Viral receptors of the gut: Insect-borne propagative plant viruses of agricultural importance

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Abstract

Insect-borne propagative plant viruses of agricultural importance are transmitted by sap-sucking insects. Although the infection routes of these viruses within the bodies of insect vectors are well established, cellular receptors on the microvilli, intercellular junctions, and basal lamina for mediating viral entry or spread in insect gut epithelium have not been identified or characterized. Recent trends in the field are opening questions on how viruses exploit actin-based tubule motility to overcome insect gut epithelium barriers after viral entry in epithelium. Advances in insect cell lines, genome sequencing, reverse genetic systems and others not yet developed technologies are needed to find and characterize the counterpart receptors in vectors and to design strategies to interfere with viral transmission.

Introduction

Propagative plant viruses of agricultural importance include plant reoviruses, plant rhabdoviruses, tospoviruses, tenuiviruses, and marafiviruses, mainly transmitted by sap-sucking insects, primarily leafhoppers, planthoppers, aphids and thrips [1, 2, 3]. These viruses infect and replicate in their insect vectors and are retained by the insects throughout their life, even after molting. While propagating in the insect vectors, viruses circulate through the insect bodies, from the gut via the hemolymph to other organs or the salivary glands, from where virions are ejected into the plant host during insect feeding [1, 2, 3]. The insect gut consists of a monolayer of epithelial cells, lined on the lumen side with protruding microvilli appearing as a brush border and covered on the outer side with the basal lamina, that are connected by intercellular junctional complexes (Figure 1) [4]. The gut barrier is the principal determinant for the ability of an insect species to transmit a virus. In general, the microvilli, intercellular junctions, and basal lamina of insect gut act as barriers for viral entry and dissemination.

Initial entry of viruses into the gut epithelium

Microvilli of the insect gut are cellular membrane protrusions of the epithelium [5]. The gut microvillar membrane contains cellular receptors for the initial attachment and entry of viruses. Propagative plant viruses, once ingested by the insects, accumulate in the gut lumen, and then infect the epithelial cells through the microvillar membranes [6, 7^{••}, 8, 9, 10]. However, only a limited number of epithelial cells contain receptors on the microvillar membranes for propagative plant viruses to attach [6, 7^{••}, 8, 9, 10]. By contrast, almost all gut microvilli contain cellular receptors for persistent nonpropagative plant viruses such as begomoviruses and luteoviruses to attach [2, 11]. In fact, the multiplication of propagative plant viruses for producing more progeny virions in initially infected epithelial cells is

essential for compensating for the insufficiency of invading viruses from the gut lumen.

Insect vector cell lines can allow us to trace the early entry process of propagative plant viruses at the cellular level. During the past 30 years, primary or continuous cell cultures derived from leafhoppers, planthoppers and aphids and thrips, originally initiated from embryonic fragments of insect eggs, have been established to support the persistent replication of propagative plant viruses [12, 13, 14, 15, 16, 17]. Rice dwarf virus (RDV), a plant reovirus, is the best-characterized plant virus regarding entry into its leafhopper vector cells, because the structures of the mature virion and minor outer capsid protein P2 have been elucidated [18^{••}, 19]. RDV enters leafhopper cells through receptor-mediated, clathrin-dependent endocytosis and is sequestered in early endosomes [20]. RDV P2, which protrudes from the surface of the outer shell of virions, is involved in initial recognition and interaction with cellular receptors [18^{••}, 21]. RDV P2 has an L-shaped flexible structure with a 10-nm-long domain that is responsible for anchoring on the surface of virion and a 15-nm-long domain for contacting with an unknown cellular receptor(s) [18^{••}]. The L-shaped structure of P2 has characteristics identical to the spike proteins of other plant reoviruses such as rice black streaked dwarf virus (RBSDV), south rice black streaked dwarf virus (SRBSDV), and rice ragged stunt virus (RRSV) [1, 2]. The spike protein of RRSV *in vitro* binds to a 32-kDa cytomembrane protein of its brown planthopper vector, and this vector protein probably serves as a potential receptor [22]. Enveloped viruses such as tospoviruses and plant rhabdoviruses encode transmembrane glycoproteins, which are responsible for mediating receptor-mediated endocytosis during viral entry into vector cells [3, 23]. Though several vector factors isolated from the gut microvilli have been identified to specifically interact with such viral glycoproteins [1, 2, 3, 24, 25, 26], whether they are the true viral receptors are unknown. Thus, identifying the cellular receptors involved in the initial recognition of propagative plant viruses by their insect vectors is still a major challenge.

