Posterior midgut and hindgut are both sites of acquisition of *Cucurbit aphid-borne yellows virus* in *Myzus persicae* and *Aphis gossypii*

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Members of the family *Luteoviridae* (‘luteovirids’) rely strictly on aphid vectors for plant-to-plant transmission. This interaction operates according to a persistent and circulative manner, which implies that the virions are being endocytosed and exocytosed across two epithelial barriers (alimentary tract and accessory salivary glands) in the vector’s body. In several luteovirid–aphid vector species combinations, the route of virions in the insect has been investigated ultrastructurally by transmission electron microscopy (TEM). Here, we used TEM to follow the route of *Cucurbit aphid-borne yellows virus* (CABYV; genus *Polerovirus*) in its two efficient vector species, *Myzus persicae* and *Aphis gossypii*. We demonstrated that CABYV particles are acquired from the gut lumen to the haemocoel through two different sites in both aphid species, i.e. the posterior midgut (as for *Beet western yellows virus in M. persicae*) and the hindgut (as for *Barley yellow dwarf virus* complex in cereal aphids). This ‘dual’ tissue specificity of CABYV represents an original situation among viruses in the family *Luteoviridae* examined so far by TEM. A variety of virion-containing structures (e.g. clathrin-coated and tubular vesicles, endosome-like bodies) are found in intestinal cells of both types in both aphids. Release of virus particles from midgut and hindgut cells into the haemolymph was confirmed by immunotrapping using CABYV-specific antibodies. In accessory salivary glands, transport of CABYV virions across the cells was similar in each aphid species, and occurred by a transcytosis mechanism involving formation of tubular and coated vesicles before release of free virions in the salivary canal.

**INTRODUCTION**

*Cucurbit aphid-borne yellows virus* (CABYV) is a member of the genus *Polerovirus* in the family *Luteoviridae* (Mayo & D’Arcy, 1999). CABYV causes severe yellowing in cucurbit crops in France (Lecoq *et al*., 1992) and in the United States (Lemaire *et al*., 1993). The isometric virion of ca. 25 nm diameter contains a single-stranded RNA of 5-6 kb which has been fully sequenced (Guilley *et al*., 1994) and for which a full-length infectious clone is available (Prüfer *et al*., 1995). Like other members of the *Luteoviridae* (‘luteovirids’; Smith *et al*., 2000), CABYV is obligately transmitted by aphids in a persistent and vector-specific manner (Herrbach, 1999). In both field and laboratory conditions, CABYV is efficiently transmitted by two aphid species, *Myzus persicae* and *Aphis gossypii* (Lecoq *et al*., 1992).

The route of several luteovirids in their aphid vector during acquisition/transmission has been followed by transmission electron microscopy (TEM). These observations have led to a model for transcytosis of virus particles by the transmission electron microscopy (TEM). Here, we used TEM to follow the route of *Cucurbit aphid-borne yellows virus* (CABYV; genus *Polerovirus*) in its two efficient vector species, *Myzus persicae* and *Aphis gossypii*. We demonstrated that CABYV particles are acquired from the gut lumen to the haemocoel through two different sites in both aphid species, i.e. the posterior midgut (as for *Beet western yellows virus in M. persicae*) and the hindgut (as for *Barley yellow dwarf virus* complex in cereal aphids). This ‘dual’ tissue specificity of CABYV represents an original situation among viruses in the family *Luteoviridae* examined so far by TEM. A variety of virion-containing structures (e.g. clathrin-coated and tubular vesicles, endosome-like bodies) are found in intestinal cells of both types in both aphids. Release of virus particles from midgut and hindgut cells into the haemolymph was confirmed by immunotrapping using CABYV-specific antibodies. In accessory salivary glands, transport of CABYV virions across the cells was similar in each aphid species, and occurred by a transcytosis mechanism involving formation of tubular and coated vesicles before release of free virions in the salivary canal.

