Evidence for Receptor-Mediated Endocytosis Regulating Luteovirus Acquisition by Aphids

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ABSTRACT

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The ability of five cereal grain aphid species to acquire four vector-specific isolates of barley yellow dwarf luteoviruses was tested. Aphids from New York clones of *Rhopalosiphum maidis, R. padi, Schizaphis graminum,* and *Sitobion avenae* acquired the New York type isolates of MAV, PAV, RMV, and RPV when fed on infected plants, regardless of the ability of each species to transmit the virus. Aphids from a California clone of *Metopolophium dirhodum* acquired MAV, PAV, and RMV, but not RPV. When *R. padi* and *M. dirhodum* were fed on 200 μg/ml of purified RPV and examined by electron microscopy, virions of RPV were observed adsorbed to the hindgut plasmalemma and intracellularly in endocytotic vesicles only in *R. padi*. In *M. dirhodum*, RPV virions were observed free in the hindgut lumen but not adsorbed to gut membranes, and virus was not acquired. Immunolabeling was used to verify the ability of *R. padi*, but not *M. dirhodum*, to acquire RPV virions. When both species were fed on purified virions of brome mosaic virus and cowpea mosaic virus, virions were observed in high concentrations in the gut lumen but were not adsorbed to the gut membrane or incorporated into gut cells. Ultrastructural evidence of acquired virions adsorbed to the hindgut plasmalemma and in endocytotic vesicles implicates receptor-mediated endocytosis as the mechanism of luteovirus acquisition. This hypothesis is supported by evidence that virions of nonacquired viruses do not adsorb to the plasmalemma. Results suggest that recognition and adsorption of virions to the hindgut membrane is a selective step in acquisition of circulatively transmitted luteoviruses by vectors.

Additional keywords: coated vesicles, insect vectors, virus transmission.

The barley yellow dwarf luteoviruses are transmitted in a circulative nonpropagative manner by aphids. The circulative route of these isometric single-stranded RNA viruses through their aphid vectors has been described (4,5). Virions are ingested by aphids feeding on phloem tissues of infected host plants and are acquired into the aphid hemocoel through the hindgut (3). The acquired virions circulate suspended in the hemolymph, which serves as a reservoir until the virions contact the accessory salivary gland. Virions may then be transported through the accessory salivary

gland to the salivary duct and be excreted into host plants during aphid feeding (7).

Each luteovirus is transmitted by aphids of specific species. This vector-specificity has been especially well documented (15,17) for the barley yellow dwarf viruses (BYDV). The aphid salivary system has been implicated as the primary site regulating BYDV vector-specific transmission. Rochow demonstrated that when infectious BYDV preparations were injected into the hemocoel of nonvector species, the aphids remained unable to transmit the virus, suggesting that the salivary gland and not the gut was the site of specificity (15). This idea was later substantiated by visualization of virions of only transmissible BYDV isolates in salivary glands of a vector species (7). At this time, the accessory salivary gland plasmalemma is believed to be the major site where vector-specificity is determined.

Little is known concerning the role of the hindgut in regulating virus acquisition and ultimately in influencing vector-specificity. Rochow and Pang (18) demonstrated that a nonvector species could acquire a BYDV isolate that it was unable to transmit. When *Sitobion avenae* (Fabricius) were fed on the RPV isolate, which they do not transmit, RPV was recovered from the hemocoel of 47 of 81 aphids tested. When *Rhopalosiphum padi* (L.) were fed on the MAV isolate, which they do not transmit, virus was recovered from five of 81 aphids. Although the gut did not act as a barrier to acquisition, there was clearly a quantitative difference in the ability of the two aphid species to acquire the nonvectored BYDV isolates. In later studies, *S. avenae* were allowed to feed on MAV- or RPV-infected oats and then observed by electron microscopy. Virions of both MAV and RPV were observed associated with the accessory salivary gland. However, only the transmissible MAV isolate was observed to be transported into the gland and released into the salivary duct (7). Recently, when the reciprocal experiment was done with *R. padi*, only the transmissible RPV isolate was observed associated with the salivary gland (*unpublished*). The nontransmissible MAV isolate was not observed in the aphid. This suggested that *R. padi* was either unable to acquire MAV or that MAV was being acquired very inefficiently and at concentrations too dilute to accumulate at the salivary gland. These observations suggested that the hindgut might play a selective function influencing luteovirus transmission. Visualization of virions budding into the hindgut through the apical plasmalemma in coated pits and vesicles (3) suggests that a receptor-mediated endocytotic mechanism (9,23) for luteovirus recognition is probably involved in virus acquisition. Additional evidence was needed to verify this hypothesis.

The objectives of this research were to determine whether plant virus recognition at the hindgut is required for virus acquisition by feeding aphids, whether the hindgut plays a role in species-specific acquisition of luteoviruses, and to determine the site of any such selective mechanism.

MATERIALS AND METHODS

The four New York type-isolates of BYDV (16,21) originally obtained from W. F. Rochow (Ithaca, NY) were used. The MAV-NY isolate was transmitted specifically by *S. avenae* and *Metopolophium dirhodum* (Walker). The PAV-NY isolate was transmitted by *R. padi* and *S. avenae*. The RMV-NY isolate was transmitted by *R. maidis* (Fitch), and the RPV-NY isolate was transmitted by *R. padi*. Virus-free colonies of the New York clones (17) of *R. padi, R. maidis, S. avenae, Schizaphis graminum* (Rondani), and a California clone of *M. dirhodum* (8) were maintained on caged Barsoy barley, *Hordeum vulgare* (L.), in a controlled environment room at 20 C, with a 24-h photoperiod under fluorescent lighting.

