In Situ Localization of Barley Yellow Dwarf Virus-PAV 17-kDa Protein and Nucleic Acids in Oats

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ABSTRACT

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Barley yellow dwarf virus strain PAV (BYDV-PAV) RNA and the 17-kDa protein were localized in BYDV-PAV-infected oat cells using in situ hybridization and in situ immunolocalization assays, respectively. The in situ hybridization assay showed labeling of filamentous material in the nucleus, cytoplasm, and virus-induced vesicles with both sense and antisense nucleic acid probes, suggesting that the filamentous material found in BYDV-PAV-infected cells contains viral RNA. BYDV-PAV negative-strand RNA was detected before virus particles were observed, which indicates that RNA replication is initiated before synthesis of viral coat protein in the cyto-

plasm. The 17-kDa protein was associated with filamentous material in the cytoplasm, nucleus, and virus-induced vesicles. The labeling densities observed using antibodies against the 17-kDa protein were similar in the nucleus and cytoplasm. No labeling of the 17-kDa protein was observed in plasmodesmata, but filaments in the nuclear pores occasionally were labeled. Since BYDV-PAV RNA and 17-kDa protein colocalized within infected cells, it is possible that single-stranded viral RNA is always associated with the 17-kDa protein in vivo. The 17-kDa protein may be required for viral nucleic acid filaments to traverse the nuclear membrane or other membrane systems.

Additional keywords: electron microscopy, luteovirus.

Barley yellow dwarf viruses (BYDVs) are members of the plant virus family Luteoviridae. They are obligately transmitted from infected to healthy plants by aphid vectors. Viruliferous aphids inject the virus into the phloem of healthy plants during the feeding process (8-10), and the viruses remain restricted to the phloem tissues (6,17). The cytopathological changes induced by BYDV strain PAV in phloem tissues have been divided into three phases: early, intermediate, and late (11-13,17). At the beginning of the early phase, densely staining material appears in the plasmodesmata, followed by an electron-opaque substance and filaments, either free or within single-membrane-bound vesicles, in the host cytoplasm. At the end of the early phase, filaments are visible in the nuclear pores. During the intermediate phase of infection, the nuclear outline becomes distorted and massive clumping of heterochromatin begins. Only in the late phase can viral particles be seen in the cytoplasm and later in the nucleus, after much of the nuclear membrane has disintegrated. Based on the length and diameter of the filaments, Gill and Chong (13) suggested that the filaments seen in the early phase contain viral RNA. However, they were unable to remove the filaments from their preparations by RNase treatment and suggested that the viral nucleic acids might be protected from RNase digestion by viral or host proteins. Filamentous materials have been observed in association with virus-induced vesicular structures in the cytoplasm of host cells infected with viruses of other taxonomic groups. In several cases, viral replication has been associated with these structures (5,14,19,20).

The protein product of the luteovirus open reading frame 4 (ORF 4), a 17-kDa protein, has been proposed to be involved in several different events in the BYDV life cycle. ORF 4 is nested entirely within ORF 3, which encodes the 22-kDa viral coat protein (CP) (22). The 17-kDa protein has been cited as potentially functioning as a genome-linked viral protein (VPg) (23,25). However, recent results demonstrating that the 17-kDa proteins of beet western yellows virus (BWYV) (30) and BYDV (24) are not required for replication suggest that the VPg hypothesis is probably incorrect. Chay et al. (3) have shown that while BYDV mutants do not express the 17-kDa protein replicate well in isolated protoplasts, they fail to infect whole plants, suggesting that the 17-kDa protein may have a role in the movement of BYDV within the phloem.

Until recently, the long-distance movement of phloem-limited viruses like luteoviruses and geminiviruses was thought to be entirely passive and independent of viral gene expression (1,15). However, geminiviruses express two proteins that are required for long-distance movement and for movement of single-stranded viral DNA into and out of the nuclei (27,28). The 17-kDa protein of potato leaf roll virus (PLRV) has been shown to bind nonspecifically to single-stranded (ss) RNA and DNA in vitro (35) and to be associated with plasmodesmata in PLRV-infected plants (31). Tacke et al. (35) suggested that the 17-kDa protein might serve to stabilize viral nucleic acids in vivo and, hence, may be responsible, at least in part, for the RNase insensitivity observed by Gill and Chong (13). These features give the product of the luteovirus ORF 4 many of the characteristics associated with movement proteins like the tobacco mosaic virus 30-K protein, including the ability to bind single-stranded nucleic acids and to bind to plasmodesmata (4,37).