Different sites of virion uptake at the intestinal level have been described, depending on the combination of virus and aphid vector under examination. Hindgut cells in cereal aphids are the site of acquisition of *Barley yellow dwarf virus*–PAV (BYDV–PAV) and –MAV (Luteovirus), as well as *Cereal yellow dwarf virus–RPV* (CYDV–RPV) (*Polerovirus*) (Gildow, 1999). Hindgut cells have also been identified as the internalization site of *Soybean dwarf virus* (SbDV; unassigned member of the family *Luteoviridae*) in *Aulacorthum solani* and *M. persicae* (Gildow *et al*., 2000). The posterior midgut of *M. persicae* is involved in the transport...
of Potato leafroll virus (PLRV; genus Polerovirus; Garret et al., 1993) and of Beet western yellows virus (BWYV; genus Polerovirus; Reinbold et al., 2001) from the gut lumen to the haemocoel. In the present study, ultrastructural observations were made to determine the route of CAYBV virions through the aphid vectors *M. persicae* and *A. gossypii*. TEM observations were undertaken to localize CAYBV particles in both gut cells and in ASG cells. These observations reveal a novel situation in which uptake of virions occurs at two distinct positions in the digestive tract.

**METHODS**

CAYBV virions, microinjected into aphids or ingested by membrane feeding, were purified from agro-infected cucumbers (Prüfer et al., 1995) or from aphid-inoculated *Montia perfoliata*. The procedure for purification of CAYBV virions was adapted from van den Heuvel et al. (1991). Purified virions were stored at −80 °C in citrate buffer (0.1 M sodium citrate, pH 6–0).

Viruses-free aphid colonies of *Myzus persicae* (Sulzer) were reared on caged pepper (*Capsicum annuum*) seedlings. Virus-free colonies of *Aphis gossypii* Glover were initiated from a specimen collected in a greenhouse in Colmar (Alsace, France) and reared on caged cucumber seedlings (*Cucumis sativus*). Both cultures were maintained in a controlled environment chamber at 20 °C with a 16 h photoperiod. For the gut observations, nymphs (third or fourth instar) or adults were given a 72 h acquisition access period (AAP) through a stretched Parafilm membrane on various concentrations of purified virions prepared in 0.1 M sodium citrate, pH 6–0, containing 20 % sucrose. Aphids fed on the artificial diet MP148 (Harrewijn, 1983) were used as non-viruliferous controls. After the AAP, some aphids were transferred to healthy *M. perfoliata* seedlings for a 4 d inoculation access period (IAP) to assess their capacity to transmit the virus. These test plants were assayed for CAYBV infection 4 weeks later by double antibody sandwich ELISA (Clark & Adams, 1977) using a rabbit polyclonal antiserum (H. Lecoq, INRA Avignon, France).

To visualize virions at the ASG level, aphids were allowed to acquire virus by membrane feeding, as described above, or purified virions were microinjected into the aphid’s haemocoel as previously described (Bruyère et al., 1997). After injection, aphids were transferred for 24 h to *M. perfoliata* before being prepared for ASG ultrastructural examination (Reinbold et al., 2001). The plants were tested for virus infection 4 weeks later by ELISA.

In order to visualize virions at the basal pole of gut cells, some aphids were microinjected with CAYBV antiserum after 72 h AAP on purified CAYBV. Aphids injected with BWYV anti-P19 polyclonal antiserum (Reutenauer et al., 1993) served as controls in these experiments. Microinjected aphids were fixed and embedded 3 to 5 h later (Reinbold et al., 2001). For ultrastructural examination, aphids were bisected, fixed and embedded in Epon/Araldite plastic as previously described (Reinbold et al., 2001). All observations were made with a Philips EM208 transmission electron microscope operating at 80 kV.

**RESULTS**

**Identification of *M. persicae* gut tissues associated with CAYBV acquisition**

In order to follow the route of CAYBV virions in *M. persicae*, aphids were membrane-fed on purified CAYBV suspensions. When aphids were fed on 100 µg virus ml⁻¹, efficient transmission occurred when 5 aphids (12 infected plants/12 challenged plants) or 1 aphid (8/12) were deposited on each test plant for a 72 h AAP. This experiment illustrates that purified virions prepared from frozen plant material retain the ability to be transmitted by aphids.

