Review

Insect vector-mediated transmission of plant viruses

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A R T I C L E   I N F O

Article history:
Received 24 January 2015
Returned to author for revisions
17 February 2015
Accepted 6 March 2015
Available online 29 March 2015

Keywords:
Capsid protein
Virus glycoprotein
Aphid
Whitefly
Thrips
Planthopper
Leaffopper
Hemipteran
Virus–vector interactions

A B S T R A C T

The majority of plant-infecting viruses are transmitted to their host plants by vectors. The interactions between viruses and vector vary in duration and specificity but some common themes in vector transmission have emerged: 1) plant viruses encode structural proteins on the surface of the virion that are essential for transmission, and in some cases additional non-structural helper proteins that act to bridge the virion to the vector binding site; 2) viruses bind to specific sites in or on vectors and are retained there until they are transmitted to their plant hosts; and 3) viral determinants of vector transmission are promising candidates for translational research aimed at disrupting transmission or decreasing vector populations. In this review, we focus on well-characterized insect vector-transmitted viruses in the following genera: Caulimovirus, Crinivirus, Luteovirus, Geminiviridae, Reovirus, Tospovirus, and Tenuivirus. New discoveries regarding these genera have increased our understanding of the basic mechanisms of virus transmission by arthropods, which in turn have enabled the development of innovative strategies for breaking the transmission cycle.

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http://dx.doi.org/10.1016/j.virol.2015.03.026
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Introduction

There are more than 2000 virus species and those affecting plants include viruses of at least 21 families and 8 unassigned genera, many of which cause important diseases of various plants that humans grow for food and/or fiber (Hull, 2014). In addition, many plant viruses have been found associated with non-cultivated plants,
and new plant viruses are being discovered every day. Virus diseases make up 47% of the new emerging diseases affecting plants (Anderson et al., 2004). Thus, plant infecting viruses are very successful. As virus hosts, plants differ from animals and even bacteria in several ways, but one that is critical in terms of virus biology is that plants are sessile. In order to survive, plant-infecting viruses must have an efficient means to move from one plant host to another. To do so, the great majority of plant viruses utilize specific vectors to ensure their ability to move from one plant host to another, and to ensure their survival, plant viruses encode for specific proteins that facilitate this process (Table 1) (Kritzman et al., 2002; Moreno et al., 2012; Moritz et al., 2004). Although there are different types of plant-associated organisms including fungi, nematodes and various types of invertebrates that serve as vectors for different plant viruses, the majority of plant viruses utilize specific plant feeding insects as their primary vector(s), and here we focus on insect-transmitted plant viruses. Proteins encoded by different plant viruses have been identified to specifically interact with their respective insect vectors and facilitate virus transmission, and there are many excellent papers and reviews on these subjects (Ammar et al., 2009; Hogenhout et al., 2008; Ng and Falk, 2006). However, recent studies suggest additional complexities for plant virus:vector relationships (Blanc et al., 2014; Gutierrez et al., 2013). Here we discuss the current state of this knowledge, but also recent exciting translational applications of fundamental knowledge of virus:insect vector interactions that has opened up new doors for plant virus and insect vector control.

### Historical perspective of modes of virus transmission by insect vectors

The biology of plant virus transmission has been studied for more than 100 years (Ando, 1910; Gutierrez et al., 2013; Takami, 1901). Since then many studies have examined the specificity of insect vector-mediated plant virus transmission and clearly demonstrated that there are specific molecular determinants required (e.g. see Pirone, 1964; Rochow, 1970; Storey, 1933). These studies led to discoveries of virus proteins that in part, determined vector-specific interactions (Table 1). Because plant virologists realized that vector transmission of

### Table 1

<table>
<thead>
<tr>
<th>Taxonomic family</th>
<th>Virus genus</th>
<th>Representative Virus</th>
<th>Type of Vector</th>
<th>Virus-encoded “transmission proteins”&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Location of virion retention or initial entry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potyviridae</td>
<td>Potyvirus</td>
<td>Tobacco etch virus (TEV)</td>
<td>Aphid</td>
<td>CP, HC-Pro</td>
<td>Stilet</td>
</tr>
<tr>
<td>Bromoviridae</td>
<td>Cucumovirus</td>
<td>Cucumber mosaic virus (CMV)</td>
<td>Aphid</td>
<td>CP</td>
<td>Stilet</td>
</tr>
<tr>
<td>Caulimoviridae</td>
<td>Caulimovirus</td>
<td>Cauliflower mosaic virus (CaMV)</td>
<td>Aphid</td>
<td>CP, P2, P3</td>
<td>Stilet, acrostyle</td>
</tr>
<tr>
<td>Clasteroviridae</td>
<td>Crinivirus</td>
<td>Lettuce infectious yellows virus (LIYV)</td>
<td>Whitefly</td>
<td>CPm</td>
<td>Foregut</td>
</tr>
<tr>
<td>Luteoviridae&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Luteovirus</td>
<td>Barley yellow dwarf virus (BYDV)</td>
<td>Aphid</td>
<td>CP-RTP</td>
<td>Midgut, hindgut</td>
</tr>
<tr>
<td>Geminiviridae&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Begomovirus</td>
<td>Tomato yellow leaf curl virus (TYLCV)</td>
<td>Whitefly</td>
<td>CP</td>
<td>Midgut, filter chamber</td>
</tr>
<tr>
<td>Bunyaviridae&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Tospovirus</td>
<td>Tomato spotted wilt virus (TSWV)</td>
<td>Thrips</td>
<td>G&lt;sub&gt;N&lt;/sub&gt;</td>
<td>Midgut</td>
</tr>
<tr>
<td>Reoviridae&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Phytoreovirus</td>
<td>Rice dwarf virus (RDV)</td>
<td>Leafhopper</td>
<td>P2*</td>
<td>Midgut, filter chamber</td>
</tr>
<tr>
<td>Rhabdoviridae&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Nuclearrhabdovirus</td>
<td>Maize mosaic virus (MMV)</td>
<td>Planthopper</td>
<td>G</td>
<td>Midgut</td>
</tr>
</tbody>
</table>