To determine if the filaments observed in infected oat cells contain BYDV RNA and to elucidate the association of the 17-kDa product of ORF 4 with BYDV RNA, virus-induced structures and host cell components, the intracellular distributions of BYDV-PAV

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minus- and plus-strand RNAs, and 17-kDa protein were determined in infected oat tissue using in situ hybridization and immunogold assays, respectively.

MATERIALS AND METHODS

Virus, vector, and hosts. An Illinois vector-nonspecific isolate of BYDV (PAV-IL) was used in these studies. Oats (*Avena byzantina* cv. Coast Black) were grown and inoculated with viruliferous aphids (*Rhopalosiphum padi* L.) as described previously (26).

Sample preparation for electron microscopy. Tissue samples for electron microscopy were prepared by conventional methods as described previously (26) or by freeze substitution. For freeze substitution, five tissue samples were collected from the midrib of inoculated primary leaves of infected plants and the corresponding leaves of noninoculated controls at 1, 2, 3, 4, 5, and 10 days post-inoculation (dpi). Leaf samples were sliced into 1-cm² pieces that were subsequently immersed in the primary fixative (3.5% paraformaldehyde, 0.5% glutaraldehyde, and 50 mM sodium cacodylate, pH 7.0). After approximately 1 h, 1-mm² pieces were cut and incubated in the primary fixative overnight at 4°C. Tissue pieces were transferred into cryoprotectant solution (1.8 M sucrose and 50 mM sodium cacodylate, pH 7.0) 2 h prior to freezing. Approximately 20 sections were transferred onto each copper grid (300 mesh, 3-mm; Electron Microscopy Sciences, Ft. Washington, PA), and cryoprotectant solution was carefully removed from the surface of the plant material and the copper grids. The copper grids were plunged rapidly into liquid nitrogen-cooled propane from which they were subsequently transferred into a metal basket within the liquid nitrogen reservoir. This process

was continued for all samples. At the end of this process, the baskets that contained the grids were transferred into a Reichert freeze substitution unit (Reichert, Vienna). The plant material was freeze-substituted with methanol that contained 1% osmium tetroxide for 72 h at -80°C, and then slowly brought up to room temperature at a rate of 3°C/h. Tissue pieces were then infiltrated with acetone and embedded in a Medcast/Spurr resin mixture (1:1; Ted Pella, Redding, CA). The resulting blocks were cured at 70°C for 20 h. Sections (80 nm) were cut with a Reichert Ultracut S ultramicrotome (Reichert) and used for in situ localization experiments.

Immunolocalization of BYDV-encoded proteins. Antiserum to BYDV-PAV particles has been described previously (26). To produce antiserum to the 17-kDa protein, a DNA segment corresponding to ORF 4 was amplified by polymerase chain reaction and inserted into pGEX-2T (Pharmacia Biotechnology, Inc., Piscataway, NJ) (33). ORF 4 was expressed in *Escherichia coli* as a fusion to glutathione-S-transferase, purified by affinity chromatography (33), and injected into New Zealand white rabbits to produce an antiserum specific for the 17-kDa protein. Antisera (diluted 1:100) were cross-adsorbed with sap of noninoculated oat plants and used to label 30 grids of BYDV-infected plant material from each sampling interval, as described previously (26).

Preparation of hybridization probes. A plasmid, pPAV410, containing a cDNA copy of BYDV-PAV-IL RNA between bacteriophage T3 and T7 promoters was used to transcribe strand-specific biotin-labeled probes for BYDV-PAV RNA (7). Transcription reactions using T3 RNA polymerase produced biotinylated probes specific for plus strands. Transcription reactions using T7 RNA polymerase produced biotinylated probes specific for minus strands. In vitro transcription reactions (50 µl) were incubated for 30 min at 37°C and contained 5 µg of cleaved pPAV 410 DNA; 40 mM Tris, pH 8.0; 8 mM MgCl₂; 2 mM spermidine; 50 mM NaCl; 30 mM dithiothreitol; 1 U of RNase-Block II (Stratagene, Inc., La Jolla, CA); 10 U of T3 or T7 RNA polymerase; and 1 mM each of ATP, CTP, GTP, and biotinylated UTP (Life Technologies, Inc., Gaithersburg, MD).