The digestive tract of *M. persicae* is divided into four successive parts: the foregut, the anterior midgut (also referred to as the ‘stomach’), the posterior midgut (or tubular midgut) and the hindgut. Our TEM observations were mainly focused on the posterior midgut and the hindgut, known to be intestinal acquisition sites of viruses of the family Luteoviridae (Gildow, 1999), although the observations were also extended to the stomach. Isolated virus particles were occasionally seen in the stomach lumen in 5 out of 9 aphids examined, but no virus-like particle was ever observed within the cytoplasm or in the basal lamina of the stomach cells (Table 1). Virions, either isolated or in rosette-like clusters of several particles, were seen free in the posterior midgut lumen, most frequently close to the microvilli of the apical plasmalemma of the epithelial cells (Fig. 1a). Single virions were occasionally observed in shallow depressions of the apical plasmalemma (Fig. 1a). Various membranous structures containing virus particles were observed inside posterior midgut cells in about one-third of the aphids examined (Table 1). Virus particles were always within membrane-bound vesicles and could be differentiated from ribosomes by their sharper outline, their larger diameter and denser staining. Virions were mainly enclosed in endosome-like vesicles such as multilamellar vesicles or multivesicular bodies (Fig. 1b). Tubular vesicles (Fig. 1b) or coated vesicles (not shown) containing virions were also present. These different structures are known to be associated with receptor-mediated endocytosis (Roth, 1993) and have already been described for posterior midgut cells of *M. persicae* fed on BWYV particles (Reinbold et al., 2001) or on PLRV particles (Garret et al., 1993). At the basal pole of these cells, isolated virus particles were observed embedded in the basal lamina in 3 out of 34 aphids observed (Table 1). Feeding aphids on higher concentrations of purified CAYBV increased the number of aphids (6 out of 11) in which virions were observed in the basal lamina but these virions were always isolated and dispersed along the basal lamina (Table 1).

When virus acquisition by aphids was followed by microinjection of CAYBV antiserum into the haemocoel, clusters of virions were observed trapped between the basal plasmalemma and the basal lamina (Fig. 1c), or aggregated in the basal lamina of the posterior midgut cells, in 65 % of aphids examined (Table 1). Microinjection of virus-specific antibodies is thought to block release of virions in the haemocoel, thus increasing the probability of detecting virus particles at the basal pole of the cell, and also providing evidence concerning the identity of the particles observed (Gildow, 1987). As a negative control, we observed that microinjection of antibodies directed against the BWYV
Table 1. Electron microscopy localization of CABYV in the gut of M. persicae and A. gossypii following membrane acquisition of purified virus

Observation of virions in the gut lumen (Lu), cytoplasm (Cyto) or basal lamina (BL) of epithelial cells of the anterior midgut (stomach), posterior midgut and hindgut of M. persicae and in A. gossypii. Observations were made following a 72 h acquisition feeding on purified virus suspended in 20% sucrose. For each aphid examined, three serial sections of the abdomen were observed. On each of them, one hindgut and two to three midgut sections were generally present. The results are presented as no. of aphids in which virus particles were observed/total no. of aphids examined.

<table>
<thead>
<tr>
<th>CABYV concn</th>
<th>Antibody microinj.</th>
<th>Anterior midgut (stomach)</th>
<th>Posterior midgut</th>
<th>Hindgut</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Lu</td>
<td>Cyto</td>
<td>BL</td>
</tr>
<tr>
<td>M. persicae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 µg ml⁻¹</td>
<td>–</td>
<td>5/9</td>
<td>0/9</td>
<td>0/9</td>
</tr>
<tr>
<td>265 µg ml⁻¹</td>
<td>–</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>265 µg ml⁻¹</td>
<td>Anti-CABYV†</td>
<td>2/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>265 µg ml⁻¹</td>
<td>Anti-P19‡</td>
<td>3/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>No virus*</td>
<td>–</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>A. gossypii</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 µg ml⁻¹</td>
<td>–</td>
<td>1/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>237 µg ml⁻¹</td>
<td>–</td>
<td>7/8</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td>264 µg ml⁻¹</td>
<td>Anti-CABYV†</td>
<td>2/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>No virus*</td>
<td>–</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

*Feeding in the control treatment was on MP148 artificial diet (Harrewijn, 1983) without virus.
†To enhance the likelihood of detecting virions, aphids were given a 72 h acquisition feeding on membranes, followed by microinjection of polyclonal anti-CABYV antibody into the haemocoel.
‡Aphids were treated as in (†), but injected with antiserum to the nonstructural P19 protein of BWYV.
§Only a few single particles were observed in these aphids.

non-structural protein P19 did not increase the number of particles observed in these locations, nor did the treatment induce clumping of virus particles. Instead, only isolated virions (identical to those observed without microinjection of specific antibodies) were sporadically observed in the basal lamina of 4 aphids out of 8 examined (Table 1).