Barley and oat plants to be used as virus source tissues for aphid feeding experiments were infested with viruliferous aphids 1 wk after planting, allowed a 3-day inoculation feeding, fumigated with DDVP (*O,O*-dimethyl-2,2-dichlorovinyl phosphate) to kill the aphids, and maintained in a greenhouse for 3 wk prior to use. Plants were tested, as previously described (8), by enzyme-linked immunosorbent assay (ELISA) to verify the infecting virus isolate identity. For virus acquisition tests, aphids of each of the five species were reared simultaneously on oats or barley infected with one of the BYDV isolates. Where possible, aphids of different species were reared on the same plants to minimize effects of virus concentration differences among plants. To begin a test, 10 apterous adults of each species were placed on virus source plants for 24 or 48 h and allowed to produce nymphs. The adult aphids were then removed, and the nymphs were allowed to feed on the plant for an additional 10 days. These aphids were then removed from the plants to test for virus acquisition.

To test for BYDV acquisition into the hemocoel, individual aphids were anesthetized with carbon dioxide gas flowing across the stage of a dissecting microscope, and one leg was removed with forceps. A drop of hemolymph formed on the severed limb that could be tested for its virus content. After collecting hemolymph for testing, the individual aphids were placed on 1-wk-old seedlings of Coastblack oats (*Avena byzantina* K. Koch) for a 5-day inoculation feeding to test for virus transmission. These seedlings were then fumigated and observed in the greenhouse over a 6-wk period for symptoms of virus infection.

Three methods were attempted to detect BYDV acquisition into the aphid hemocoel. In one test, 0.1-μl samples of hemolymph were collected in microcapillary tubes and diluted into 50 μl of ELISA sample buffer. These samples were then transferred to microtiter plate wells coated with the appropriate detecting antiserum and tested as previously described (8). A second method consisted of an immunospecific test (immunosorbent electron microscopy) done by placing the diluted hemolymph onto 300-mesh Formvar-carbon-coated grids coated with the appropriate detecting antiserum, incubating the grids for 30 min, rinsing for 30 s in water, and negative staining with uranyl acetate. Replicated grids from each aphid were then examined with a Phillips 300 transmission electron microscope for the presence of attached virions. A third test consisted of an injection-recovery bioassay. Hemolymph from the aphid to be tested (donor) was drawn into a microcapillary needle, and approximately 0.02 μl was injected into a third-instar nymph (recipient) of the appropriate efficient vector species for the BYDV isolate being used. Recipient aphids used to recover MAV, PAV, RMV, and RPV were *S. avenae, R. padi, R. maidis,* and *R. padi*, respectively. Two nymphs were injected with hemolymph from each individual donor aphid. These recipient aphids were then given a 5-day inoculation feeding on 1-wk-old Coastblack oat seedlings, which were then fumigated and observed for symptom development over a 4-wk period. These plants were then tested by ELISA to confirm infection.

Virions of viruses used for membrane feeding experiments were purified by powdering tissues in liquid nitrogen, homogenizing in 0.1 M phosphate buffer (pH 7.0), clarifying in chloroform/

TABLE 1. Recovery of barley yellow dwarf virus (BYDV) from hemolymph of each of five aphid species acquisition fed 10–14 days on oats or barley infected with one of four vector-specific BYDV isolates

Aphid species[a]	No. of aphids (of 60) from which virus isolate was recovered			
	MAV	PAV	RMV	RPV
Metopolophium dirhodum	14	4	6	0
Rhopalosiphum padi	20	26	8	20
Sitobion avenae	36	16	2	18
Schizaphis graminum	6	22	10	12
R. maidis	43	43	32	32

[a] Twenty aphids of each species were selected from each of three source plants infected with each BYDV isolate. *M. dirhodum*, *R. padi*, and *S. avenae* were reared simultaneously on the same Coastblack oat source plants. *Schizaphis graminum* was reared independently on oats. *R. maidis* was reared independently on infected Barsoy barley, because it would not survive and feed well on oats. BYDV was detected in hemolymph of acquisition-fed apterous adult aphids by injection-recovery bioassay (IRB). For IRBs, approximately 0.02 μl of hemolymph was recovered from each aphid and microinjected into each of two virus-free, third-instar nymphs of the appropriate efficient vector for each isolate. Injected aphids were allowed a 5-day inoculation feeding on 7-day old oat seedlings. After 4 wk, these plants were tested by ELISA to verify infection.

butanol (4:1), and centrifuging at low speed. Virions in the resulting supernatant were precipitated overnight in 6% polyethylene glycol in 0.15 M sodium chloride. The precipitate was resuspended in 0.01 M phosphate buffer and then pelleted at 114,000 *g* for 2 h. High-speed pellets were resuspended overnight in 0.01 M phosphate buffer and centrifuged through a 10–40% sucrose gradient at 72,000 *g* for 1.5 h. Gradients were fractionated, and absorbance peaks at 260 nm were collected, pelleted at 114,000 *g* for 3 h, resuspended in 0.01 M buffer, and observed by electron microscopy (EM) to verify virion content. Viruses and host plants used consisted of RPV-NY in Coastblack oats, brome mosaic virus (BMV) in Barsoy barley, and cowpea mosaic virus (CPMV) in cowpea (*Vigna unguiculata* L.).

To test for aphid acquisition of purified viruses, aphids were allowed to feed through stretched Parafilm membranes for 24 or 48 h on various concentrations of purified virions diluted in 20% sucrose in 0.01 M phosphate buffer. Following the membrane feeding, the aphids were given a 24-h feeding on healthy oats or barley before being fixed for EM. Methods for immunologically labeling virions in the aphid hemocoel and for fixing and preparing aphids for EM observations have been described (3,4,7).

RESULTS

In a preliminary experiment, virus-free *S. avenae* and *R. padi* were reared together for 2 wk on the same oat plants infected with either MAV or RPV, or on healthy plants as controls. Five aphids from each treatment were then sectioned for electron microscopy, and the accessory salivary glands were examined for an association with the virus on which the aphids had fed. Both the vectored MAV and nonvectored RPV were acquired by *S. avenae*. Virions of both isolates were observed embedded in the basal lamina surrounding the accessory salivary gland and adjacent to the basal plasmalemma in all five *S. avenae* examined from each treatment. Only in *S. avenae* fed on MAV, however, were virions observed intracellularly in association with the salivary canal. In contrast, when *R. padi* were examined, only virions of the transmissible RPV isolate were observed associated intracellularly in the accessory salivary gland in all five aphids examined. No virions of the nonvectored MAV isolate were observed associated with the salivary gland basal lamina in any of five *R. padi* examined. It was assumed that either aphids of this clone of *R. padi* did not acquire this isolate of MAV, or acquisition through the hindgut was very inefficient, and the concentration of MAV in the hemolymph was too dilute to be detectable at the salivary gland by EM (*data not shown*).

