Vector Relations

Coated-Vesicle Transport of Luteoviruses Through Salivary Glands of *Myzus persicae*

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ABSTRACT


Beet western yellows virus (BWYV) or potato leaf roll virus (PLRV) was visualized by electron microscopy in basalm lamina and plasmalemma
invaginations of accessory salivary glands in each of 61 aphids that had fed
on infected plants, but never in any of 15 control aphids reared on healthy
plants. Virions were identified as BWYV or PLRV by indirect labelling with
ferritin-antibody. Virus particles were frequently observed in tubular
vesicles and coated vesicles in cytoplasm near salivary canals, and in coated
pits connected to the canal membrane. Following injection of anti-PLRV
antibody into the aphid hemocoel, PLRV particles accumulated in the
accessory gland basalm lamina and aggregated in the salivary duct. These
results help identify the route of luteoviruses through the accessory salivary
gland to the salivary duct. A cellular mechanism, involving coated-vesicle
transport of virions from tubular vesicles to the salivary canal, is suggested
as a model for transport of luteovirus through accessory gland cytoplasm.
The relation of vector-specific transmission of luteoviruses to this model is
discussed.

Beet western yellows virus (BWYV), potato leaf roll virus
(PLRV), and barley yellow dwarf virus (BYDV) are icosahedral
RNA luteoviruses that replicate only in phloem tissue of host plants
and are transmitted only by aphids in a persistent-circulative
manner (21). Recent studies indicate luteoviruses do not replicate
in aphid vectors. Aphids failed to transmit PLRV (5) or BYDV (15)
following attempts to pass the virus serially in vectors by injection,
indicating eventual loss of virus from the aphid hemocoel. Tests
utilizing enzyme-linked immunosorbent assays (ELISA) to
measure PLRV concentration in viruliferous aphids placed on
immune plants (28) and BYDV in aphids maintained by membrane
feeding (W. F. Rochow, personal communication) support this
idea. The apparent failure of luteoviruses to replicate in vectors
makes the luteovirus-aphid system ideal for studies of virus
penetration and transport through vector cells. One can avoid the
complications of virus replication and resulting possible effects on
vector cell structure and physiology.

The importance of salivary glands in circulation of luteoviruses
through aphids was suggested from earlier work on vector-
specificity (22) and detection of BYDV in salivary glands (18).
Harris et al (10) first demonstrated a specific association between
aphid accessory salivary gland and a persistently transmitted plant
virus. A vectored isolate of pea enation mosaic virus (PEMV)
accumulated in and penetrated the extracellular basalm lamina
surrounding the accessory salivary gland of the aphid,
*Acyrthosiphon pisum* (Harris). Subsequent identification of

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vector-specific isolates of BYDV in accessory gland basali lamina and plasmalemma invaginations of *Stobion avenue* (Fabricius) supported the idea that the accessory gland was involved in persistent virus transmission (7). In addition, virons of a transmitted BYDV isolate were observed within the secretory cell cytoplasm in lysosomelike vesicles and coated vesicles, and in the lumen of the secretory canal. Viron of a nontransmitted isolate were not observed in the cytoplasm or canal (7). This suggested that selection of virus isolates occurred at the plasmalemma of the accessory gland, and indicated potential routes for luteoviruses out of the aphid.

The purpose of this work was to study the role of the accessory salivary gland in aphid transmission of other luteoviruses and determine the cellular route for virus movement through accessory gland cells.

**MATERIALS AND METHODS**

The green peach aphid, *Myzus persicae* (Stal), originally collected at Salinas, CA, by J. E. Dufus, was maintained in virusfree colonies on individually caged radiish plants, *Raphanus sativus* L. *White Icicles*, in a growth chamber at 15 C with a 24-hr photoperiod. The BYWV isolate used was the originally described BYWV isolate and subsequently designated as ST-1 (4). The PLRV isolate obtained from E. Syluster at Berkeley, CA, has been previously studied (5). These two isolates were distinguished by symptom production on *Physalis floridana* Rydb. and shepherd’s purse, *Capsella bursa-pastoris* (L.) Medic., and by serological infectivity neutralization tests (4).