In situ hybridization. All steps were performed in autoclaved and baked glass petri dishes. To avoid evaporation of solutions at higher incubation temperatures, petri dishes were kept in a moist chamber. As in the immunolocalization studies, about 20 ultrathin sections were placed on each carbon-coated Formvar nickel grid. Sections were pretreated with protease K (10 mg/ml in 10 mM Tris-NaOH, pH 7.4, and 2 mM CaCl₂) for 60 min at 37°C, and then incubated for 20 min at 42°C on 50-µl droplets of prehybridization solution (50% formamide, 6× SSPE [20× SSPE is 3.0 M NaCl, 0.2 M sodium phosphate, and 0.02 M EDTA, pH 7.4], 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, and 200 µg of yeast RNA per ml). The temperature was kept constant using a water bath. Grids were then transferred onto 50-µl droplets of hybridization solution (1 ng of biotinylated minus- or plus-strand in vitro transcript probe in 500 µl of prehybridization solution) and allowed to hybridize at 42°C. After 5 h, the following steps were all performed at room temperature. Grids were removed and washed four times for 5 min on 25-µl droplets of 5× SSC (20× SSC is 3.0 M NaCl and 0.3 M sodium citrate, pH 7.0), once for 15 min on 0.1× SSC, and three times for 5 min on 2× SSC. Nonspecific binding sites were then blocked with 3% bovine serum albumin in TBS-Tween (100 mM Tris-NaOH, 150 mM NaCl, and 0.05% Tween-20, pH 7.5) for 5 min. Hybridized DNA probes were detected with goat anti-biotin antibodies conjugated to 10-nm-diameter gold particles (Ted Pella, Redding, CA) at a 1:25 dilution in TBS-Tween. After 30 min, sections were washed twice in droplets of TBS-Tween and twice in distilled water. Sections were stained for 60 min on droplets of 4% aqueous uranyl acetate and viewed with an Hitachi H-600 transmission electron microscope (Nissei Sangyo America, Ltd., Mountainview, CA) at 75 kV. Thirty grids were analyzed from each sample.

Fig. 1. The presence of filaments was a common cytopathological change in barley yellow dwarf virus strain PAV-infected oat phloem cells 2 to 4 days post-inoculation. Filaments (F) were typically seen first in the cytoplasm (Cy) and later in the nucleus (Nu) and nuclear pores (NP). Large areas of condensed filaments (CF) were often seen in the cytoplasm during later stages of infection. The 17-kDa protein was detected (arrows indicates gold label) in association with filaments first in the nucleus and later in the cytoplasm of infected cells. $\times 42,000$.

RESULTS

Ultrastructure. BYDV-PAV-induced cytopathological changes similar to those reported by others (11–13,17) were observed in this study. Filamentous material was first seen in the cytoplasm of infected phloem parenchyma and phloem companion cells as early as 1 dpi. Typically, filaments were abundant in the cytoplasm at 3 to 4 dpi, where they often formed circular aggregates with diameters of up to several hundred nanometers (Fig. 1). These aggregates will be referred to as condensed filamentous material. Filaments at the outer edges of these aggregates were frequently associated with ribosomes and virus particles. In only 1 out of over 30 samples were large amounts of filaments observed in the nucleus at 1 dpi when no filaments were visible in the cytoplasm of infected cells. In all other samples, filaments were observed in the nucleus and in the nuclear pores at 2 to 4 dpi. At late stages of infection (>5 dpi), cells were often completely filled with virus particles and condensed filamentous material.

Localization of 17-kDa protein. Using an antiserum raised against recombinant 17-kDa protein, gold labeling was observed

TABLE 1. Label densities for the 17- and 22-kDa proteins of barley yellow dwarf virus strain PAV-IL associated with filaments in the nucleus and cytoplasm and condensed filaments in the cytoplasm

	Nucleus	Cytoplasm	
	Filaments	Filaments	Condensed filaments
17 kDa	7.7 ^a (4.4) ^b	8.8 (4.9)	3.6 (0.49)
22 kDa	1.4 (1.1)	5.1 (3.6)	0.0
Control	0.0	0.0	0.0

^a Gold particles/square micrometer; values are averages from three experiments in which five independent micrographs, each containing four to five cells, were examined.

^b Values in parentheses are standard deviations.