The above observations, together with ultrastructural studies (3) showing BYDV endocytosis into aphid hindgut cells by means of coated pits, suggested that the hindgut might play a selective role in regulating luteovirus acquisition into the hemocoel and ultimately affect virus transmission. To determine whether luteovirus recognition might be occurring at the hindgut, a series of experiments was done to test for BYDV acquisition into the hemolymph of vector and nonvector aphid species. In the first series of three tests, *S. avenae* and *R. padi* were reared together for 2 wk on MAV- or RPV-infected oats, and their hemolymph was tested for the presence of virus by the injection-recovery bioassay (IRB) technique. Attempts to utilize ELISA and immunospecific EM to detect BYDV in 0.1 μl of hemolymph were inconsistent when compared with the IRB. Therefore, the IRB technique was utilized for all additional tests. Considering the combined data from all three experiments involving 56 aphids per treatment, RPV was detected in 45% of the *R. padi* and 20% of the *S. avenae*. MAV was detected in 32% of the *R. padi* and in 71% of the *S. avenae*. These results verified the earlier work of Rochow and Pang (18) and indicated that these two aphid species could acquire into the hemocoel a BYDV isolate that they do not vector. The above results indicated that, if luteovirus recognition occurred at the hindgut, then it must be less specific than the type of recognition occurring at the accessory salivary gland.

In order to determine whether or not BYDV recognition at the hindgut might play a role in limiting acquisition of some BYDV isolates by some aphid species, a series of experiments was conducted to test for virus acquisition of four BYDV isolates by aphids of five species. Aphids of *R. padi, R. maidis, M. dirhodum, S. avenae,* and *Schizaphis graminum* were allowed to feed 2 wk on Coastblack oats or Barsoy barley (*R. maidis* only) infected with the RPV, RMV, MAV, or PAV isolates. Combined results of two separate experiments (Table 1) indicated that aphids of all five species were able to acquire into the hemocoel all four BYDV isolates, with one exception. RPV was not recovered from any of 60 *M. dirhodum* tested. By comparison, RPV was recovered from 30 and 20%, respectively, of *S. avenae* and *Schizaphis graminum* (nonvectors). No significance was placed on the apparent differences in virus recovery from other aphid species, because of limited sample size and limited replication.

To verify the inability of *M. dirhodum* to acquire RPV, a second experiment was designed to compare RPV acquisition between *R. padi* and *M. dirhodum* reared together for 2 wk on RPV-infected oats and barley. RPV was recovered from 35% of the *R. padi* fed on oats and from 60% of the *R. padi* fed on barley, but it was not detected in any of the *M. dirhodum* fed on either host plant (Table 2). This suggested that some type of recognition system must function at the aphid hindgut that regulated virus transport and acquisition.

To aid in determining a site and cellular mechanism for virus recognition, an ultrastructural study was designed to compare hindgut cell interactions with the RPV isolate in *R. padi* and *M. dirhodum*. In one treatment, aphids of both species were reared together for 2 wk on a 3-wk-old oat seedling infected with RPV. In a second treatment, aphids of both species were fed for 24 h on 200 μg/ml of purified RPV virions in 20% sucrose through Parafilm membranes. Following the acquisition feedings and prior to fixation for EM, some aphids from each treatment were injected with polyclonal RPV or MAV antiserum to immunolabel virions released into the hemocoel.

Preliminary light microscope observations of 0.5-μm thick sections, done to localize internal organs, indicated that the anatomy of *M. dirhodum* was similar to that described for other aphid species (3,4,7,13). Tissues examined for BYDV associations included the epithelial linings of the anterior midgut (stomach), the posterior midgut, and the hindgut (Fig. 1). The midgut tissues were characterized by single-celled epithelial layers, which ranged in thickness from 30–50 μm at the anterior midgut to 5–13 μm at the posterior midgut (Fig. 2) in second- and third-instar nymphs. The apical plasmalemma bordering the gut lumen was highly convoluted and formed a dense lining of microvilli extending into the gut lumen. In addition, the basal plasmalemma invagi-

TABLE 2. Results of injection-recovery bioassays to compare *Metopolophium dirhodum* and *Rhopalosiphum padi* fed on RPV-infected oats, on RPV-infected Barsoy barley, or on healthy Coastblack oats, for their ability to acquire RPV into the hemocoel

