassembly in tobacco mosaic virus (Wilson & Watkins, 1986), and appropriate coat protein subunits already present in a cell infected with homologous or closely related virus may do the same. If so, the efficiency of protection between virus strains could be influenced by capsid subunit structural characteristics that also govern phenotypic mixing. Evidence against this, however, is that some degree of phenotypic mixing was found in mixed infections of NY-RPV and NY-RMV, though no cross-protection was observed in earlier work (Wen *et al.*, 1991). Cross-protection and phenotypic mixing appear to exhibit some parallels, but are constrained by different features of relatedness.

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