Text in blue indicates plant viruses that are non-circulative in their respective insect vectors, they do not enter the body as part of the transmission process. Text in red indicates viruses that do enter the body as part of the transmission process and either circulate (indicated by superscript 1) or circulate and replicate (indicated by superscript 2) within the insect vector body. CP—capsid protein or major capsid protein; HC-Pro—helper component proteinase, P2—non-virion helper component protein, P3—protein anchored in the CaMV virion CPm—minor capsid protein, CP-RT—capsid protein readthrough domain, G<sub>N</sub>—glycoprotein N, P2*—outer capsid protein encoded by RDV segment 2,G—glycoprotein (indicated by superscript 3).
plant viruses is specific, and well before we were able to analyze virus genomes so readily by contemporary sequencing and bioinformatics analysis, vector transmission properties were used as critical criteria for plant virus taxonomy. Today’s sequence data-based taxonomy largely supports these earlier findings. Within a given plant virus genus, all species utilize the same type of vector and show the same transmission relationship; for example, all members of the genus *Potyvirus* are transmitted by various aphid vectors in a non-circulative (non-persistent) manner. However, virus species of other genera even within the same virus family may have other types of vectors. Other examples from the *Potyviridae* are the Ipomoviruses and Tritimoviruses that are transmitted by whiteflies and eriophyid mites, respectively. Table 2 shows as an example the vectors for viruses within the different genera of the family *Potyviridae*.

While some plant viruses may be transmitted by several different vector species (e.g. aphids and the non-persistent transmitted potyviruses and *Cucumber mosaic virus* (CMV)), other viruses are highly specific, being transmitted perhaps by a single species of insect vector, as shown by the circulative-propagative transmitted rhabdoviruses. Early research into the biology of insect transmission of plant virus:vector interactions gave rise to terms describing transmission relationships based on acquisition and inoculation thresholds, as well as retention of the virus by its vector(s). Thus, four basic types of insect vector:plant virus transmission relationships were described: non-persistent; semi-persistent; persistent:circulative and persistent:propagative (see Ng and Falk, 2006). More recent terminology emphasizes how plant viruses interact with their insect vectors (Blanc et al., 2014). Some bind to specific insect vector cuticular locations without entering cells (noncirculative) and others enter the insect gut and circulate or replicate within the insect vector body (circulative; see Fig. 1 and Table 1).

### Noncirculative transmission

Noncirculative plant viruses are retained in the stylet or foregut. Early studies with potyviruses and *Cauliflower mosaic virus* (CaMV) first demonstrated that aphid transmission of these viruses results not from mere contamination of virions on aphid stylets, but from specific interactions. Pirone (1964) showed that by using high concentrations of two noncirculative aphid-transmitted viruses, *Cucumber mosaic virus* (CMV) and *Alfalfa mosaic virus* (AMV), that aphids could acquire these viruses from solutions held between two paraffin membranes and subsequently transmit these viruses to plants. However, similarly high concentrations of infectious *Tobacco mosaic virus* (TMV) and the potyvirus, *Turnip mosaic virus* (TuMV) could not. CMV, AMV and TuMV were known to be aphid-transmitted in nature, so what explained the non-transmissibility of TuMV in these types of feeding experiments? We now know that viruses such as CMV contain virus-encoded aphid transmission determinants as part of the virion capsid protein (Perry et al., 1998, 1994), but potyviruses and CaMV require intact virions plus additional non-structural virus protein(s). Gower and Kassanis (1974a, 1974b) used simple, but elegant experiments to show that more than virions were needed for aphid transmission of *Potato virus Y* (PVY), and they coined the term “helper component” as the “virus-induced” factor that aphids must acquire simultaneously with, or prior to acquiring virions of PVY. Since these seminal discoveries the potyvirus-encoded helper component, or HCPro, has been very well studied (Valli et al., 2014) and its role in potyvirus transmission by aphids is well known (Blanc et al., 1998, 2014; Ng and Falk, 2006; Peng et al., 1998). Thus, it is now understood that two strategies are recognized for viruses that are transmitted by insects (not just aphids) in a noncirculative manner. These are the capsid strategy and the helper strategy (reviewed in Blanc et al., 2014; Ng and Falk, 2006). However, what is still in its infancy is our ability to identify the insect vector receptors that are involved in interacting with “transmission proteins” to allow for successful virus transmission. But some recent studies using powerful, contemporary imaging technologies have in some cases given much greater understanding of at least where the hypothetical receptors are located, and in some cases dynamic interactions that are necessary for vector-mediated virus acquisition and transmission to occur (Blanc et al., 2014).

#### Non-circulative, stylet-borne transmission

CaMV is a para-retrovirus (family *Caulimoviridae*) and virions have isometric *T*=7 capsids of approximately 52 nm in diameter, composed primarily of the P4 (gag or capsid) protein (Fig. 1). An elaborate and intricate tritrophic interaction determines aphid transmission of CaMV. CaMV can be acquired by aphids probing, transient puncturing of the epidermal, mesophyll, parenchyma cells of infected leaf tissues, but also by when aphids feed on phloem tissues, i.e. long periods of sustained ingestion from the phloem (Palacios et al., 2002). As alluded to above, CaMV uses a helper strategy to achieve aphid transmission (Lung and Pirone, 1974), but unlike for aphid transmission of potyviruses, the aphid transmission of CaMV is more complex. CaMV aphid transmission requires interactions between three CaMV-encoded proteins, one of which, P2, also interacts with the aphid stylet. Another CaMV-encoded protein, P3, is anchored within the virion capsid shell, and CaMV-encoded P2 is the non-virion helper component protein which binds by its N-terminus to the aphid stylet, but its C-terminus also binds to the N-terminus region of P3 (Blanc et al., 2014; Hoh et al., 2010; Plisson et al., 2005).

CaMV replicates in the plant cell cytoplasm, and large (up to 4.5 μM in diameter), electron dense distinct viroplasms composed mostly of the CaMV-encoded P6 protein and progeny virions are visible by light and electron microscopy as inclusion bodies in the cytoplasm (Drucker et al., 2002; Shalla et al., 1980). But CaMV-infected plant cells also contain another type of cytoplasmic inclusion body, the electron-lucent IB (elIB; Espinoza et al., 1991). The primary component of these elIBs is the CaMV-encoded P2 protein (Drucker et al., 2002; Espinoza et al., 1991; Khelifa et al., 2007). Because the elIBs contain essentially all of the P2 within the cell and because P2 is essential for binding CaMV to viroms and aphid stylets, the elIBs are referred to recently as transmission bodies (TBs; Bak et al., 2013; Martiniere et al., 2013). Recent, work by this group demonstrated that CaMV acquisition by aphids results from dynamic plant cell and virus responses to aphid activity.