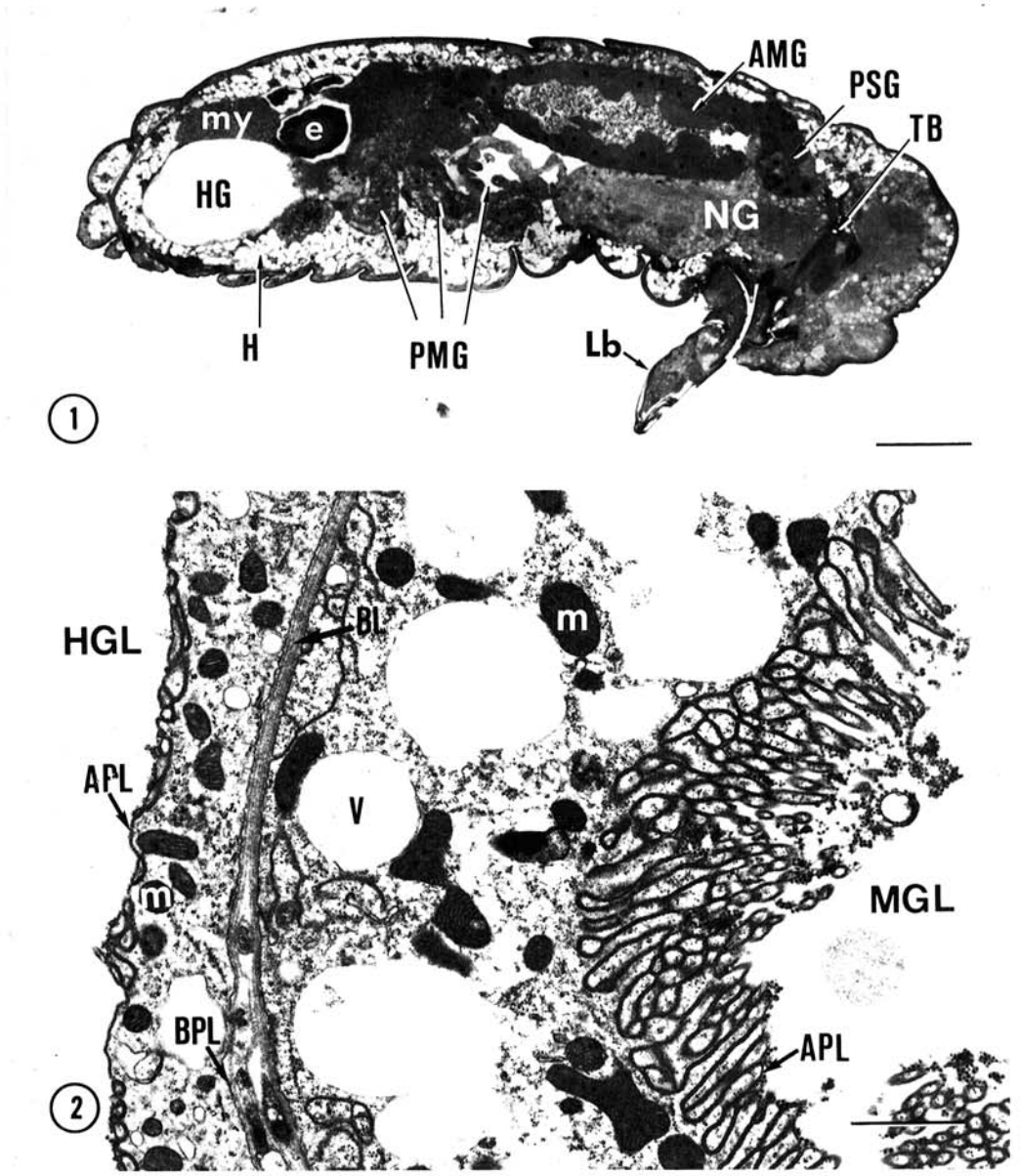
Virus source[a] Aphid species	No. of aphids (of 20) from which RPV was recovered
RPV from oats	
M. dirhodum	0
R. padi	7
RPV from barley	
M. dirhodum	0
R. padi	12
Healthy oats	
M. dirhodum	0
R. padi	0

[a] Aphids were reared together on the same source plants for a 2-wk virus acquisition feeding. Apterous adult aphids were then selected for injection-recovery bioassays in which approximately 0.02 μl of hemolymph was recovered from each adult donor aphid and injected into each of two virus-free, third-instar nymphs of *R. padi*. The injected *R. padi* was then given a 5-day inoculation feeding on 7-day old Coastblack oat seedlings. After 4 wk, these plants were tested by ELISA to verify infection and isolate identity.

nated into the cytoplasm, forming membrane channels. The midgut cytoplasm contained a normal complement of organelles, including Golgi bodies, rough and smooth endoplasmic reticulum, lysosomal vesicles, numerous mitochondria, and endocytotic and exocytotic coated vesicles and pits at the apical and basal plasmalemma. In comparison, the thin-walled hindgut (Fig. 2) varied from 0.5 to 3 μm in thickness and was characterized by a lack of apical microvilli and invaginations of the basal plasmalemma. The apical plasmalemma was lined extracellularly in the gut lumen with a single row of cylindrical tubules approximately 25 nm in diameter. These structures occur only in the hindgut and aided tissue identification. The composition of these tubules is unknown at this time, but they have been previously described as extracellular microtubules (12).

When *R. padi* were fed on RPV-infected oats, virions of RPV were only occasionally observed in the lumen of the midgut in very low numbers and were never observed associated with the midgut cell membranes or intracellularly within the cytoplasm. In contrast, RPV virions were identified consistently in the hindgut lumen and within membrane vesicles in the hindgut cells in all 12 aphids examined (Table 3). Labeled virions were observed after having been released from the hindgut cells into the hemocoel in all five anti-RPV injected aphids examined. In contrast, RPV was detected in the hindgut lumen of only three of 12 *M. dirhodum*. More significantly, virus was not detected in the cell cytoplasm of *M. dirhodum*, and no labeled virions released from the hindgut into the hemocoel were detected by anti-RPV injection.

Feeding *R. padi* and *M. dirhodum* on high concentrations of purified RPV virions through Parafilm membranes was done to enhance the visualization of the RPV-membrane interaction. High concentrations of RPV were readily observed in the hindgut lumen of all 12 *R. padi* and *M. dirhodum* examined (Table 3), indicating that aphids of both species readily ingested the virion suspension. In *R. padi*, virions were detected lining the gut cell apical plasma-



Figs. 1 and 2. Structure of midgut and hindgut in a second-instar nymph of *Metopolophium dirhodum*. **1,** Light micrograph of a 0.5-μm thick section showing a longitudinal view of the anterior midgut (AMG), transverse sections through the posterior midgut (PMG), and a partial section through the hindgut (HG). Position of alimentary canal is shown in relation to tissues of embryo (e), labium (Lb), hemocoel (H), mycetocytes (my), nerve ganglion (NG), principle salivary gland (PSG), and tentorial bar (TB). Bar = 0.1 mm. **2,** Electron micrograph comparing ultrastructure of adjacent hindgut and midgut cells shown in transverse section. Hindgut cells varied between 1 and 3 μm in thickness and were characterized by a smooth apical plasmalemma (APL) forming few or no microvilli and lined extracellularly with a single layer of 25-nm diameter tubules exposed to the hindgut lumen (HGL). Midgut cells varied from 5 to 10 μm in thickness and were characterized by a highly convoluted apical plasmalemma forming microvilli extending into the midgut lumen (MGL) and a basal plasmalemma (BPL), which invaginated to form membrane-lined channels. All gut tissues were surrounded by an extracellular basal lamina (BL). Mitochondria (m), vacuoles (V). Bar = 1 μm.

lemma (Fig. 3) and in various membrane invaginations, suggesting that the virions were attached to the membrane. Virions were also observed in coated pits, coated vesicles, receptosomes, and tubular vesicles, which are structures associated with receptor-mediated endocytosis (9,23,24). In *M. dirhodum*, virions were only observed suspended in the lumen of the hindgut (Fig. 4). Virions were never observed in close proximity to the gut cell membrane or apparently attached to the membrane. No virions were observed in vesicle structures within the cytoplasm of gut cells, and no labeled virions were observed released into the hemocoel of the five anti-RPV injected aphids examined.