Plants to be used as virus sources were infested with viruliferous aphids for a 3-day inoculation feeding, after which the aphids were killed with nicotine sulfate. The plants were used 3 wk after inoculation for virus acquisition feeding. Seedlings of *P. floridana* and radish were inoculated 1 wk after emergence. Older seedlings of shepherd’s purse were inoculated, however, since BYWV tended to kill young seedlings.

To obtain aphids for study, 10 virusfree adult aphids were allowed to produce nymphs for 24 hr on either BYWV- or PLRV-infected plants or on healthy plants as controls. After removing adults, the nymphs were allowed to feed and mature on the plants for 10 days. Individual aphids were arbitrarily selected for microscopic examination. After the remaining aphids, five aphids were placed on each of four seedlings of *P. floridana* (for PLRV) or shepherd’s purse (for BYWV) to verify virus acquisition from source plants, and the noninfected condition of control plants and the original aphid colony.

For ultrastructural examination aphids were immersed in fixative and bisected transversely with a thin double-edge razor blade. Fixative consisted of 1% formaldehyde, 2% gluteraldehyde, 0.01% CaCl2, and 0.05% sodium azide in 0.01 M phosphate buffer (pH 7.0). Aphids were fixed overnight at 4 C, rinsed three times in 0.1 M sodium cacodylate buffer (pH 7.2), and fixed 2 hr in 2% osmium tetroxide in cacodylate buffer. Following two rinses in buffer and distilled water the aphids were stained in 2% aqueous uranyl acetate for 1 hr. Tissue were rinses three times in water, dehydrated in an acetone series over a 2-hr period, and infiltrated over 24 hr with PolyBed 812 (Polysciences Inc., Warrington, PA 18976) - araldite mixture (11). For light microscopy, sections 0.25- 

electron microscope.

Indirect ferritin-antibody labelling of BYWV and PLRV was initiated by removing aphid heads with fine dissecting needles in a depression slide filled with 0.01 M phosphate buffer. The exposed subesophageal nerve ganglion and adjacent salivary gland complex was then freed from the remaining head tissue (Fig. 4). Isolated glands were incubated overnight at 4 C in rabbit IgG antibody (0.1 mg/ml) made against BYWV (4), the MAV isolate of BYD (20), or PLRV (25), or in buffer as a control. Following five rinses of 15 min each, the glands were incubated for 3 hr in ferritin-conjugated goat antirabbit IgG (Miles Laboratories Inc., Elkhart, IN 46514) diluted to 0.15 mg/ml in buffer. Tissues were then rinsed five times over 2 hr in buffer and fixed as previously described. All procedures were performed at 4 C or on ice.

Aphids to be injected were anesthetized with CO2 gas and kept on ice prior to injection with IgG. Injections were done with needles drawn from 1-mm thin-walled capillary tubing on a commercial needle puller. Needles were filled by capillarity to a volume of about 0.02 pl and emptied into aphids by air pressure. Viruliferous aphids were injected with IgG or buffer between the junction of the thorax and abdomen on the dorsal surface and then allowed to feed overnight on infected plants before being fixed.