Fig. 3. Filaments (F) were observed in nuclear pores (NP) of barley yellow dwarf virus strain PAV-infected oat phloem cells 2 to 4 days postinoculation. The 17-kDa protein (arrow indicates gold label) was occasionally associated with these filaments. Cy = cytoplasm and Nu = nucleus. ×138,000.

Fig. 2. Immunolocalization of the barley yellow dwarf virus strain PAV 17-kDa protein 2 to 4 days postinoculation. **A**, In nuclei of infected oat phloem cells, the 17-kDa protein (arrowhead indicates gold label) was commonly associated with filaments (arrow) in the nuclear euchromatin (Eu). Hc = heterochromatin. **B**, In the cytoplasm (Cy), the 17-kDa protein (arrowheads indicate gold label) was most often associated with intermediate regions of filaments (arrow) and only rarely associated with filament termini. Nu = nucleus. **A**, ×49,000. **B**, ×48,000.

during all stages of the infection cycle. Before cross-adsorbing the 17-kDa antiserum, some nonspecific labeling was observed over cell walls. After cross-adsorption, the polyclonal antibodies specifically labeled only infected plant samples. The 17-kDa protein was observed as early as 1 dpi in nuclei in association with euchromatin in infected cells (Fig. 2A). When the 17-kDa antiserum labeled filamentous material, gold particles were sometimes bound to one terminus of the filaments, but more often were found along the length of the filaments (Fig. 2B). Labeling with the 17-kDa antiserum was dependent on the presence of filaments and was detected in both the cytoplasm and the nucleus at 2 to 4 dpi (Fig. 1). The 17-kDa protein occasionally was associated with filaments seen within nuclear pores (Fig. 3). Areas of condensed filamentous material often were labeled (Fig. 1). However, the label densities were higher over areas that contained free filaments and were higher than those produced using antibodies specific for virus particles (Table 1). Gold-label densities for the 17-kDa protein were similar in the nucleus and the cytoplasm (Table 1). Labeling densities with the 17-kDa antiserum was lower in the cytoplasm in areas

with condensed filaments. The 17-kDa protein was rarely associated with viral particles. In BYDV-infected cells at the late stage of infection, the condensed filamentous material was labeled (Fig. 4). The 17-kDa protein was never seen associated with oat phloem plasmodesmata.

In situ hybridization. Both plus- and minus-strands of BYDV-PAV RNA were detected throughout the infection cycle. Protease pretreatment was essential for successful in situ localization of viral nucleic acids (data not shown). Nonspecific labeling occasionally was seen in control samples over cell walls, mitochondria, and nuclear heterochromatin. In contrast, specific labeling was most often associated with filamentous material in the cytoplasm (Fig. 5), nucleus (Fig. 6), and virus-induced vesicles (Fig. 7). Labeling often occurred as rows of three or more gold particles (Fig. 5). Minus-strand RNA was detected first in the nucleus and slightly later in the cytoplasm (Fig. 6). Gold labeling from RNA probes was associated with filamentous material that could easily be discerned in conventionally fixed sections; for example, in vesicles (Fig. 7). Labeling was abundant in freeze-substituted sections, but filaments

Fig. 4. In the late stage of barley yellow dwarf virus strain PAV infection in oat phloem cells (5 to 10 days postinoculation), the 17-kDa protein (arrow indicates gold label) was associated with condensed filaments (CF). Cells contained mainly degenerated organelles and viral particles (V). CW = cell wall. $\times 42,500$.

were difficult to distinguish, so that the association of gold label with the filaments was not as easy to confirm (Fig. 8). During the late stage of infection (>5 dpi), areas that contained large amounts of condensed filaments were labeled with both viral RNA probes (Fig. 9). In contrast, areas of cells in the late stage that contained large numbers of virus particles contained predominantly plus-strand RNA (Fig. 10).

DISCUSSION

In this study, we localized the 17-kDa protein and RNA of BYDV-PAV in infected oat cells. Both were associated with filamentous material in the cytoplasm and the nucleus. Because the BYDV-PAV biotin-label RNA probes specifically bound to the filamentous material in the *in situ* hybridization assay, we suggest that the filamentous material contains BYDV RNA. Nonspecific labeling in noninfected control samples was rare, further confirming the specificity of the assay. However, *in situ* hybridization with BYDV-PAV probes was successful only after pretreatment of the ultrathin sections with protease K, which suggests that viral RNA is associated with protein, possibly the 17-kDa protein, BYDV CP, host cellular proteins, or a combination of these proteins. This result is consistent with the observations of Gill and Chong (11–13), who were unable to remove the filaments by RNase treatment and suggested that proteins bound to the viral nucleic acids might prevent RNA digestion.