Biological data clearly demonstrated that the three CaMV-encoded proteins, P2, P3 and P4, are required for CaMV acquisition by aphids, but detailed microscopic examination of CaMV-infected plant cells shows that these proteins are not co-localized (Bak et al., 2013; Drucker et al., 2002; Espinoza et al., 1991; Khelifa et al., 2007). However, when aphids explore cells via probing activity they produce minute wounds that quickly heal. Upon probing,
CaMV-induced TBs “sense” cell wounding and almost immediately respond by dissociating and redistributing P2 onto cellular microtubules (Bak et al., 2013; Martiniere et al., 2013). The TB dissociation and P2 relocalization occurs in cells only very near to aphid probing, and can be induced by some chemical treatments or cell wounding (Martiniere et al., 2013). Furthermore, the relocalization of P2 is temporary and reversible for when chemical inducers are removed, or after aphids were removed from leaves, P2 redistributed back into TBs (Martiniere et al., 2013). The authors interpreted their data suggesting that TBs “sense” aphid activity and reorganize as an active response to increase the probability of CaMV transmission by aphid vectors (Martiniere et al., 2013). They proposed naming this activity as “virus perceptive behavior” (Martiniere et al., 2013), and additional work shows that CaMV virions from viroplasms also relocalize, probably to be able to interact with P2 and be transmitted by aphid vectors (Bak et al., 2013).

Uzest et al. (2010, 2007) used creative and high resolution approaches to identify the aphid stylet binding sites for the CaMV-encoded P2. They created P2:GFP and P2 mutant:GFP fusion proteins by recombinant baculovirus expression in Sf9 cells (Uzest et al., 2007). They then performed aphid stylet in vitro binding assays with these proteins and used epifluorescence microscopy to carefully examine aphid stylets for GFP fluorescence. Several important findings emerged from these experiments. First, GFP fluorescence was seen...
only when styles were tested with P2:GFP fusion proteins, not when they were tested with GFP only. Second, the P2-GFP bound to vector aphid styles but not the stylet of non-vector aphids. Third, a mutant P2-GFP with a Q to Y substitution at amino acid 6 in P2 (P2Rev5-GFP) did not bind to vector aphid styles. This mutation had previously been shown to disable the biological activity of P2, rendering it unable to support CaMV aphid transmission (Moreno et al., 2005). Most important, the fluorescence for P2-GFP was not randomly distributed, or scattered along the styles, but was localized to a unique and tiny region at the aphid stylet tip (Uzest et al., 2007). Furthermore, the ability of stylets to bind the P2-GFP was abolished by proteinase K, but not by trypsin, pronase E, n-hexane, chloroform:methanol or sodium metaperiodate (Uzest et al., 2007). The interpretation from these data was that there is a specific stytel region containing specific non-glycosylated proteinaceous receptors. These receptors are most likely embedded in chitin within the salivary canal extremity stylet. More recent ultrastructural examination showed that this region is conserved among aphids (Uzest et al., 2010). These authors referred to this unique anatomical feature to be called the “acrostyle” (Uzest et al., 2010), and suggested that due to its uniqueness, this is an ideal place for receptors for “CaMV and perhaps those of other noncirculative viruses” (Uzest et al., 2007).

**Non-circulative, semipersistent transmission**

While non-circulative, nonpersistent transmission of plant viruses is only so far found among viruses transmitted by aphid vectors, several aphid, whitefly and leafhopper-transmitted viruses show a non-circulative, semipersistent transmission relationship (Ng and Falk, 2006). Viruses showing this type of transmission relationship are retained by viruliferous vectors for longer time periods than are viruses transmitted in a nonpersistent manner (Ng and Falk, 2006), and they are lost by viruliferous vectors during molting. The latter property supports the hypothesis that these viruses are not internalized within insect vector guts, but are likely retained in chitin-lined areas that are lost during vector insect molting. Attempts to localize several viruses transmitted in a non-circulative semipersistent manner suggested that they were not borne at the tips of the vector styles, but definitive localization and correlation with the vectors’ ability to transmit viruses with these properties has been mostly lacking (Blanc et al., 2014). Recent findings with the *Bemisia tabaci*-transmitted *Lettuce infectious yellows virus* (LIYV, genus Crinivirus, family Closteroviridae) have identified not only the LIYV-encoded protein determining its transmission by *B. tabaci*, but also where in the whitefly the LIYV virions are retained (Chen et al., 2011a; Stewart et al., 2010; Tian et al., 1999), thereby giving some suggestion as to the type of vector behavior involved in transmitting LIYV back to plants.

Tian et al. (1999) showed that the filamentous LIYV virions were structurally complex, composed of at least four LIYV-encoded proteins, and that the purified virions could be acquired in vitro, and subsequently transmitted to plants by *B. tabaci*. This work demonstrated for the first time that LIYV has a capsid and not helper strategy for its whitefly vector transmission. The two major virion proteins are the CPm and CP (minor capsid protein and major capsid protein, respectively). The CPm covered only about 10% of the virion from one end, thus showing that virions were morphologically polar (Tian et al., 1999, see Fig. 2). As purified virions were transmissible by vector whiteflies, they performed experiments in attempts to identify which of them are likely to be vector transmission determinants. They first incubated purified LIYV virions with antibodies specific to each of the four virion proteins separately, and then after centrifugation fed the supernatant to vector whiteflies and tested their ability to transmit LIYV to plants. Only antibodies to the LIYV CPm neutralized the ability of *B. tabaci* to acquire and transmit LIYV to plants. Even though antibodies to the CP cover most of the LIYV virion, CP-treated LIYV virions were still very efficiently transmitted by *B. tabaci*. These results strongly supported the hypothesis that the CPm is a LIYV-encoded protein involved in the transmission of LIYV by *B. tabaci*. Subsequent mutations in the CPm which did not affect the ability of LIYV to form virions or to systemically infect *N. benthamiana* plants, did abolish the ability of the mutant LIYVs to be transmitted by *B. tabaci* (Stewart et al., 2010).

Chen et al. (2011a) combined biology and molecular biology, coupled with contemporary imaging technologies in attempts to identify LIYV virion binding sites in vector whiteflies. They fed vector and non-vector whiteflies (*B. tabaci* A and B biotypes, respectively) sequentially on artificial diets containing virions or LIYV virion capsid proteins produced by expression in *E. coli*, followed by solutions containing different antibodies (including fluorescent-labeled antibodies) to bind to LIYV virions or capsid proteins in whiteflies. Then whiteflies were examined by both wide field fluorescence microscopy and confocal laser scanning microscopy to visualize where the fluorescent antibodies could be found. They showed that fluorescence was localized only in a precise region of the foregut, the anterior foregut region (cibarium) of the A biotype *B. tabaci* vector whitefly. Essentially no binding, as assessed by fluorescence, was seen when non-vector B biotype *B. tabaci* fed on the same preparations. This strongly suggested that LIYV virions bound to this specific location in vector whiteflies (Fig. 1B).