Observations were also carried out on the aphid hindgut, which can be easily differentiated from the posterior midgut by its typical flat and long cells, by the low abundance of membrane invaginations formed by the apical plasmalemma and by the presence of tubules lining the apical plasmalemma (O’Loughlin & Chambers, 1972). Virus particles, both free and in rosette-like aggregates (Fig. 2a), were frequently seen in the lumen of the hindgut, as well as close to the apical plasmalemma. Linear arrays of virions in shallow depressions or in deep invaginations on the surface of the plasmalemma were also routinely observed (Fig. 2a). Whatever the virus concentration delivered to the aphids, a high percentage of the aphids observed contained virions within hindgut cells (Table 1). Virions were occasionally seen clustered in larger, spherical vesicles (not shown) but they were mainly present in tubular vesicles and in small circular vesicles containing one or more virions (Fig. 2a, b). When M. persicae was fed on CABYV particles at a concentration of 100 µg ml⁻¹, some hindgut epithelial cells were heavily charged with virions, as shown in Fig. 2(b), but virus particles were never observed in the basal lamina of these cells (Table 1), although tubular vesicles were sometimes observed in very close proximity to the basal pole of the cell (Fig. 2c). Virions were seen between the basal plasmalemma and the basal lamina or embedded in the basal lamina of hindgut cells only when aphids were fed on higher virus concentrations or when CABYV antiserum was microinjected subsequently (Table 1). In this latter treatment, virions were always observed in clusters (not shown). In contrast, only isolated virions were seen after microinjection of BWYV P19 antiserum (Table 1). Virus-like particles were never observed in any cellular compartment in M. persicae fed on artificial medium lacking virus (Table 1).

Identification of A. gossypii gut tissues associated with CABYV acquisition

The ultrastructure of the alimentary canal of A. gossypii has not been previously studied but our observations revealed that its anatomy was not notably different from that observed for M. persicae (Ponsen, 1977) and for other previously described aphid species (Gildow, 1985, 1993). When A. gossypii was allowed to acquire CABYV particles by membrane feeding, transmission occurred with moderate to high efficiency when 5 aphids (12 infected plants/12 challenged plants) or 1 aphid (6/12) were deposited on each test plant after a 72 h AAP. Results of TEM observations of CABYV particles in the A. gossypii digestive tract after
Fig. 1. Transmission electron micrograph showing ultrastructure of *M. persicae* posterior midgut cells after membrane acquisition of purified virus. (a) CABYV virions mostly in rosette-like aggregates close to microvilli (mv) in the lumen (lu). Note the presence of a single virion in an invagination of the apical plasmalemma (arrow). (b) In the cytoplasm of midgut cells, virus particles in tubular vesicles (tv) and in endosome-like structures of various types [multilamellar vesicles (mlv), multivesicular bodies (mvb)] in the cytoplasm. (c) The arrow indicates a cluster of four virus particles between the basal plasmalemma (bpl) and basal lamina (bl) following microinjection of CABYV-specific antibodies into the haemocoel. Bar, 150 nm.
**Fig. 2.** Electron micrograph of ultrathin sections through the hindgut of *M. persicae* after acquisition of purified virus. (a) CABYV virions free or in rosette in the hindgut lumen (lu), lining the apical plasmalemma (apl) in shallow depressions of the membrane (arrow) or in an invagination of the plasmalemma (arrow). In the cytoplasm, virions arranged in a long tubular vesicle (tv). (b) Numerous tubular vesicles in the cytoplasm of a hindgut cell. Some virions are singly enclosed in vesicles (arrows). (c) A tubular vesicle containing virions in close proximity to the basal plasmalemma (bpl) next to basal lamina (bl). r, ribosomes. Bar, 150 nm.
membrane feeding are presented in Table 1. Even though virus particles were frequently observed in the lumen, no virus particles were ever observed inside the stomach cells, whatever the concentration of virus delivered to the aphids (Table 1). Using a virus concentration of 100 μg ml⁻¹ for acquisition, virus particles were observed in the lumen of both the posterior midgut and the hindgut (Table 1). However, only a few isolated virus particles were observed in the cytoplasm of posterior midgut epithelial cells in 1 out of 11 aphids examined (Table 1) and no virus-like particle was detected in the basal lamina (Table 1). When the virus concentration delivered to aphids was increased, many virus particles were observed in the lumen (Fig. 3a), in small depressions of the plasmalemma (not shown),