When the five *R. padi* injected with anti-RPV antiserum were examined, unlabeled virions were typically observed in the gut lumen apparently adsorbed or attached to the apical plasmalemma (Fig. 5). The antibody molecules do not penetrate the cell membranes and can only react with the virions once they are released into the hemocoel. Ferritin-labeled virions were observed trapped between the basal plasmalemma and the basal lamina facing the hemocoel in four of the five aphids (Fig. 6). This indicated that virions had been transported through the hindgut and liberated into the hemolymph. Virions in cytoplasmic tubular vesicles and receptosomes in anti-RPV injected *R. padi* remained unlabeled (Fig. 7). Attempts to label RPV in sections have not been successful. Virions were not labeled in anti-MAV-injected aphids previously fed on RPV, providing positive identification of the particles as RPV.

For a positive control, *R. padi* and *M. dirhodum* were reared together on a MAV-infected oat plant, and 12 aphids of each species were examined by EM. MAV was recovered from the hemolymph of both species by IRB (Table 1). This MAV isolate is efficiently transmitted by this clone of *M. dirhodum* but not by *R. padi*. Five aphids of each treatment were microinjected with polyclonal anti-MAV immunoglobulin G (IgG) and goat ferritin-conjugated antirabbit IgG to detect MAV virions released into the hemocoel. MAV was detected in the hindgut cytoplasm in 83% of the vector *M. dirhodum* and in 50% of the nonvector *R. padi*. MAV was also observed immunologically labeled (Fig. 8) in the hemocoel of both species, verifying the IRB results. Virus particles were not observed in any of five aphids of each species reared on healthy oats and examined as controls.

Of the dozens of plant viruses that various aphids probably ingest while feeding on infected plants, only the luteoviruses and pea enation mosaic virus are known to be transmitted in a nonpropagative circulative manner. To study how other nontransmitted viruses interact with the aphid hindgut, *R. padi* were fed for 48 h on Parafilm membranes containing BMV at 1.5 mg/ml, CPMV at 1.0 mg/ml, or on 20% sucrose without virus (as a control). Aphids were then fixed and embedded for EM. No virions were observed in the hindgut lumen of any of five aphids fed on the sucrose control. High concentrations of virions were observed in the gut lumen of nine of 10 aphids fed on BMV (Fig. 9) and in four of five aphids fed on CPMV (Fig. 10). However, virions were observed only free in the lumen and were never observed attached to the gut membrane or in cytoplasmic locations in any of two sections on a minimum of two grids examined for each aphid. When *M. dirhodum* were fed for 48 h on the CPMV membrane, similar results were obtained. CPMV was observed in the hindgut lumen of all five aphids examined but never attached to the membrane or in the gut cells. Results suggest that these two viruses are not recognized by the hindgut membrane, do not bind at the plasmalemma, and are not internalized.

TABLE 3. Electron microscopic visualization of the MAV and RPV isolates of barley yellow dwarf virus in hindgut tissue of *Metopolophium dirhodum* and *Rhopalosiphum padi* fed on RPV-infected oats, on purified RPV, on MAV-infected oats, or on healthy Coastblack oats

Virus source[a] Aphid species	No. of aphids in which virus was observed in hindgut location indicated/ total no. examined		
	Lumen	Cytoplasm	Hemocoel
RPV from oats			
M. dirhodum	3/12	0/12	0/5
R. padi	12/12	12/12	5/5
RPV from membrane			
M. dirhodum	12/12	0/12	0/5
R. padi	12/12	12/12	4/5
MAV from oats			
M. dirhodum	10/12	10/12	2/5
R. padi	8/12	6/12	3/5
Healthy oats			
M. dirhodum	0/5	0/5	0/5
R. padi	0/5	0/5	0/5

[a] Both aphid species were reared together on the same virus source plants for a 2-wk acquisition feeding immediately prior to preparation for electron microscopy. Aphids were fed 24 h on 200 μg/ml of purified RPV suspended in 20% sucrose in 0.01 M phosphate buffer, pH 7.

DISCUSSION

The failure to detect RPV attaching to and being transported into cells of the hindgut of *M. dirhodum* following feedings on RPV-infected oats or barley, or on high concentrations of purified RPV, supports the hypothesis that luteovirus recognition occurs at the hindgut membrane. The fact that neither BMV nor CPMV were observed attached to gut membranes of *R. padi* or *M. dirhodum* also suggests that virus recognition regulating membrane attachment is a precursor of virus acquisition. This might explain why aphids transmit so few viruses in a circulative nonpropagative manner. Apparently the gut does not indiscriminately transport plant viruses into the hemocoel or ingest them as nutrient sources.

The failure to detect a similar gut barrier in any of 19 other BYDV-aphid combinations may be due to the fact that the recognition system at the gut is of a very general nature and recognizes capsid epitopes or amino acid sequences that are similar or shared by a range of related BYD luteoviruses.

Current evidence indicates that the accessory salivary gland is acting as the major site determining the high level of vector-specificity observed in BYDV transmission. Apparently, the salivary gland membrane is recognizing a different set of attachment or recognition sites on the virus capsids. The initial failure to detect virions associated with the salivary glands of *R. padi* fed on MAV-infected oats suggested that hindgut acquisition might play a role in limiting transport of MAV into the aphid hemocoel. Subsequent tests, however, revealed that *R. padi* was able to acquire MAV into the hemocoel (Table 1). Why I was unable to observe MAV at the *R. padi* accessory salivary gland is unclear. Perhaps MAV is transported into *R. padi* at concentrations sufficient to detect by the sensitive bioassay but occurs at concentrations too low to detect visually at the salivary gland by EM. Alternatively, other unknown mechanisms for specificity may be functioning to limit survival or movement of specific luteoviruses within nonvector aphid species.