Of the LIYV capsid proteins evaluated (CP, CPm, HSP70h, IF59), only CPm was found to bind within the anterior foregut and again, only in vector Biotype A *B. tabaci*. They also used a recombinant LIYV CPm mutant, which was not transmissible by A biotype *B. tabaci*, and showed that it also did not bind, but when the mutation was restored to give wildtype CPm, they observed LIYV transmission and specific binding in the anterior foregut. Like the work with CaMV, the work by Chen et al. (2011a) demonstrated the specificity of binding only in vector species, and only at a precise location, for LIYV this is within the stylet/foreguts. Furthermore, unlike CaMV which is inoculated to plants by aphid probing, LIYV is transmitted to, and acquired from plants by *B. tabaci* feeding, i.e. sustained periods of ingestion from plant vascular tissues. Because CaMV virions are located in the vector aphid acrostyle, inoculation to plants likely occurs when aphids salivate during probing, the saliva flows through the acrostyle region. However because LIYV is in the foregut, the work of Chen et al. (2011a) suggests that release of LIYV from the anterior foregut during inoculation to plants most likely can occur during egestion or regurgitation by the viruliferous whitefly, and not merely by salivation. The authors noted that due to virion binding in the anterior foregut, which is physically separated from the maxillary stylet and salivary duct, LIYV virions thus cannot be released during salivation (2011a), but that salivation could serve to release viruses like CaMV which are in the acrostyle at the stylet tip (Uzest et al., 2010), a location where the salivary and food canals are confluent.

**Introduction to the biology of persistent, circulative virus transmission**

Circulative viruses, by definition, enter the insect body and disseminate to various tissue systems prior to their transmission to plant hosts. Circulative viruses include both those that disseminate but do not replicate in the body of the insect (non-propagative) and those that replicate (propagative) in different tissues. The precise route of dissemination from point of entry (Gutierrez et al., 2013) to the salivary glands has been well-described, with some variation, for different types of circulative viruses (Fig. 1). Virus dissemination is a major defining feature of vector competency, and as such, has been a
primary focus of vector–virus research. Examination of various interactions between vectors and circulative viruses has revealed commonalities and unique aspects pertaining to pathways to the salivary glands, viral determinants of transmission, and in some systems, vector components that respond to or interact directly with viral proteins.

**Circulative, non-propagative transmission**

The luteoviruses are transmitted by aphid vectors in a non-propagative, circulative manner. The dissemination pathway and associated interactions between virus and vector are well-characterized for luteovirus transmission by aphid vectors (Gray et al., 2014). Luteovirids are acquired when the aphid feeds on the phloem tissues of infected plants. These viruses are relatively small, simple icosahedral virions that enter the insect body through the alimentary canal. The majority of species of luteovirids cross the hindgut, however, in a few species, the point of entry is the midgut (Garret et al., 1996; Gildow, 1993; Reinbold et al., 2003). The virions interact with molecules on the surface of the gut epithelial cells, enter in a receptor-mediated endocytic fashion and traverse the epithelial cell layer without uncoating (Gildow, 1993). Subsequently, virions are delivered to the space between the basal

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**Fig. 2. Virion structures and viral attachment proteins.** TEM and immunogold labeling analysis of partially purified LIYV virions (A and B). The virion in (A) was labeled using antiserum to the LIYV capsid protein (CP). Virions in (B) were labeled using antiserum to the LIYV CP minor (CPm), the determinant of insect transmissibility. Bars represent 224 nm. Arrows in (B) indicate LIYV virion termini labeled using CPm antiserum. Arrow in (A) indicates a virion terminal region unlabeled with LIYV CP antiserum. Panels A and B were reproduced and modified from Tian et al. (1999). Structural model of the CaMV virion (C). The cryo-EM reconstruction shows the capsid protein (P4) in yellow and the P3 ectodomain (gray) decorating the surface of the virion. The helper component protein (P2) is not a structural protein but binds to the aphid stylet and P3-decorated virion to enable transmission. (D) Enlarged view of P3 (gray) in the cryo-EM difference map surrounded by three adjacent capsid hexamers (yellow). The ectodomains of 3 antiparallel dimeric coiled-coil P3 molecules are displayed as ribbons. Panels C and D reproduced and modified from Hoh et al. (2010) with permission from American Society for Microbiology. Drawing of an icosahedral luteovirid showing the coat protein (CP) in beige and the read through domain (RTD) in blue (E). The RTP is produced via translational readthrough of a leaky stop codon at the end of the CP gene. The RTD is predicted to be highly disordered and the CP:RTP stoichiometry in luteovirids is not known (Chavez and Cilia et al. 2012). The CP and N-terminus of the RTD are essential for aphid transmission (modified from DelBasio et al., in press). Diagram of TSWV virion (F). A double-layered membrane of host origin (blue) is shown with the viral-encoded proteins Gn and Gc (green) projecting from the surface in monomeric and dimeric configurations. The genomic RNA is presented as ribonucleoprotein (RNP) complex created by its association with many copies of N protein (peach). A few copies of the virion-associated RNA-dependent RNA polymerase (RdRp or L) are shown (purple) in association with the RNPs. Panel F is reproduced from Whitfield et al. (2005a).
plasmalemma and basal lamina via the exocytic pathway. This endo- and exocytic virion transport process is termed transcytosis. The mechanism for virus movement through the basal lamina is not well understood. Virions reach the hemocoel, where bacterial endosymbionts are hypothesized to enhance vector transmission efficiency (Gray et al., 2014). Once the virions enter the hemocoel they must circulate to and penetrate the accessory salivary glands (ASG) to be inoculated. For the majority of luteovirids characterized, the gut is not a major barrier to virus entry and it is common for viruses to enter the hemocoel of non-vector aphids (Gray and Gildow, 2003). The basal lamina and basal plasmalemma of the ASG are both significant barriers to transmission for various luteovirid-aphid species combinations and the major determinants of vector competence (Gray et al., 2014; Gray and Gildow, 2003; Peiffer et al., 1997). As these viruses do not replicate in the vector, higher virus accumulation in plants and/or longer feeding periods increase the amount of virus harbored by the aphid and the efficiency of transmission (Gray et al., 1991).