Although filaments appeared typically in the cytoplasm before the nucleus, one sample showed abundant labeling of filaments in the nucleus, and filaments were absent from the cytoplasm. It is possible that, in this case, filament clusters in the cytoplasm were in an area that was not sectioned or accessible for viewing. Phloem cells are relatively long (several hundred micrometers) and filaments in the cytoplasm may have been absent in the few sections

examined. In all other samples, filaments were seen only in the cytoplasm or in both the nucleus and cytoplasm.

The site of viral RNA replication could not be unequivocally determined, because localization of neither double-stranded (ds)

Fig. 6. *In situ* localization of barley yellow dwarf virus strain PAV plus-strand RNA in the nucleus of infected oat phloem cells at the early stage of infection (1 to 2 days postinoculation). Labeling (arrows) at the early stage was sparse, but often observed first in euchromatic regions (Eu) of the nucleus (Nu) and later in the cytoplasm (Cy). Hc = heterochromatin and Nm = nuclear membrane. $\times 85,000$.

Fig. 5. Localization of barley yellow dwarf virus strain PAV plus- and minus-strand RNA in infected oat phloem cells 3 days postinoculation by *in situ* hybridization. **A**, Plus-strand and **B**, minus-strand RNAs were both observed in the cytoplasm (Cy). Gold labeling often occurred in rows of three or more particles (arrows). **A**, $\times 122,400$. **B**, $\times 125,000$.

Fig. 7. In situ localization of barley yellow dwarf virus strain PAV plus- and minus-strand RNAs in conventionally fixed oat phloem cells 3 to 4 day postinoculation. **A**, Plus-strand and **B**, minus-strand RNAs (arrows indicate gold label) were often associated with filaments and sometimes seen in virus-induced vesicles (Ve). CW = cell wall. **A**, $\times 111,000$. **B**, $\times 111,000$.

Fig. 8. In situ localization of barley yellow dwarf virus (BYDV)-PAV RNA in freeze-substituted cells. Labeling with BYDV-PAV minus-strand probes (arrows) was abundant in freeze-substituted cells. However, filaments, especially within vesicles (Ve), were more difficult to discern than in conventionally fixed sections (Fig. 7). $\times 84,000$.

RNA nor viral replicase was undertaken. Negative-strand RNA was detected at early infection stages, first in the nucleus and slightly later in the cytoplasm. As reported previously (26), CP was not detected in infected cells until near the end of the intermediate stage of infection. Hence, RNA synthesis occurs before the accumulation of detectable amounts of CP and before virus particles are observed. The BYDV CP and 17-kDa protein are expressed from a subgenomic RNA that is thought to be transcribed from a negative-sense copy of the genomic RNA (22). The appearance of negative-sense RNA before CP and virus particles is consistent with this expression strategy. Minus- and plus-strand RNAs were associated with vesicular structures in the infected plant cells, and it is possible that virus-induced vesicles are involved in viral replication. In other RNA viruses, viral replication has been associated with membranous structures (2).

In previous analyses of the accumulation of plus- and minus-strand RNAs of plant viruses in protoplasts, minus-strand RNA synthesis was observed a few hours before plus-strand RNA accumulation was detected (16,18,21). Minus-strand RNA accumulation ceased relatively early in infection, and the amount of minus-strand RNAs began to decrease. At the same time, the levels of plus-strand RNA increased rapidly and became stable late in infection. The localization of BYDV plus- and minus-strand RNAs in this study mirrors these findings. Both RNA strands were detected early in infection (Figs. 5, 7, and 9), but, near the end of the late stage of infection, plus-strand RNA was abundant, while minus-strand RNA was difficult to detect (Fig. 10).

The 17-kDa protein was associated with filamentous material in the cytoplasm and the nucleus. The densities of labeling were not significantly different in the nucleus and cytoplasm, unlike the labeling of the BYDV-PAV CP, which was observed primarily in the cytoplasm (26). Free filaments and groups of filaments in the nuclear pores were labeled occasionally by the 17-kDa antiserum. Slightly lower labeling densities were observed over the condensed

Fig. 9. In situ localization of barley yellow dwarf virus strain PAV RNA late in infection of oat phloem cells (>5 days postinoculation). **A**, Plus-strand and **B**, minus-strand RNAs (arrows indicated gold label) were often associated with condensed filamentous material (CF). CW = cell wall and V = viral particles. **A**, $\times 28,000$. **B**, $\times 28,000$.