**RESULTS**

Aphid accessory salivary glands. The salivary glands of *M. persicae* (Fig. 1) were as described by Ponsen (19) and consisted of a set of accessory and principal glands located above the subesophageal nerve ganglion on both sides of the aphid. The four celled accessory glands were located immediately posterior to the optic lobes of the brain. Ultrastructurally the accessory gland (Fig. 2) was similar to that of *S. avenue* (7). The secretory cells were surrounded by an extracellular basal lamina, which appeared as a fibrous meshwork believed to consist of a complex of collagen, protein, and carbohydrates (12). The plasmalemma invaginated into the cell cytoplasm and was closely associated with many mitochondria. Below this region were many secretory vesicles, lysosomes, and multivesicular bodies. Typical lamellar Golgi bodies were not recognized in the accessory gland, but did occur in abundance in the principal gland. A system of microvilli-lined canals branched throughout the secretory cell. Vesicles of various types were observed fused to the canal membrane. The canals joined the chitin-lined salivary duct at the apical end of the secretory cell (Fig. 3). From this point secretions liberated into the canal lumen pass through the salivary duct (Fig. 4) to the salivary syringe and out of the aphid. It should be noted that the microvilli-lined canals described are the same as the striated intracellular canaliculii described by Ponsen (19) by light microscopy. The membrane of the microvilli is continuous with the apical plasmalemma of the secretory cell and therefore the lumen of the canal is extracellular. Virulike particles, approximately 25 nm in diameter, were consistently observed in the basal lamina of accessory salivary glands from 61 aphids fed on BYWV or PLRV-infected plants in three experiments done over a 10-mo period. Particles were observed only in basal lamina of the optic lobe nerve tissue, muscle tissue, or connective tissue adjacent to the accessory gland, or of the principal salivary gland. None of 15 aphids reared on uninfected plants as controls possessed similar particles.

Indirect ferritin labelling. Small isometric particles of unknown origin have been reported in vector tissues (2). Therefore, positive identification of virus particles in the aphids was essential. Identification of the particles observed in the accessory gland basali lamina as BYWV and PLRV was done by ferritin-antibody labelling of dissected salivary glands (Fig. 4). Dissected glands from four aphids reared on BYWV-infected shepherd’s purse or PLRV-infected *P. floridana* were first incubated in rabbit IgG to BYWV, the MAV isolate of BYD, or PLRV, or in buffer as a control, before incubation in ferritin conjugated goat antirabbit IgG. Grids prepared from four aphids in each treatment were examined for labelled particles in the basal lamina. Particles in aphids fed on BYWV were positively labelled by antiserum to BYWV (Fig. 5) and
Figs. 1-4. Aphid accessory salivary gland structure. 1, Longitudinal section through *Myzus persicae* showing dorsal view of accessory salivary glands (AG), principal salivary gland (PG), optic lobes of the protocerebrum (OL), and eyes (E) by interference contrast light microscopy. Bar = 100 μm. 2, Electron micrograph of the basal region of an accessory gland secretory cell showing the basal lamina (bl), plasmalemma invaginations (pl), mitochondria (m), secretory vesicles (sv), and microvilli-lined canal (C). Bar = 1 μm. 3, Electron micrograph of the apical portion of an accessory gland showing a microvilli-lined canal (C) opening into the thick walled salivary duct (SD). Bar = 3 μm. 4, Interference contrast light micrograph of a whole unstained dissected accessory gland (AG), principal gland (PG), and subesophageal nerve ganglion (NG), and showing the chitin-lined salivary ducts (SD), which lead to the salivary syringe. Bar = 50 μm.
Figs. 5–13. Indirect labelling with ferritin-conjugated goat antirabbit IgG (f) of BWYV and PLRV (v) embedded in accessory gland basal lamina (bl). 5–8, Particles of BWYV in aphids fed on BWYV-infected shepherd’s purse treated with IgG against 5, BWYV; 6, the MAV isolate of BYDV or 7, PLRV; and 8, buffer only as a control. 9–12, Particles of PLRV in aphids fed on PLRV-infected Physalis floridana treated with IgG made against 9, BWYV; 10, the MAV isolate of BYDV or 11, PLRV; and 12, buffer as a control prior to treatment with ferritin-conjugated goat antirabbit IgG. Bar = 200 nm. 13, Basal region of accessory salivary gland cell from an aphid reared on PLRV-infected Physalis floridana. Note virus particle in coated vesicle (cv), and in the plasmalemma invagination (pi) and basal lamina (bl) (arrows). Also shown is rough endoplasmic reticulum (er) and an invagination that is continuous with a multivesicular body (mvb). Bar = 0.5 μm.
PLRV (Fig. 7), but not to BYDV (Fig. 6) or in the buffer control (Fig. 8). Particles in aphids fed on PLRV were also positively labelled by antiserum to both BWYV (Fig. 9) and PLRV (Fig. 11), but not to BYDV (Fig. 10) or buffer (Fig. 12). These results indicated that the observed particles were BWYV and PLRV. However, the technique could not discriminate between these two ecologically related luteoviruses.