![Fig. 3. CABYV virions in the midgut of A. gossypii following membrane feeding on purified virus. (a) Virions in the lumen (lu) free or less frequently in rosettes close to the microvilli (mv). (b) In the cytoplasm, virus particles in tubular vesicles or in circular vesicles containing one virion (arrow). (c) CABYV virions embedded in a cluster in the basal lamina (bl) after their release from the cytoplasm of the cell (cyto) following microinjection of CABYV-specific antiserum into the haemocoel. bpl, basal plasmalemma; r, ribosomes. Bar, 150 nm.](image-url)
adsorbed on the surface of the apical plasmalemma (Fig. 4a) or in deep invaginations observed on the apical plasmalemma of hindgut cells (Fig. 4b) in the process of being endocytosed. In the cytoplasm of posterior midgut epithelial cells, virions were present in 3 of 17 aphids examined and in hindgut epithelial cells in 8 of 17 aphids examined (Table 1). In midgut epithelial cells, the virions were enclosed in endosome-like vesicles (not shown), and in vesicles containing one or more virions (Fig. 3b). Virions were also observed arranged in one or two rows in tubular vesicles (Fig. 3b). Similarly to what was observed in *M. persicae* hindgut cells, virions were mostly present in tubular vesicles (Fig. 4c). Release of virions into the haemocoel from these intestinal locations could only be visualized after microinjection of CABYV antiserum (Table 1). Following this treatment, aggregated virions were observed with confidence in the basal lamina of posterior midgut epithelial cells (in 3 aphids out of 8 examined; Fig. 3c) and of

![Image](http://vir.sgmjournals.org) 3479

**Fig. 4.** CABYV virions in the hindgut of *A. gossypii* after delivery of purified virus to aphids by membrane feeding. (a) Virions free in the lumen (lu) or adsorbed in a linear array on the surface of the apical plasmalemma (apl) (arrow). (b) In the cytoplasm (cyto), a deep invagination (arrow) of the apical plasmalemma (apl). (c) In the cytoplasm (cyto), a tubular vesicle (tv) containing virions in very close proximity to the apical plasmalemma (apl). r, ribosomes. Bar, 150 nm.
hindgut epithelial cells (in 4 aphids out of 7 examined; not shown). When *A. gossypii* was microinjected with BWYV P19 antiserum there was high aphid mortality and poor conservation of the gut tissue, and observations at the basal pole of the cells were only made on 1 aphid in which no virions could be visualized outside the cell in the posterior midgut. Nonviruliferous *A. gossypii*, examined as a control, was free of virus-like particles (Table 1).

**Localization of CABYV in accessory salivary glands cells of *M. persicae* and *A. gossypii***

When *M. persicae* was fed on a purified virus suspension (130 µg ml$^{-1}$) or when the same concentration was microinjected into the haemocoel, virions were only occasionally observed in the basal lamina surrounding the ASG cells or in invaginations of the basal plasmalemma (Table 2). The virus concentration for microinjection was therefore raised to 350 µg ml$^{-1}$, which allowed us to observe numerous virions embedded in the basal lamina in half of the aphids examined (Table 2, Fig. 5a). Virus particles in invaginations of the basal plasmalemma (Fig. 5a) in tubular vesicles and coated vesicles (not shown) were also seen in the cytoplasm of the ASG cells (Table 2). Free virions were detected in the salivary canal, presumably in the process of being excreted with saliva (Table 2).

The same experimental conditions were used for observation of CABYV virions in *A. gossypii* ASG cells. The ultrastructure of these cells did not differ from that of the other aphid species examined previously (Gildow, 1982; Gildow & Rochow, 1980; Ponsen, 1977). The results of our observations are summarized in Table 2. Many particles were observed specifically embedded in the basal lamina surrounding the ASG (Fig. 5b). Virions were also frequently seen either in shallow pits of the basal plasmalemma (Fig. 5b) or enclosed in deep and narrow invaginations of this membrane (Fig. 5b). Virus particles were likewise visualized in the salivary canal, along the apical plasmalemma or free in the canal lumen (Fig. 5c). None of the virus-free *M. persicae* and *A. gossypii* sampled from the stock colony showed virus particles near or inside ASG cells (Table 1).