The luteovirus virion is an icosahedral, T=3 structure that is composed primarily of the capsid protein (CP). The minor component of the virion is the CP-readthrough protein (CP-RTP), generated by translational readthrough of the CP stop codon resulting in a C-terminal extension of the CP (Fig. 2). Numerous studies provide evidence that the CP and CP-RTP are the major determinants of insect acquisition and transmission of luteoviruses, and no other luteovirid proteins have been implicated in these coordinated processes (Brault et al., 2000; Gray et al., 2014; Liu et al., 2009; Peter et al., 2008). Virions made of the CP alone can transcytose through the gut, indicating that the CP is sufficient to deliver the virus to the hemocoel. The RTP contains a highly conserved N-terminal region and a variable C-terminal region and these domains have distinct functional roles. The N-terminal half of the RTP is important for association with the ASG and the C-terminal region is dispensable for transmission but plays a role in accumulation and tissue tropism in plants (Brault et al., 2000; Bruyere et al., 1997; Peter et al., 2009). The full length RTP is detectable in plant tissues, but in purified virion preparations, the protein is significantly smaller due to proteolytic processing of the C-terminal region (Brault et al., 1995; Wang et al., 1995). The CP-RTP also has a proline hinge domain that is important for incorporation of CP-RTP into virions. The N-terminus of the RTP is required for aphid transmission and is thought to mediate interactions with the salivary glands (Peter et al., 2008; Brault et al., 1995, 2000). Mutations in CP-RTP have been shown abrogate transmission but have no apparent effect on persistence in the vector as long as the mutant CP-RTP is incorporated into virions (Peter et al., 2008).

Much like the family Potyviridae, the virus genera in the family Geminiviridae are transmitted by vectors in a virus genus-specific manner. For example, whiteflies transmit viruses in the genus Begomovirus, e.g., Tomato yellow leaf curl virus (TYLCV), while leafhopper vectors transmit viruses in the genus Mastrevirus, e.g., Maize streak virus (MSV). Like luteovirids, the route of the Begomovirus begins with the insect feeding on phloem sap of infected plants and virions are ingested and travel through the alimentary canal (reviewed in Gray et al., 2014). The virions traverse the gut at the midgut region and the majority accumulate in the filter chamber region (Cicero and Brown, 2011; Chanin et al., 2001). In most cases, begomoviruses do not replicate in their vectors and virions move through the gut via a transcytotic pathway much like the luteovirids. Virions are released from gut cells, travel through the insect hemocoel, and reach the primary salivary glands, moving through different salivary gland physical barriers for transmission to occur (Cicero and Brown, 2011; Chanin et al., 2001). Like luteovirids, it is hypothesized that bacterial endosymbionts residing in bacteriocytes, specialized cells in the hemocoel, may play a role in virus transmission (Gray et al. 2014). A recent study of Wheat dwarf virus (WDV, genus Mastrevirus) movement in the leafhopper vector Psammotettix alienus, reported that the entire process from virus acquisition to transmission occurred in 5 min, following the same dissemination route as other geminiviruses but deviating remarkably from the 8-h latent period required for TYLCV in whiteflies and the 6–12-h latent period for MSV in the leafhopper Cicadulina mbila (Ghanim et al., 2001; Storey, 1928; Wang et al., 2014).

For members of the Geminiviridae, several lines of evidence implicate the viral CP as the sole determinant of insect transmissibility. The CP comprises the twinned icosahedral particles that identify geminiviruses. In elegant recombinant chimera virus experiments, exchanging CP ORFs between Beet curly top virus (BCTV, leafhopper vector) and African cassava mosaic virus (ACMV, whitefly vector) resulted in vector ‘switching’, defining role of the CP in vector specificity (Briddon et al., 1990). Other experiments provide direct evidence that the CP serves as the viral attachment protein (VAP) to insect vector guts, a first step in acquisition. A recombinant CP of TYLCV fed to whiteflies bound to the midgut, and in competition assays with wildtype virus reduced the amount of virus in whiteflies (Wang et al., 2014). Likewise, feeding leafhopper (P. alienus) vectors recombinant WDV CP localized the recombinant CP to the midgut, i.e., site of entry, and sequentially feeding leafhopper antibodies raised against wildtype WDV CP and virus reduced the proportion of insects harboring the virus in various tissues along the route of dissemination and virus accumulation in the vector (Wang et al., 2014). Collectively, these findings support the hypothesis that although geminiviruses are transmitted by diverse vector species, they use a similar route of dissemination in the vector and the viral CP is the viral determinant of this process.

Circulative, propagative transmission

The family Reoviridae is a large virus family with 15 genera that infect humans, animals, plants, insects, and fungi (Attoui et al., 2012). Members of the Reoviridae have genomes composed of multiple (9–12) segments of linear double-stranded RNA (dsRNA) that are encased in a non-enveloped particle (Attoui et al., 2012). Reoviruses have icosahedral symmetry with a diameter of approximately 60–85 nm made up of one or more structural layers of capsid protein(s). There are three plant-infecting genera of the Reoviridae: Fijivirus, Phytoreovirus, and Oryzavirus that are transmitted in a persistent-propagative manner by planthopper (Hemi- ptera: Delphacidae) or leafhopper (Hemiptera: Cicadellidae) vectors. Despite the similarities in vector transmission of reoviruses, there are some variations in reovirus tissue tropism and dissemination routes in their vectors that have been recently documented. Vector transmission of plant-infecting reoviruses is well characterized due to several tools and features of these viruses: 1) vector cell monolayers (VCM) are available for studying virus infection at the cellular level with a synchronous infection; 2) the viral genome is segmented which enables viral gene function to be studied by RNA interference (RNAi), despite the lack of an infectious clone system and 3) the leafhopper and planthopper vectors are amenable to RNAi for reducing transcript abundance of potent host proteins that are part of the virus infection cycle. Application of these technologies to the study of reovirus–vector interactions has enabled significant advancements in understanding initial virus entry into cells, movement between cells, mechanisms of dissemination and viral protein function.