Fig. 10. In situ localization of barley yellow dwarf virus strain PAV RNA in the late stage of infection (>5 days postinoculation) in oat phloem cells. **A**, Plus-strand RNA (arrow indicates gold label) was commonly detected in cells that were mostly filled with viral particles (V) and degenerated organelles such as mitochondria (Mi). **B**, Minus-strand RNA (arrows indicate gold label) was rarely observed in cells at this stage. CW = cell wall. **A**, $\times 35,000$. **B**, $\times 35,000$.

filamentous material that was found most commonly at the late infection stages. The filaments in these areas may be degraded or may be composed predominantly of dsRNA, which does not bind the 17-kDa protein efficiently. Interactions between the antibodies and the 17-kDa protein in condensed filamentous material also may be sterically hindered. These results suggest that the filaments

consist of viral ssRNA that is associated with 17-kDa protein in vivo and are consistent with in vitro studies that reported that the 17-kDa protein of PLRV (pr17) possesses RNA-binding properties (35). It is possible that the 17-kDa protein is required for movement of viral RNA across the nuclear membrane. However, the significance of the nuclear localization is uncertain given the

observation that the 17-kDa protein is not required for replication of BYDV-PAV RNA (3,24).

The observation of the BYDV-PAV 17-kDa protein in the nucleus of infected cells agrees with the results of Tacke et al. (36), who found the PLRV 17-kDa protein associated with a subcellular fraction containing nuclei. Nuclear transport is usually a very selective process involving recognition of the protein by receptors in the nuclear pores and then the active transport of the protein across the nuclear membrane (32). The association of 17-kDa protein on both sides of the nuclear envelope with filaments that extend through nuclear pores suggests that the 17-kDa protein may contain amino acid sequences that are recognized as nuclear localization signals. The 22-kDa CP and the carboxyl terminus of the 17-kDa protein contain basic amino acids that usually form nuclear localization signals. However, CP is not found in the nucleus until a very late stage, when the nuclear membrane is beginning to disintegrate (26). The accumulation of the 17-kDa protein in the nucleus may be a by-product of its role in facilitating the movement of viral RNA across membranes. The observations of plus- and minus-sense BYDV-PAV RNA in association with membrane vesicles in the cytoplasm suggest that, like many RNA viruses, some steps of BYDV-PAV RNA replication occur in the cytoplasm. Other RNA viruses presumed to replicate in the cytoplasm (e.g., potyviruses) accumulate nonstructural proteins in the nucleus (29).

It had been proposed that the 17-kDa protein could function as a VPg (23,25). However, observations that mutations in the ORFs expressing the 17-kDa proteins of BWYV or BYDV-PAV did not affect replication of the viruses (3,24,30) do not support this hypothesis. Labeling of filaments with 17-kDa antibodies along their length, rather than only at termini, also fails to support the VPg hypothesis.

While luteoviruses and geminiviruses are capable of long-distance movement through vascular tissues, they usually cannot move laterally out of the vascular tissues. Schmitz et al. (31) localized pr17 in PLRV-infected and transgenic potato plants expressing the pr17 gene, which showed strong labeling of plasmodesmata (31) similar to that observed with the movement proteins of tobacco mosaic virus and alfalfa mosaic virus (34,37). In sharp contrast, no labeling of plasmodesmata was observed with anti-17-kDa antiserum in this study. It is possible that the association between the 17-kDa protein and plasmodesmata, if one exists, may be more transient in BYDV-PAV-infected oat cells. BYDV-PAV-infected cells, like PLRV-infected cells, show rapid degradation of cellular structures and organelles. The association of the BYDV-PAV 17-kDa protein with plasmodesmata may have been overlooked in these highly disrupted cells, but, given the large number of samples examined, this seems unlikely. The cytopathological changes induced by luteoviruses (including BYDV-PAV) and poleroviruses (including BYDV-RPV and PLRV) are similar, but consistently different. For example, no filaments are observed in the nuclear pores or nuclei of oat cells infected with BYDV-RPV until very late in infection (12). These differences in cytopathology and labeling patterns may reflect functional differences in the mechanisms by which the proteins mediate movement of virus nucleic acids within cells, among cells, or both.

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