**Virus in accessory gland secretory cells.** In addition to labelled virus in the basal lamina and plasmalemma invaginations, particles were occasionally observed in coated and uncoated vesicles in the cytoplasm near the basal plasmalemma (Fig. 13). That these 25 nm hexagonally shaped particles observed in the cytoplasmic structures were luteoviruses was suggested by the observation that they did not occur in cells of aphids reared on healthy plants, that their shape and staining characteristics were distinct from ribosomes, and that they appeared identical to virus labelled in the basal lamina. Further examination of cells indicated that coated vesicles may originate from coated pits at the plasmalemma adjacent to the basal lamina, and from the membrane invaginations deep within the cytoplasm (Fig. 14). Occasionally particles were observed at the ends of invaginations (Fig. 15). Virus particles were frequently found in elongate membrane structures (Figs. 16 and 17).

**Figs. 14-21.** Electron micrographs of sections through accessory salivary gland cells of *Myzus persicae* fed on either BWYV (14 and 15) or PLRV (16-21)-infected plants. 14, Coated pit (cp) forming on a plasmalemma invagination adjacent to a virus particle. Note three virions within invagination (arrow). Bar = 200 nm. 15, Tip of membrane invagination, which appeared to be forming a vesicle containing two particles (arrow). Bar = 100 nm. 16 and 17, Tubular vesicles (tv) containing virions. Bar = 200 nm. 18 and 19, Plasmalemma invaginations (pi) suggesting continuity with rough endoplasmic reticulum (er) (arrow). Bar = 200 nm. 20, Tubular vesicles containing particles (arrows) near a microvilli-lined canal with a coated pit (cp). Bar = 200 nm. 21, Coated pit (cp) formed on a tubular vesicle or endoplasmic reticulum containing a virion (arrow). Bar = 200 nm. Particles associated with similar structures occurred in aphids fed on either BWYV- or PLRV-infected plants.
similar in appearance to tubular vesicles described in other cell systems (3,13), which are involved in protein transport. The tubular vesicles were similar in appearance to smooth endoplasmic reticulum. Plasmalemma invaginations occasionally appeared to be continuous with the rough endoplasmic reticulum (Figs. 18 and 19). Coated pits were observed associated with tubular vesicles and particles were frequently observed within these vesicles (Figs. 20 and 21). Coated vesicles containing particles were observed near tubular vesicles (Fig. 22) and the canal membrane (Fig. 23). Coated vesicles were a common structure in accessory gland cells in aphids fed on healthy plants. Apparently the coated vesicles found in large numbers associated with microvilli-lined canals are part of a normal mechanism for protein transport in the accessory gland. In aphids fed on BWVV- and PLRV-infected plants, the coated pits frequently contained virus particles, which appeared to have been in the process of liberation into the canal when the aphids were fixed (Figs. 24–26).