In parallel, transmission of virus to plants by microinjected aphids was monitored by transferring 5 aphids to each test plant. When *M. persicae* nymphs were microinjected with a purified suspension of CABYV at 130 µg ml$^{-1}$, half of the test plants became infected (5 infected plants out of 10 plants analysed). We observed that less than half of the microinjected aphids survived the treatment (22 survivors out of 50 injected aphids 24 h after microinjection). When the virus concentration delivered into the haemocoel of *M. persicae* was raised to 350 µg ml$^{-1}$, no virus transmission to test plants was observed. However, we noticed that this treatment had a dramatic effect on aphid survival (4 survivors out of 40 injected aphids), which could explain the observed loss of virus transmissibility. Microinjection of 350 µg ml$^{-1}$ CABYV suspension into *A. gossypii* also resulted in a reduction of aphid survival (5 survivors out of 40 microinjected), but we still observed transmission of virus to test plants for 3 plants out of 8 analysed. The reason for elevated aphid mortality following microinjection of the concentrated virus solution is not known but could be due to the presence in the purified viral suspension of a plant compound having a toxic effect when delivered directly to the haemocoel at sufficiently high concentration.

**DISCUSSION**

In the present paper we describe the transport of CABYV in its two efficient vector species, *M. persicae* and *A. gossypii*. To our knowledge, these are the first data concerning ultrastructural analysis of virus uptake in the alimentary system of *A. gossypii*. Concerning the latter point, our findings indicate that the anatomy of the midgut, hindgut and ASG of *A. gossypii* is not notably different from the corresponding structures, described in the literature, of *M. persicae* or of other aphid species that transmit luteovirids. With respect to acquisition of luteovirid particles,

<table>
<thead>
<tr>
<th>Virus acquisition</th>
<th>CABYV concentration</th>
<th>Sites of virus particle visualization</th>
</tr>
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<tbody>
<tr>
<td><em>M. persicae</em></td>
<td>Membrane feeding</td>
<td>BL      INV  TV  CV  SC</td>
</tr>
<tr>
<td></td>
<td>130 µg ml$^{-1}$</td>
<td>1/6 1/6 0/6 0/6 0/6</td>
</tr>
<tr>
<td>Microinjection</td>
<td>130 µg ml$^{-1}$</td>
<td>3/4 1/4 0/4 0/4 0/4</td>
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<tr>
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<tr>
<td></td>
<td>Aphid control*</td>
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</tr>
<tr>
<td><em>A. gossypii</em></td>
<td>Microinjection</td>
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</tr>
<tr>
<td></td>
<td>Aphid control*</td>
<td>6/6 6/6 4/6 4/6 6/6</td>
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*Apiphid controls in these experiments were directly taken from the virus-free stock colony.
Fig. 5. For legend see page 3482.
our results with CABYV reveal a novel type of ‘dual’ tissue specificity for virion transport across the gut of the vector. For other combinations of virus and aphid species that have been studied, the site of acquisition into the haemocoel has been shown to be either the posterior midgut or the hindgut, but acquisition has never been reported to occur at both locations for the same virus species. Thus, the hindgut of cereal aphid species (Rhopalosiphum padi, R. maidis, Schizaphis graminum, Sitobion avenae) is the acquisition site for BYDV-PAV and -MAV (genus Luteovirus) and for CYDV-RPV (genus Polerovirus) (Gildow, 1999). PLRV and BWYV (genus Polerovirus) are acquired via the posterior midgut of M. persicae (Garret et al., 1993; Reinbold et al., 2001), whereas SbDV (not currently assigned to a genus) is acquired through the hindgut of M. persicae and Aulacorthum solani (Gildow et al., 2000). For the moment, CABYV represents the only member of the Luteoviridae which can be transcytosed into the haemocoel at two histologically different levels along the alimentary tract, the posterior midgut and the hindgut. It should also be noted that, taken together, the aforesaid observations indicate that the site for luteovirid particle acquisition is not fixed for a given aphid species. Thus, virus uptake in M. persicae has been shown to occur through either the posterior midgut (PLRV and BWYV), the hindgut (SbDV), or both simultaneously, as shown here for CABYV.