The Phytoreovirus, Rice dwarf virus (RDV) and its vector, Nephotettix cincticeps, is the best characterized virus–vector system within this family. The generalized route of dissemination for plant-infecting Reoviruses starts with virus entry into the alimentary canal and entering cells of the epithelial cells of filter
chamber region and then the anterior midgut (Chen et al., 2011b). Virus traverses the basal lamina to infect the muscle cells, and travels to the salivary glands via a hemolymph route. The study of RDV entry and movement in VCMs enabled the discovery of a unique tubule-mediated mechanism of virus delivery to surrounding cells (Fig. 1). Initial entry into VCMs and midgut tissues is mediated by the viral structural protein P2, a minor component of the outer capsid of RDV (Omura et al., 1998). The virion binds to cells and is taken up by clathrin-mediated endocytosis (Wei et al., 2007). P2 is also a fusion protein that facilitates virion release from the endocytic vesicle and upon release from the vesicle the virus replication cycle begins (Zhou et al., 2007). After assembly of progeny virions, newly generated RDV virions can associate with tubule structures or accumulate in multi-vesicular bodies in the vector cells (Wei et al., 2009). RDV-induced tubule structures were first observed in VCMs and are composed primarily of a nonstructural protein, Pns10 (Wei et al., 2006, 2008). TEM examination of tubules revealed that RDV virions are encased within the structure in a narrow row. The Pns10 tubules are associated with actin-based filopodia, protrude from the surface of cells, and are capable of penetrating neighboring cells (Wei et al., 2006). Experiments demonstrated that RDV exploits these tubules to move into adjacent cells without release outside of the cell (Wei et al., 2006). Pharmacological experiments with VCMs led to the hypothesis that the endomembrane system and myosin motors associated with actin filaments are required for tubule-mediated transport of RDV to adjacent cells (Wei et al., 2008). A direct interaction between cytoplasmic actin of the vector, N. cincticeps, but not actin of an inefficient vector indicates that the ability to interact via tubules through specific interactions with Pns10 and actin is a determinant of vector specificity (Chen et al., 2015). In vivo studies with N. cincticeps, provide evidence that the tubule movement strategy facilitates RDV movement in the microvilli of the gut epithelial cells and in the muscle cells encircling the gut (Chen et al., 2012). RNAi knockdown of Pns10 in vector feeding experiments inhibited formation of tubules, prevented intercellular spread, and reduced leafhopper transmission efficiency of the virus (Chen et al., 2012). These data conclusively show that the Pns10 tubules facilitate the intercellular spread of RDV in the leafhopper vector.

In contrast to RDV tubule-mediated transport within microvilli of midgut epithelial cells and visceral muscles, analysis of the Fijivirus, *Southern rice black-streaked dwarf virus* (SRBSDV) in the vector *Sogatella furcifera*, revealed that tubules are involved in virus escape through the basal lamina. SRBSDV-induced tubules are comprised of the P7-1 protein (Liu et al., 2011). RNAi experiments demonstrated that P7-1 and the associated tubules are required for virus spread but knockdown did not affect virus replication (Jia et al., 2014). A primary difference between SRBSDV and RDV tubules is that the SRBSDV tubules crossed the basal lamina and appear to provide a route for rapid movement of the virus from gut epithelial cells into visceral muscle cells. The basal lamina is a major barrier to virus escape from gut cells. Correspondingly, the latent period for SRBSDV is shorter than the RDV latent period, 6 to 9 days for SRBSDV in contrast with 2 to 3 weeks for RDV (Honda et al., 2007; Pu et al., 2012). The discovery of tubule structures in insect vectors and the role in virus movement along the actin cytoskeleton provides new insight into virus movement in vectors and highlights similarities of the virus lifecycle in insect and plant hosts. Additionally, these findings for reoviruses emphasize the importance of understanding interactions for each virus–vector combination within a virus family.

Tospoviruses are members of the family Bunyaviridae and like all viruses in this family, they have enveloped virions that encapsidate three-sRNA genome segments that have helical symmetry and are covered in nucleocapsid protein (N). All tospoviruses are transmitted in a circulative, persistent-propagative manner by thrips, small insects in the order Thysanoptera (Whitfield et al., 2005a). The type member, *Tomato spotted wilt virus* (TSWV), is transmitted efficiently by western flower thrips, *Frankliniella occidentalis*, and the virus vector interactions for this virus and vector species combination has been well characterized. The acquisition and transmission of tospoviruses is an insect-stage specific process. Larval thrips acquire TSWV and virus enters the anterior midgut epithelial cells. The virus spreads to surrounding gut cells and traverses the basal lamina to infect the circular and longitudinal muscle cells. In contrast to reoviruses, tospoviruses have not been observed in tubule-like structures in insect vectors despite the ability for these viruses to induce tubule formation in insect tissue culture cells (Storms et al., 1995). Virions have not been observed in the hemocoel of thrips and it is thought that virus moves from the midgut to the salivary glands through connective structures (i.e., tubular salivary glands, ligaments) and/or directly between tissues during insect stages when the midgut and salivary tissues are in close contact (Kritzman et al., 2002; Montero Astúa, 2012; Moritz et al., 2004). The timing of virus movement from the initial site of entry, the midgut, to the site for virus replication and delivery to plants, the salivary glands is the latent period and at 24°C is 109 h (Wijkamp and Peters, 1993). Virus can reach the salivary glands during the second larval stage, but the primary transmitters are adults because they are winged and are more mobile. Once the insect is infected, the virus persists for the duration of the lifespan. The pupal stages do not feed on plants so they neither acquire nor transmit virus. Adult insects can feed on infected plants and sustain midgut infections, but they do not transmit the virus (Assis Filho et al., 2004). It is thought that a developmental specific barrier or other changes in the vector prevents virus from reaching the salivary glands of adult thrips. The development of transcriptome and proteome resources for thrips are now available for *F. occidentalis*, and research aimed at identifying and characterizing vector molecules that interact with and respond to TSWV is beginning to describe the vector–virus interactome (Badillo-Vargas et al., 2012; Rotenberg and Whitfield, 2010).