No differences in cell structure or intracellular location of virus particles were observed between aphids fed on BWVV- or PLRV-infected plants. Table 1 summarizes data from one experiment on occurrence of virus particles in various regions of the aphid accessory salivary glands. It is especially important that particles

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Figs. 22–29. Sections of accessory salivary gland cells from *Myzus persicae* fed on plants infected with BWVV (23–25) or PLRV (22 and 26–29). 22, PLRV particles in coated vesicles (cv) near a tubular vesicle (tv) and particles (arrows) in the microvilli-lined salivary canal (c). Bar = 200 nm. 23, Virus (arrows) in cytoplasmic vesicles adjacent to canal with coated pits (cp). Bar = 100 nm. 24 and 25, Particles of BWVV (arrows) apparently released into the canal lumen following fusion of a coated vesicle with canal membrane to form a coated pit. Bar = 200 nm. 26, Particle of PLRV (arrow) at point of release into canal. Bar = 100 nm. 27, Basal lamina (bl) of accessory gland (AG) and principal gland (PG) from aphid fed on PLRV and injected with buffer. Note relative density of particles. Bar = 100 nm. 28, Basal lamina of accessory gland (AG) and nerve ganglion (NG) from aphid fed on PLRV and injected with anti-PLRV antibody. Note density of particles relative to Fig. 27. Bar = 100 nm. 29, Section through the salivary duct (SD) at base of accessory gland from an aphid fed on PLRV and injected with anti-PLRV antiserum. Note aggregation of particles. Bar = 100 nm.
were always observed in aphids reared on infected plants, but never in any aphids reared on healthy plants.

**Aphid injection with anti-PLRV antibodies.** In one experiment aphids reared on PLRV-infected *P. floridana* were injected with anti-PLRV IgG or 0.1 M phosphate buffer. The accessory gland cell ultrastructure of injected aphids remained similar to that of noninjected aphids. The number of virus particles observed in basal lamina of buffer-injected aphids was similar to that of uninjectected aphids (Fig. 27). Aphids injected with anti-PLRV, however, showed a great increase in basal lamina-embedded particles (Fig. 28), indicating that antibodies may trap particles in the basal lamina. The specific association of virus with accessory gland basal lamina was also observed. No viroplasms were seen in basal lamina of the principal salivary gland (Fig. 27) or nerve tissue (Fig. 28). Large numbers of viroplasms were consistently observed aggregated in the lumen of the chitin-lined salivary duct at the base of the accessory gland in aphids injected with anti-PLRV (Fig. 29). Apparently antibody was transported through the accessory gland to the duct where it reacted with virus particles. This indicates that PLRV is transported to the salivary duct by way of the accessory salivary gland. No particles were observed in salivary ducts of noninjected aphids or aphids injected with buffer. It is assumed that the relatively small numbers of viroplasms that reach the duct from the canals are rapidly flushed through the system. No unusual structures were observed in cells as a result of injection, however, and further more particles were observed in the tubular vesicles and coated pits of aphids injected with anti-PLRV when compared to aphids injected with buffer.

**DISCUSSION**

These observations support earlier studies (7,10) that indicated the accessory salivary gland is a route for nonpropagative circulative plant viruses through aphid vectors. The consistent visualization of BWVV and PLRV in specific propogative structures suggests possible mechanisms for cellular transport of luteoviruses through gland cells to the salivary duct (Fig. 30). Movement of virus-containing coated vesicles to microvilli-lined canals and subsequent fusion of vesicles to the canal membrane, to form coated pits with concomitant release of virus into the canal, is a reasonable assumption, based on the static images observed. Once virus enters the canal it may move unimpeded into the salivary duct and out of the aphid. Observation of virus-antibody aggregates in the duct supports this idea. How the luteoviruses become enclosed in coated vesicles is less certain. Coated vesicles are distinct organelles consisting of a membrane-bound vesicle surrounded by a network of protein which in cross section appears as radiating spikes (30). Coated vesicles play a major role in endocytosis and exocytosis of proteins (16,23). My observations suggest that coated vesicles can arise from the plasmalemma or from tubular vesicles that may be identical with or derived from endoplasmic reticulum. The small number of coated vesicles observed near the basal plasmalemma, relative to the number of particles observed in coated vesicles near the canals, suggests that most coated vesicles probably are derived from tubular vesicles. How virus particles become enclosed in these tubular membrane structures is unknown. Since some plasmalemma invaginations may be continuous with the endoplasmic reticulum, it is possible that viroplasms deep within invaginations become incorporated into the endoplasmic reticulum, which gives rise to tubular vesicles.