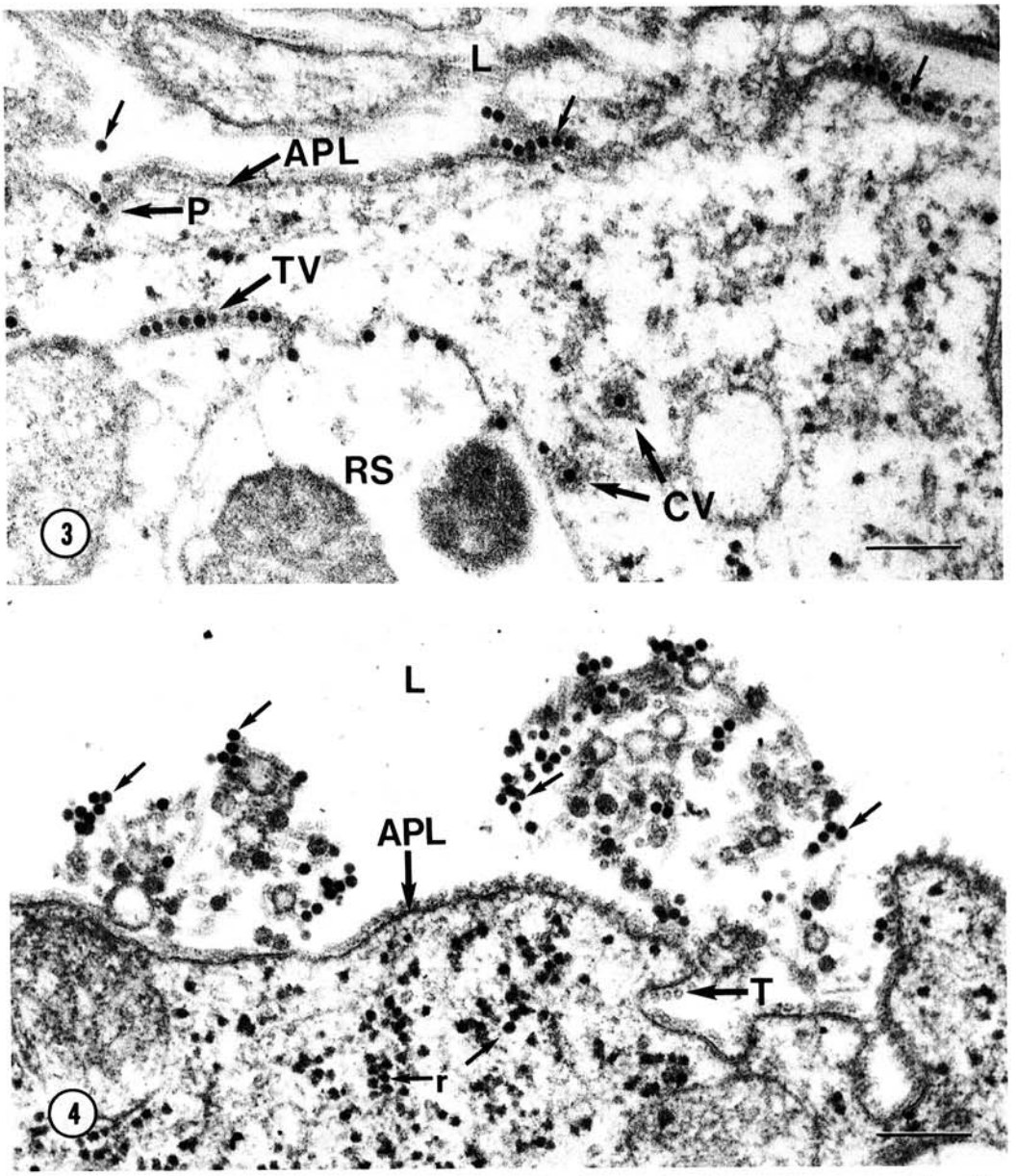
When *R. padi* and *M. dirhodum* were fed together on RPV-infected oats, virions were more frequently observed in the *R. padi* hindgut lumen (Table 3). This difference does not necessarily indicate a reduced level of feeding or lesser amounts of RPV ingested by *M. dirhodum*. Virions observed in the gut lumen are almost always particles observed in contact with the gut cell apical plasmalemma, as in Figure 5. Virions are very rarely observed free in the gut lumen in aphids fed on virus-infected plants. Therefore, it is possible that virions that are to be acquired into the hindgut attach to the membrane, resulting in sequestering and concentrating virus. This would aid visualization of virus at this site. If virions did not attach to the membrane, they would not be concentrated at this site and might be too dilute to visualize free in the gut lumen.

Of the 20 luteovirus-aphid combinations tested, only the RPV-*M. dirhodum* combination demonstrated selectivity functioning to prevent virus acquisition through the hindgut. However, I do not think this should be interpreted to mean that the hindgut is not selective. Based on the ultrastructural evidence of receptor-

mediated endocytosis, the inability of *M. dirhodum* to acquire RPV and CPMV, and the inability of *R. padi* to acquire BMV or CPMV, it seems reasonable to assume that the hindgut is recognizing luteoviruses as a prerequisite to acquisition. However, the type of recognition occurring is less selective than that which occurs at the accessory salivary gland limiting transmission.