The major tospovirus determinants of thrips transmission are the viral glycoproteins that project from the surface of the virion (Fig. 2). Several research groups observed that serial mechanical inoculation of TSWV to plants led to development of virus populations with decreased thrips transmissibility (Nagata et al., 2000; Resende Rde et al., 1991; Sin et al., 2005). Analysis of transmission-deficient virus populations revealed changes in the medium genome segment, the segment that encodes the glycoproteins. To specifically map the genome segment that encodes the determinants of transmissibility, a virus genome reassortment study was conducted between thrips transmissible and non-transmissible isolates (Sin et al., 2005). The ability to be transmitted by thrips was always associated with virus isolates that contained an M segment from the thrips-transmissible isolate. Further analysis of single lesion isolates derived from a serially-mechanically passaged virus further mapped the transmission to the glycoprotein ORF. Using a different approach to study the role of viral proteins in transmission, the C\textsubscript{N} protein was expressed in insect cells using a recombinant baculovirus and feeding experiments with the protein demonstrated that C\textsubscript{N} could bind to thrips midguts and block entry into midguts (Whitfield et al., 2004). The C\textsubscript{N} protein was capable of binding to midguts in the absence of other viral proteins indicating that it is a VAP or an important component of the viral attachment complex. The C\textsubscript{G} protein of other Bunyaviruses has been shown to be involved in fusion with host membranes and the TSWV C\textsubscript{G} protein has similar characteristics supporting the hypothesis that it also plays a similar role in entry of virus into thrips (Garry and Garry, 2004; Whitfield, Ullman, German, 2005b).

The Tenuiviruses are non-enveloped viruses that replicate in their planthopper and leafhopper vectors. The dissemination route of these
viruses in their vectors have been described in detail, and initial studies found that tenuiviruses infect organs including the digestive and respiratory tracts, Malpighian tubules, leg muscles, fat bodies, brain, salivary glands, and reproductive tracts of both sexes (Nault and Ammar, 1989; Zheng et al., 2014). The generalized dissemination route for Tenuiviruses begins with entry into and replication in the midgut epithelial cells, traversing the basal lamina, and infection of the visceral muscles surrounding the gut. The virus then occurs in the salivary glands, and specifically. *Rice stripe virus* (RSV) has been observed in the reproductive organs of both sexes of the small brown planthopper (Wu et al., 2014). However, there are some key differences between *Tenuivirus* species with regards to tissue tropism. For example, *Rice grassy stunt virus* (RGSV) in the small brown planthopper, *Nilaparvata lugens*, spreads intercellularly in the midgut epithelium and then traverses the basal lamina and infects the principal and accessory salivary glands (Zheng et al., 2014). This virus is not found in neutral tissues or ovarioles (Zheng et al., 2014). In contrast, RSV in *Laodelphax striatellus* is found in the ovarioles, spreads between epithelial cells and is found in the principal salivary glands and absent in the accessory salivary glands (Deng et al., 2013; Wu et al., 2014).

The *Tenuivirus* genome is composed of four to six negative strand RNA segments coated in nucleocapsid protein, and the virions are ribonucleoprotein structures with helical symmetry. Based on sequence comparisons, the tenuiviruses are most closely related to viruses in the family *Bunyaviridae*; however the lack of an envelope and the greater number of genome segments precludes taxonomic placement of this genus into the *Bunyaviridae*. Interestingly, the Tenuiviruses encode membrane glycoproteins like those of the Tospoviruses but enveloped virus particles have not been found in plants or insect vectors. In a protein localization study with plants, tenuivirus glycoproteins of RSV were documented to be processed into two proteins, designated P2-C and P2-C based on their location at the N- and C-termini of the polyprotein, and localized in the plant cell in a similar manner as the TSWV glycoproteins (Yao et al., 2014). The N-terminal protein was targeted to the Golgi and the C-terminal protein to the ER. When expressed together, the N-terminal protein and C-terminal protein co-localize to the Golgi. In insect cells, both proteins were found in the ER (Zhao et al., 2012).

Notably, there are currently no described roles for these glycoproteins in plants and insects and it appears that Tenuiviruses are efficiently acquired and disseminated in insect vectors without the envelope that is required for Tospoviruses. In their study, Yao et al. (2014) hypothesized that the RSV glycoproteins may function as helper components that assist in virus acquisition by vectors much like the helper components of non-circulative viruses. This hypothesis is yet to be tested but could be an explanation for the retention of the genes that encode these glycoproteins in the tenuivirus genome. Understanding the role of the glycoproteins in the *Tenuivirus* infection cycle will be an interesting pursuit for these viruses because it is highly unlikely that these viruses have retained a “vestigial” gene. One emerging theme for the propagative plant viruses that lack envelopes is that they induce the formation of tubules in the insect vector. The tubule dissemination strategy may be a unique mechanism of dissemination in vectors for propagative viruses that lack membranes.

As with other circulative plant viruses, non-structural proteins play essential roles in *Tenuivirus* dissemination in the insect vector body and transovarial transmission. In the case of RSV in *L. striatellus*, ribonucleoprotein (RNP) interactions with NS4, a non-structural protein, facilitates tissue tropism in the insect vector (Wu et al., 2014). In the insect gut, RNPs co-localized with fibrillary inclusions of NS4 and knockdown of NS4 by RNAi slowed virus spread and reduced transmission efficiency (Wu et al., 2014). The ability to disseminate to and infect reproductive tissues is a prerequisite for transovarial transmission of viruses. Tenuivirus species display differences in reproductive tissue tropisms that are associated with the ability of a species to be transovarially transmitted. For RSV, the mechanism of transovarial transmission was documented using a yeast two hybrid assay to identify virus-host protein interactions and functional validation of the interaction using RNA interference (RNAi) methods. The RSV major nucleocapsid protein, pC3, interacted with vitellogenin, the major yolk protein precursor of egg-laying animals, in yeast two-hybrid assays (Huo et al., 2014). Functional analysis using RNAi to knockdown vitellogenin transcripts resulted in a significant reduction in virus in the ovariole and demonstrated the importance of the protein in transovarial transmission. These findings support the hypothesis that RSV directly binds to vitellogenin and appropriates the vitellogenin transport route to enter *L. striatellus* oocytes.

**Translational outcomes derived from basic virus–vector research**

Exploiting the binding and functional properties of viral determinants of virus acquisition is an exciting and real possibility for the development of new interdictive strategies that mitigate pathogen dispersal and disease. For the majority of plant virus–vector interactions described in this review, viral components directly involved in virus acquisition are well defined. The identification and functional analysis of VAPs in vector–virus relations have enabled innovative strategies for virus transmission disruption and vector pest control. Recombinant VAPs can be used to block binding of native virus to vector molecules that coordinate virus entry into gut tissues, culminating into reduced acquisition and viral loads, and subsequently prevention of transmission. Another use is engineering VAPs to deliver insecticidal chemistries to the insect hemolymph by way of the natural dissemination route from point of entry, the gut epithelium. The bottom line is that these strategies rely on molecular interactions between VAPs and points of entry. Here, we discuss new frontiers in transmission disruption strategies facilitated by *Tospovirus* and *Luteovirus* VAPs.