Secondary dissemination of viruses from initially infected gut epithelium

After viral particles enter the gut epithelium, the microvilli, intercellular junctions, and basal lamina act as barriers to virus dissemination. The core of the insect gut microvilli is composed of bundles of parallel actin filaments [5]. Intercellular junctions are physical structures, termed tight junctions, which use a variety of transmembrane proteins that are coupled with the actin cytoskeleton to connect adjacent cells, thereby forming intercellular seals [4]. The basal lamina is a tightly interwoven, noncellular matrix that lines the gut epithelium [1]. Cellular receptors on these tissue or membrane barriers mediate the dissemination of viruses from the infected gut epithelium. Plant reoviruses are the best characterized of plant viruses in terms of the interactions between viruses and cellular receptors on insect gut barriers. Here, we propose a mechanism based on what we call actin-based tubule motility to explain the mechanism by plant reoviruses to overcome insect gut barriers.

The replication of propagative plant viruses in the insect gut epithelium induces the formation of various cytopathological inclusions composed of viral nonstructural proteins [1]. Such virus-induced inclusions are essential for viral replication or spread in insect vectors [7^{••}, 8, 10, 15]. Through the use of continuous insect cell lines, we have shown that plant reoviruses exploit the tubules formed by viral nonstructural proteins to move along actin-based cellular protrusions and then enter neighboring cells, facilitating viral cell-to-cell spread [7^{••}, 8, 27, 28]. Viral nonstructural proteins such as P7-1 of SRBSDV or RBSDV, and Pns10 of RDV have the capacity to assemble into the tubules [7^{••}, 28, 29]. Components of the insect actin–myosin motility system can directly interact with these tubular proteins [9, 28, 30]. It is apparent that actin-myosin motility system provides the power and track for the tubules to break through tissue or membrane barriers they may encounter. We have called this

phenomenon actin-based tubule motility.

Use of this actin-based tubule motility to overcome insect gut epithelium barriers is widespread adapted by plant reoviruses for dissemination from the insect gut epithelium. For example, RDV exploits virus-induced tubules to move along actin filaments into the microvillus, and then propels the elongation of the microvillus to form a membrane protrusion towards the lumen, finally breaking through the microvillar membranes (Figure 1) [8]. This is a new pathway for viral dissemination from the insect gut epithelium in virus research. SRBSDV depends on virus-induced tubules to move along actin filaments, finally breaking through the basal lamina to disseminate from the gut into the hemocoel (Figure 1) [7^{••}]. This model also reveals a new pathway adapted by the virus for rapid dissemination of virions from the insect gut. Such tubules can also move along actin filaments to break through the tough intercellular junctions between epithelial cells (Figure 1), representing a new mechanism for viral cell-to-cell spread among epithelial cells. Thus, the direct interaction of virus-induced tubules and actin-myosin motility system is the driving force for the virus to break through the tough barriers in insect gut epithelium. Whether such behavior is a passive or active consequence of actin-based tubule motility, however, is still unclear. Because direct attachment of free virions to the microvilli, intercellular junctions, or basal lamina of insect gut epithelium has never been observed [7^{••}, 8], cellular receptors on these barriers for direct contact with intact viral particles may be nonexistent or weak. It is possible that actin-myosin motility system could act in place of cellular receptors for mediating the secondary dissemination of plant reoviruses from the initially infected gut epithelium. In fact, other propagative plant viruses may also exploit various virus-induced inclusions to traffic along the cellular cytoskeleton or other motility machineries for breaking through insect gut barriers. For example, viral inclusions formed by nonstructural proteins NSs of tomato spotted wilt virus, a tospovirus, or NS4 of rice stripe virus (RSV), a tenuivirus, play important roles in

viral spread in insect gut epithelial cells [10, 31]. NS3 of RSV can hijack the 26S proteasome of the small brown planthopper via direct interaction with the regulatory particle non-ATPase subunit 3 [32], suggesting that such interactions may facilitate viral spread in insect gut epithelial cells. Unfortunately, thus far, the main bottleneck is confirming whether insect candidates interacting with viral components are the receptors of gut epithelium barriers to mediate the dissemination of viruses.