The identity and nature of the putative hindgut receptors recognizing and binding to BYDV isolates are unknown. Several animal viruses are known to utilize different types of host membrane-associated proteins as receptors. Some retroviruses utilize transmembrane permease-like proteins as cellular receptors (19). Reoviruses and influenza viruses are recognized by specific sialic acid residues of oligosaccharides on cell surface glycoproteins (10). Human immunodeficiency virus, rhinoviruses, and picornaviruses utilize cell surface glycoproteins as receptors (1,10). Molecules utilized by viruses as cell attachment receptors are normally occurring components of the cell membrane surface with various functions related to healthy cell physiology and metabolism. Single amino acid substitutions of several such receptor proteins have been shown to alter host specificity or eliminate virus binding. Some viruses, such as human immunodeficiency virus, however, are capable of utilizing alternative binding sites on potential host cells (10). Therefore, it is possible that different sets of luteovirus-recognizing receptor molecules function at the aphid hindgut and salivary gland membranes, resulting in differential binding of BYDV isolates.

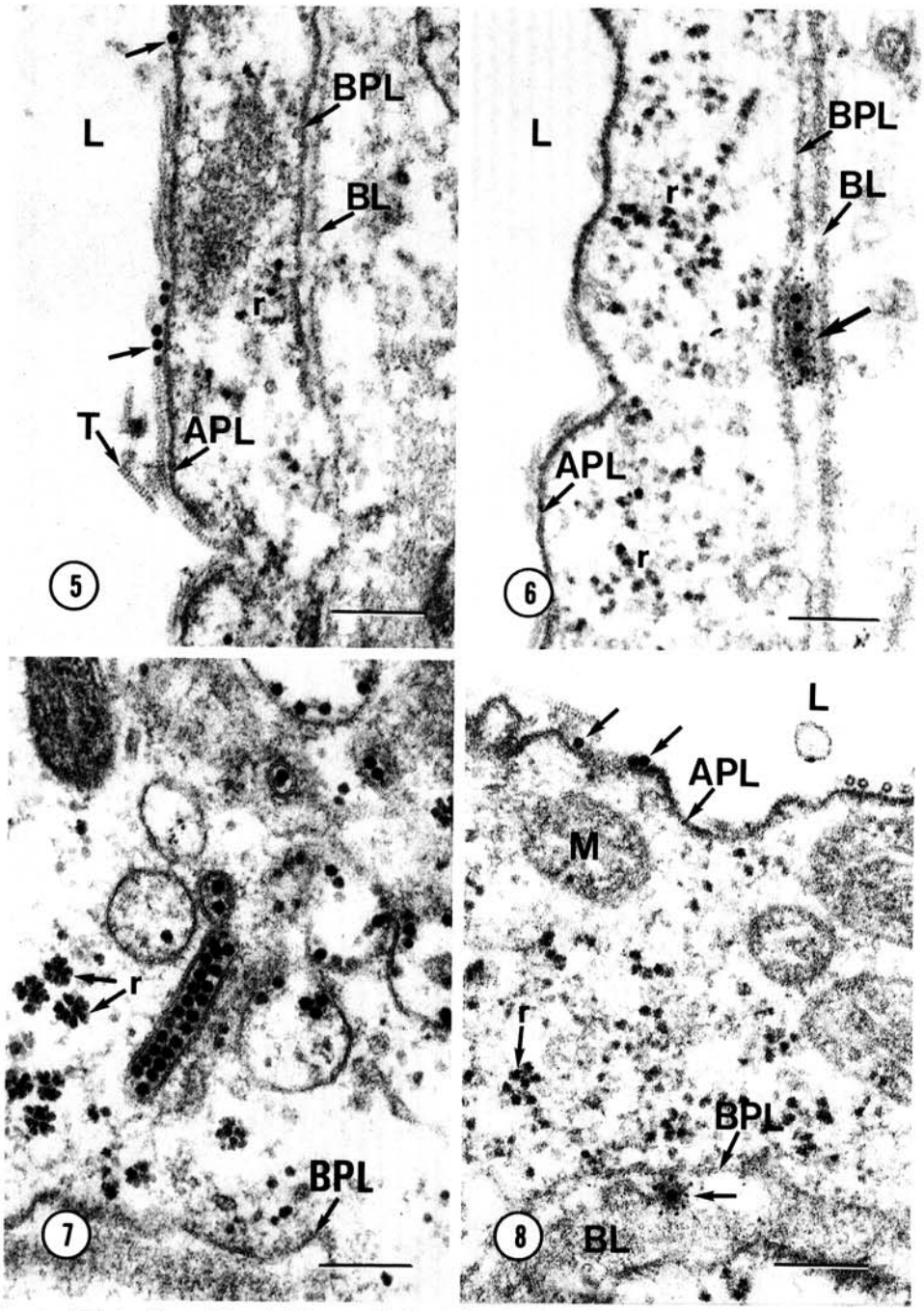
Little is known about the receptor recognition sites on the virion capsid or the possible capsid-associated virus attachment proteins that have been hypothesized to exist on the BYD luteovirus capsid (14,20,22). Peptide sequences of amino acids with the appropriate molecular dimensions, charge, and degree of hydrogen bonding in specific sites of the capsid protein are believed to be responsible for regulating virus-receptor recognition and attachment (2). The receptor recognition sites are highly conserved among the virus strains that have been studied. At least 78 rhinovirus strains utilize a common attachment site on host cells, suggesting all strains