**Blocking transmission**

TSWV G<sub>0</sub> is a one of two viral transmembrane-bound structural proteins decorating the envelope of the virion and it plays an essential role in the attachment of the virus to thrips midguts. A soluble recombinant form of G<sub>0</sub> (G<sub>0</sub>-S) expressed from a baculovirus-SF21 cell-culture system has been the workhorse for TSWV acquisition and transmission disruption studies. Demonstration of the capacity of purified G<sub>0</sub>-S to specifically bind larval thrips guts, block TSWV acquisition and to reduce virus accumulation (Whitfield et al., 2004) and subsequent transmission (Whitfield et al., 2008) when applied exogenously to western flower thrips (WFT), led to the development of a proof-of-concept tomato transgenics-based strategy to determine if ingestion of plant-expressed G<sub>0</sub>-S could inhibit TSWV acquisition and transmission by WFT (Montero-Astúa et al., 2014). TSWV accumulation (titer) in young larval thrips exposed to TSWV-infected transgenic plants for a 24-h AAP was significantly reduced compared to those exposed to infected non-transgenic plants. Interestingly, non-transgenic and transgenic plants supported similar titers, making it unlikely that reduced larval acquisition/titer resulted from exposure to source plants harboring low levels of virus. It appears that the G<sub>0</sub>-S-transgenic plants interfered with the infection of larval thrips by TSWV. The reduction in titer persisted through the adult stage and transmission efficiency (number of transmitting adults) was significantly reduced, indicating that the initial virus inoculum dose is important for vector competence. These studies show that blocking or even significantly reducing the amount of virus acquired and accumulated in the body can be an effective transmission reduction
strategy for tospoviruses (Montero-Astúa et al., 2014; Rotenberg et al., 2009; Whitfield and Rotenberg, in press).

Vector population suppression

PEMV, a member of the genus *Enamovirus* within the family Luteoviridae, is transmitted by *Acrystosiphon pisum*, the pea aphid. PEMV CP is the primary constituent of the luteovirus virion and a critical determinant of acquisition and transmission by the aphid vector. In the absence of other PEMV proteins, CP has the capacity to bind to and enter the aphid hindgut to travel to the hemocoel via transcytosis (Liu et al., 2009). In addition, entry of luteovirids into the aphid hemocoel is not vector specific, implying that the PEMV CP can target aphids that are not vectors. Taking advantage of these characteristics of CP movement along the natural dissemination route to the hemocoel and the proline hinge region of the CP-RTP, a feature determined to be critical for protein solubility and efficient transport of fused foreign sequences to the hemocoel, a PEMV CP (plus proline hinge) – neurotoxin fusion protein was engineered to determine if the CP could serve as a vehicle to transport neurotoxic chemistries to the aphid hemocoel (Bonning et al., 2014). The neurotoxin of choice was a spider-derived insect-specific toxin that, in its native form, is not orally harmful to insects; it is toxic if delivered artificially to the hemocoel (Pal et al., 2013). Feeding CP-toxin fusion protein to aphids via a membrane apparatus, delivered the toxin to the hemocoel and efficaciously resulted in high mortality of PEMV-vector (*A. pisum* and *Myzus persicae*) and non-vector (*Rhopalosiphum padi* and *Aphis glycines*) aphids belonging to different tribes (*Aphidini* and *Macrosiphini*). This finding supports the idea that the gut is not the major barrier for acquisition and transmission by the aphid hemocoel (Bonning et al., 2014). Highly-expressing *Arabidopsis* plants were assayed for their ability to suppress aphid populations. After seventeen days, *M. persicae* populations on control plants were ~10 fold higher than those reared on the CP-toxin plants, and aphid feeding damage was observed on control plants, but CP-toxin-expressing plants appeared healthy. With relevance to biosafety and potential application in agricultural systems, the spider toxin is not harmful to mammals (Fletcher et al., 1997), enabling further testing to determine the feasibility of deploying the CP-toxin as a broad spectrum aphicide or by way of crop transgensics. Moving beyond aphids, another profitable possibility would be to adopt the CP-toxin fusion strategy to VAPs of other viruses to target other agronomically-important vectors and crops pest, including thrips, whiteflies, leafhoppers, and planthoppers (Whitfield et al., 2014).

Summary

Vector transmission is an essential step in the infection cycle of most plant viruses. The study of diverse virus–vector interactions has revealed commonalities in transmission strategies. For all plant viruses studied, one or more of the structural virion proteins is required for virus transmission. For some viruses, the capsid protein (s) is sufficient for transmission and other viruses require a “helper” protein(s) to facilitate retention by serving as a bridge between binding the surface of the vector and the virus. The circulative viruses (non-propagative and propagative) generally follow a similar acquisition route by vectors and this begins in the gut of the insect. After entry into gut epithelial cells the paths for virus movement between cells and dissemination to other tissues diverge, sometimes even for viruses within a family. The identification of virus intercellular movement in vectors through tubule structures composed of non-structural virus proteins and the role of the actin cytoskeleton in movement highlights similarities between the virus infection cycle in insect and plant hosts, and in at least one case, the tubule has been documented to be a new strategy for escape through midgut barriers in the insect. Another new discovery for vector transmission of plant viruses was the use of the vitellogenin uptake pathway for virus invasion of insect eggs, and this pathway for transovarial transmission of viruses is parallel to transovarial transmission of other insect-associated microbes. While the virus components of the interaction are well-defined in most economically important systems, the identification of vector molecules that interact and respond to virus are just beginning to be characterized and we expect that the use of new research technologies will enable the functional analysis of these vector components in the virus transmission process. For now, the commonalities in the role of viral structural proteins and initial sites of virus entry or retention indicate that these are candidate targets for disrupting virus transmission by a wide range of vectors. The successful use of viral CP to deliver toxins to vectors and use of viral proteins to prevent transmission provide hope that the basic knowledge of virus binding and entry to vectors can provide a platform for development of a new control strategies for viruses and their vectors, a situation where current control options are often limited and ineffective.

Acknowledgments

We thank Aurie Bak for helpful comments and Michelle Cilia for the Luteoviridae drawing. This work was supported by the USDA NIFA/AFRI Grant numbers 2012-68004-20166 (DR and AEW) and 2007-35319-18326 (AEW) and by the National Science Foundation CAREER Grant IOS-0953786 (AEW); and in part by the University of California, and a USDA NIFA grant to BWE. Contribution no. 15-316-J from the Kansas Agricultural Experiment Station.

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