Acquisition and transmission of CABYV were observed to occur similarly in its two vector species. It should be noted, however, that in order to visualize virions in the digestive tract, we were obliged to use a higher virus concentration for A. gossypii than for M. persicae, although we found that these species did not differ in transmission efficiency when fed with the same virus concentration. An opposite situation pertained at the ASG level, where virions were more numerous in cells of A. gossypii than of M. persicae when microinjected with equivalent virus concentrations. The relative efficiency of transcytosis thus appears to differ between the aphid species and between the two epithelial barriers. These observations may reflect differences in transcytosis rates or may be related to the frequency of the putative virus receptors at the corresponding epithelium. Further work using quantitative RT-PCR will help to compare the dynamics of virus transport across each type of epithelium in the two vector species (Lett et al., 2002).

Our ultrastructural observations detected virion-containing membrane-bound organelles in intestinal cells similar to those described by previous workers for other luteovirids and in accordance with the ‘Gildow model’ for acquisition (Gildow, 1999). The observed virus-containing structures included clathrin-coated pits and vesicles, multilamellar and multivesicular endosome-like bodies, and tubular vesicles. Moreover, the transport of CABYV virions across the ASG cells does not seem to differ notably from previous reports for other virus–vector combinations. At the cellular level, the transcytosis mechanism at the intestinal and salivary epithelia is therefore very likely to be the same for all members of the Luteoviridae and all vector species. Transcytosis is an important pathway for membrane trafficking which allows selective and rapid transcellular vesicular transport from the apical to the basolateral pole of epithelial cells (Mostov et al., 2000), and viruses have no doubt hijacked this pathway for their own ends. Specificity of luteovirid entry into epithelial cells is thought to be mediated by receptors associated with the cell membrane, followed by endocytosis of virions, according to the so-called ‘receptor-mediated endocytosis’ (Pastan & Willingham, 1985) mechanism, which is also widely used by animal viruses to enter host cells (for review, see Sieczkarski & Whittaker, 2002). This step is generally followed by virus release into and replication in the cytosol, in contrast to luteovirids in their vectors (Eskandari et al., 1979; Tamada & Harrison, 1981). However, acquisition of luteovirids has many points in common with the manner in which human immunodeficiency viruses traffic through the gastrointestinal wall without any uncoating and multiplication (Bomsel, 1997).

In the midgut cells of viruliferous M. persicae and A. gossypii, typical virus-laden clathrin-coated and tubular vesicles were observed as well as endosome-like bodies. The hindgut cells of both species, on the other hand, rarely harboured multivesicular bodies; instead, tubular vesicles were predominant. This observation may reflect physiological differences in the transcytosis process between midgut and hindgut and could be related to the presence of different receptors on the two types of membranes.

Release of virions from intestinal cells into the haemolymph was confirmed after microinjection of CABYV antiserum into the haemocoel. Following this treatment, aggregated particles were seen trapped by the antibodies between the basal plasmalemma and the basal lamina or within the basal lamina. No virion-containing vesicle was ever observed fused to the basal plasmalemma, presumably indicating that, once virions have attained this site, their release from the cell occurs rapidly.

The significance of the dual tissue specificity during acquisition of CABYV virions in terms of virus receptors...
is not understood. One possible hypothesis is that internalization of different poleroviruses such as CABYV and BWYV relies on the same biochemical ‘partner’ (i.e. a receptor or receptor complex), which would be present in both intestinal locations and in both aphid species. In this hypothesis, the inability of BWYV to enter hindgut cells could be explained by different ‘environmental’ conditions in the hindgut (pH, for instance) that would inhibit the virion–receptor interaction for BWYV but not for CABYV (due to putative differences in the physico-chemical properties of the virions). Alternatively, CABYV and BWYV could utilize different receptors. The receptors would differ not only in their binding affinity for the different poleroviruses but also in their distribution in the midgut and hindgut. Whatever the case, our data support the existence of a tight association between receptors on the apical surface of aphid gut cells and determinants borne on the surface of the virus particle.

The nature of the viral determinants governing tissue specificity during acquisition is not known. Sequence comparisons of structural proteins of viruses that are acquired at the posterior midgut, at the hindgut, or at both levels, do not reveal any obvious sequence motif which could be correlated to tissue specificity (for amino acid comparisons, see Guilley et al., 1994; Mayo & Ziegler-Graff, 1996). Further information on this question could be gained using chimeric viruses created by exchanging structural protein genes between viruses with different intestinal acquisition sites. Such chimeras between BWYV and CABYV have recently been obtained and their transmissibility of passage for potato leafroll virus from the gut lumen to the haemocoel in the aphid vector, *Myzus persicae* Sulz. *Arch Virol* 131, 377–392.


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