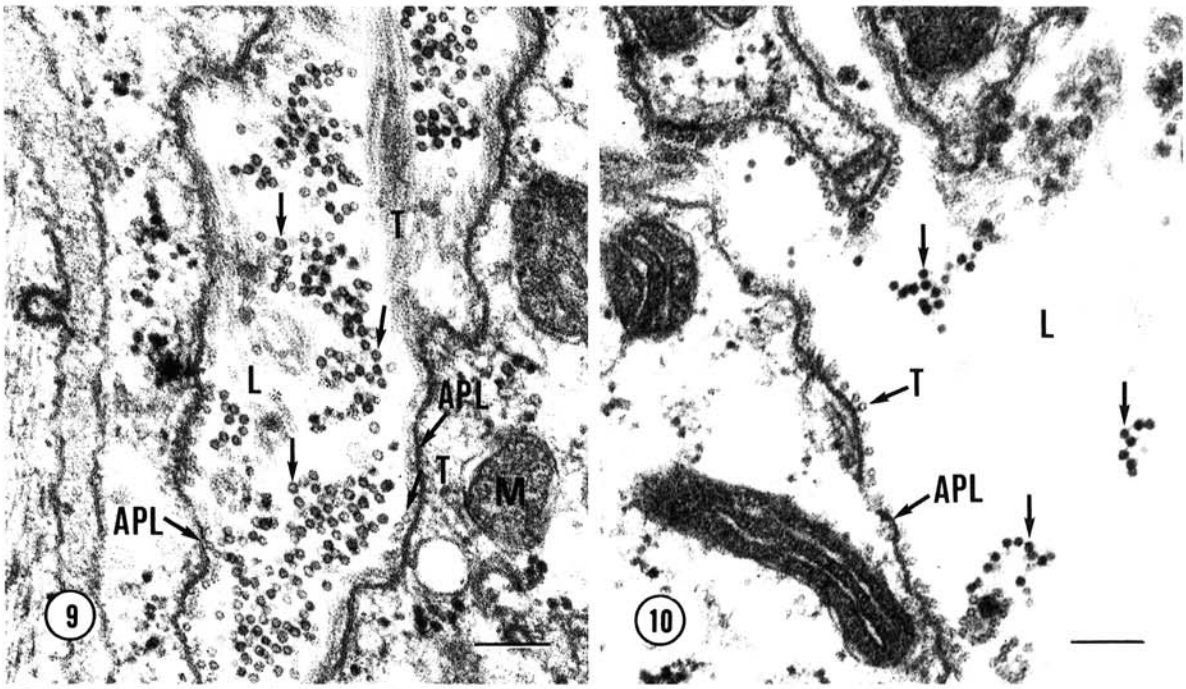
Figs. 3 and 4. Electron micrographs of hindgut from representative aphids fed 24 h on Parafilm membranes containing 200 μg/ml of the RPV isolate of barley yellow dwarf virus. **3,** Hindgut of *Rhopalosiphum padi* showing RPV virions (unlabeled arrows) concentrated along the apical plasmalemma (APL) in the gut lumen (L), and in an invagination forming a coated pit (P). Virions in the cytoplasm occur only in coated vesicles (CV), receptosomes (RS), and tubular vesicles (TV). **4,** Hindgut of *Metopolophium dirhodum* showing ingested RPV virions (unlabeled arrows) aggregated in the hindgut lumen (L). Virions were not observed associated with the apical plasmalemma (APL) or intracellularly. All particles free in the cytoplasm are ribosomes (r). Note transverse sections of 25-nm extracellular tubules (T) lining the cell membrane. Bars = 200 nm.

share similar receptor recognition sites. The amino acid sequences making up these sites are believed to occur in protected depressions or canyons on the capsid surface (2). Specificity of these sites was demonstrated by site-directed substitution of four amino acids located at the bottom of the canyon. Single amino acid substitutions reduced virus attachment to host cells by 50–90%. Other workers have reported receptor recognition sites occurring on exposed peptide loops extending from the virion surface (11). However, the sequence of these loops is also highly conserved among strains of the same virus, and alteration of amino acid sequences altered host range of the viruses (10). Conserved receptor recognition sites on BYDV luteoviruses recognized by similar hindgut receptors common to the five aphid species tested could explain the ability of these aphids to acquire a wide range of BYDV isolates.

For picornaviruses, the receptor recognition site is believed to involve complex interactions among different capsid proteins. Binding sites are easily disrupted, suggesting that tertiary structural interactions are involved (1). A similar situation may exist for BYDV serotypes. Monoclonal and polyclonal antibodies often do not react with denatured virions resulting from pH- or ultraviolet-induced alterations of capsid structure (6,14). This suggests



Figs. 5–8. Electron micrographs of hindgut from *Rhopalosiphum padi* microinjected with anti-RPV (5–7) or anti-MAV (8) antibodies for immunolabeling following acquisition feedings on Parafilm membranes containing purified RPV, or on oats infected with the MAV isolate of barley yellow dwarf virus. **5,** Ingested, unlabeled RPV virions (arrows) in the hindgut lumen (L) adsorbed to the apical plasmalemma (APL). Note longitudinal views of extracellular tubules (T), ribosomes (r), basal plasmalemma (BPL), and basal lamina (BL). **6,** Ferritin-labeled RPV virions (arrow) captured between the basal plasmalemma (BPL) and basal lamina (BL) upon release from the hingut cell into the hemocoel. Apical plasmalemma (APL), hindgut lumen (L). **7,** Unlabeled RPV concentrated in receptosome-like vesicles and in a tubular vesicle adjacent to the basal plasmalemma (BPL) and basal lamina. Ribosomes (r). **8,** Unlabeled virions of MAV (arrows) in the hindgut lumen (L) adjacent to the apical plasmalemma (APL), and an anti-MAV-labeled virion adjacent to the basal plasmalemma (BPL) in the nonvector *R. padi* following transport to the hemocoel. Basal lamina (BL), mitochondria (M), ribosomes (r). Bars = 200 nm.



Figs. 9 and 10. Electron micrographs of *Rhopalosiphum padi* hindgut following a 24-h acquisition feeding on Parafilm membranes containing purified **9,** brome mosaic virus or **10,** cowpea mosaic virus. Note virions (unlabeled arrows) free in the hindgut lumen (L). Virions of these nontransmitted viruses were not observed attached to the apical plasmalemma (APL) or in vesicles suggesting transport to the hemocoel. Extracellular tubules (T), mitochondria (M). Bars = 200 nm.

that these antigenic sites are conformational epitopes requiring maintenance of specific secondary and tertiary bonding interactions. Similar sites could be involved in acquisition and transmission specificity of luteoviruses, making identification of receptor recognition sites based on linear amino acid sequences difficult.

The illustration that luteovirus recognition systems involving receptor-mediated endocytosis probably function to control virus acquisition at the hindgut suggests that this area of the aphid would lend itself to experimental studies of virus-membrane interactions. The ability to membrane-feed aphids on various luteoviruses, on viruses with genetically altered capsid proteins, and on a range of monoclonal antibodies and antiidiotypic antibodies should aid in our understanding of how aphids regulate virus transmission and might give clues useful in developing mechanisms to interfere with this process.

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