Association of virus with tubular vesicles in vector cells has been previously described. In a study of nonpropagative PEMU, Shikata et al (27) described a stringlike arrangement of viroplasms in tubular membranes in both infected pea plant and aphid vector cells. These structures are very similar to those shown in Figs. 16 and 17. Viruses that replicate in insect vectors have also been shown to aggregate within tubular membranes (26,29), or cisternae of endoplasmic reticulum (6). Tubular vesicle transport of proteins through cells is well documented (3,23), and evidence suggest that tubular vesicles may derive from endoplasmic reticulum (13).

**TABLE 1. Visualization of beet western yellows virus (BWVV) and potato leaf roll virus (PLRV) in accessory salivary glands of *Myzus persicae* reared 10 days on BWVY- or PLRV-infected plants or healthy plants**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Source</th>
<th>No. of aphids in which virus was observed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Apophids observed (no.)</td>
</tr>
<tr>
<td>1</td>
<td>BWVV</td>
<td>Healthy</td>
</tr>
<tr>
<td>2</td>
<td>BWVV</td>
<td>Healthy</td>
</tr>
<tr>
<td>3</td>
<td>PLRV</td>
<td>Healthy</td>
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*Apophids were reared from birth to 10 days on BWVY-infected radish (exp. 1), shepherd's purse (exp. 2), or PLRV-infected *Physalis floridana* (exp. 3), or on healthy plants of the same species for controls. A minimum of two sections on each of two grids from each aphid were scanned for the presence of virus particles in each region of the gland.*

**Fig. 30. Diagram showing proposed routes of luteovirus through the accessory salivary gland of aphid vectors.** 1) Virus may be taken up by coated pits on the plasmalemma and transported directly to the microvilli-lined salivary canal by coated vesicles. 2) Virus deep within plasmalemma invaginations may become enclosed in the lumen of endoplasmic reticulum, which gives rise to tubular vesicles. Coated vesicles then transport the virus from the tubular vesicle to the canal and form coated pits that release the virus into the canal. 3) Virus particles may penetrate the plasmalemma directly without forming vesicles and become enclosed in tubular or coated vesicles. BL = basal lamina, PL = plasmalemma, V = luteovirus, RER = rough endoplasmic reticulum, SER = smooth endoplasmic reticulum, C = salivary canal, TV = tubular vesicle, SD = salivary duct.
coated vesicles in accessory gland cells, but a nontransmissible isolate (RV) was observed only extracellularly. This suggested the plasmalemma as the selective site. If virus-specific receptors regulate luteovirus uptake, they could function at the basal plasmalemma, associated with coated pits forming from tubular vesicles, or at a point preventing movement of virus into tubular vesicles. Tubular vesicles were not readily observed in the BYDV study, suggesting they were not preserved by the fixative used and may be labile structures.

Results of this study point out the high degree of specificity between luteoviruses and the accessory gland basilar lamina. The failure of virus particles to associate with basilar lamina of other organs in aphids injected with anti-PLRV antiserum was surprising. I assumed some antibody would contaminate basilar lamina of tissues near the accessory gland and would bind some particles. The failure of this antibody to suggest an inherent difference between accessory gland basilar lamina and basilar lamina of other organs which allowed antibody and virus to accumulate only in the accessory gland. Structural differences between basilar lamina of different tissues and the ability of basilar lamina to select and filter out molecules by size and charge is well documented. In the luteovirus system it would seem the accessory gland basilar lamina may attract virions and facilitate their uptake into the gland. These results substantiate the role of the aphid accessory salivary gland in luteovirus transmission and suggest a cellular mechanism for transporting luteoviruses out of the gland cells. Questions concerning how virus enters tubular vesicles, where these vesicles originate, and where the site determining vector-specificity occurs remain to be answered.

LITERATURE CITED