Final dissemination of viruses from gut visceral muscles into the hemocoel

The gut visceral muscles lining the basal lamina include external, longitudinal and internal, circular muscle fibers, which act as final barriers preventing viral dissemination into the hemolymph [7^{••}, 8, 33, 34]. For plant reoviruses, after the tubules cross the basal lamina, they first associate with the internal, circular muscle fibers, then traffic along the external, longitudinal muscle fibers throughout the whole gut [7^{••}, 8]. The actin-myosin motility system again acts as a cellular receptor to propel the movement of virus-induced tubules along visceral muscle fibers [7^{••}, 8]; thus, the actin-based tubule motility is used by plant reoviruses to overcome visceral muscle barriers. From the visceral muscle tissues, the virions are apparently disseminated directly into the hemocoel and then into the salivary glands [7^{••}, 8, 33, 34].

Constraints and perspectives

Insect-borne plant propagative viruses cause significant agricultural losses. While blocking propagative plant virus transmission would allow for crop protection, virus receptors in insect vectors are still unknown. Recently, a receptor protein, membrane alanyl aminopeptidase N, has been identified in the gut of the pea aphid as responsible for the entry of the nonpropagative pea enation mosaic virus into the vector [35[•]]. However, the *in vivo*

evidence for showing this protein could mediate the initial viral recognition and entry into the microvilli membrane of vector midgut is still lacking. Thus, a major challenge in virus-insect interaction is undoubtedly identifying cellular receptors on insect gut microvilli for initial recognition with plant viruses. Because only a limited number of gut epithelial cells contain cellular receptors for the initial recognition with propagative plant viruses, it is impossible for us to identify viral receptors on gut microvillar membrane using current protein–protein interaction technologies. Continuous insect cell lines support uniform, synchronous viral infection, thus enabling us to enrich receptors from purified clathrin-coated endocytic vesicles at an early stage of infection. Identification of insect receptors is particularly important because disrupting viral recognition with such receptors represents a nonchemical strategy for controlling insect-transmitted plant viruses in future [35[•]].

Another major challenge is elucidating how receptors on the microvilli, intercellular junctions, and basal lamina could facilitate the spread of propagative plant viruses. The development of insect vector cell lines, RNA interference, immunofluorescence, and electron tomography have allowed us to advance our understanding of the mechanism enabling viral spread in the gut epithelium. New technologies will provide new opportunities to more fully unravel mechanisms in such important insect–vector interactions. For example, plasmids with vector promoters have been successfully constructed [36], providing an opportunity for us to observe the real-time motility of viral components. The availability of complete genomes of brown planthopper [37] and ongoing sequencing efforts of other insect vectors for propagative plant viruses will provide crucial genomic resources for insect-virus interaction. Another major breakthrough has been the recent development of a plasmid-based reverse genetics system for a plant rhabdovirus [38^{••}]. We hope the plasmid-based CRISPR/Cas9 technology for insect genome editing will be used in sap-sucking insects in the near future. These technologies and others not yet developed will be important for unraveling molecular

mechanisms of viral transmission by insect vectors, especially for identifying the true receptors for propagative plant viruses in fine detail.

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19 in-time observation of viral motility in insect gut.

20
21 **Figure legends:**

22 Figure 1. Plant reoviruses exploit actin-based tubule motility to overcome gut epithelium
23 barriers in the vector insects. (A) Model for the motility of virus-containing tubules in insect
24 gut epithelium. Initially, viral particles recognize and bind to cellular receptors on the
25 microvillar membrane, and then enter the gut epithelium. The viruses then propagate in the

1 viroplasm for assembly of progeny virions and synthesis of virus-containing tubules. These
2 tubules can pass through microvilli of the epithelium into the lumen or across the basal lamina
3 from the epithelium into the visceral circular muscle, then spread through the external
4 longitudinal muscle, or cross the tight junctions between epithelial cells to facilitate viral
5 cell-to-cell spread. Blue arrows indicate the process of viral infection. (B) Electron
6 micrograph showing the elongated tubule-associated microvillus (arrow) has formed
7 membrane protrusion toward the gut lumen in RDV-infected leafhopper. (C) Transverse
8 section of microvilli of about 100 nm in diameter, with virus-containing tubules (arrows)
9 inside in RDV-infected leafhopper. Arrowheads indicate actin filaments within the microvilli.
10 (D) Electron micrograph of the enclosure of a tubule of RDV by actin filaments (arrow) in
11 leafhopper gut epithelium. (E) Electron micrograph of the association tubules of RDV with
12 the tight junctions between epithelial cells. bl, basal lamina; ge, gut epithelium; mv, microvilli;
13 gl, gut lumen; tj, tight junction; cm, circular muscle; lm, longitudinal muscle; Vp, viroplasm;
14 Tu, tubule. Bars, 100 nm.

Figure 1