Hijack it, change it: how do plant viruses utilize the host secretory pathway for efficient viral replication and spread?

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INTRODUCTION

Like any other eukaryotic cells, plant cells are characterized by an elaborated secretory pathway composed of a complex network of organelles including the endoplasmic reticulum (ER), the Golgi apparatus, the trans-Golgi network (TGN), various endosomes, and vacuoles (Hanton et al., 2005; Bassham et al., 2008). This pathway is involved in the synthesis, modification and transport of proteins, lipids and polysaccharides (Bassham et al., 2008). Proteins made in the ER can be transported, via Golgi, toward the plasma membrane; on the other hand, proteins outside of the cells can also be internalized, via endocytosis. The proper organization and the dynamics of the secretory pathway are vital for normal cell development and physiology (Hanton et al., 2005).

In animal cells, it is known that efficient replication of many viruses involves a modification of the secretory pathway in host cells that generates a membrane structure designated as viroplasm, virus inclusion, virus factory, or viroplasm (for review, see Novoa et al., 2005; Netherton et al., 2007; Miller and Krijnse-Locker, 2011). Most animal viruses are membrane enveloped and it is known that the modification of the secretory pathway in host cells is also crucial for viral assembly (Netherton et al., 2007). Intracellular and intercellular movement of viral complexes (Brandenburg and Zhuang, 2007), and inhibition of cellular secretion to reduce host immune response (Netherton et al., 2007) are important for cell-to-cell spread (Laliberte and Sanfacon, 2010; Schoelz et al., 2011; Verchot, 2011).

In this review, we will first highlight the recent progress in the organization and dynamics of the plant secretory pathway, we will then present the recent findings on how plant viruses may utilize the host secretory pathway for replication and intracellular and intercellular transport, with a focus on the recent advances on how the secretory pathway may be utilized by tobacco mosaic virus (TMV) and turnip mosaic virus (TuMV) for successful virus infection.

THE ORGANIZATION AND DYNAMICS OF THE ER–GOLGI INTERFACE IN PLANT CELLS

The ER is an extensive membrane network that extends throughout the cytoplasm. The ER is the largest membranous structure in the eukaryotic cell; it is the site where proteins and lipids are synthesized and modified. The Golgi apparatus, with cis-Golgi facing the ER and trans-Golgi away from the ER, is the central station in the secretory pathway. In Golgi, proteins and lipids received from the ER will be further modified and sorted to the proper destination in the secretory pathway. Despite the fact that in animal cells the ER is mainly aligned with microtubules (Du et al., 2004) and in plant cells with microfilaments (Boevink et al., 1998), the morphology of the ER in plants is quite similar to that in animal cells. The ER in both mature animal and plant cells exhibits a labyrinth-like morphology composed of membranous tubules and cisternae (Hu et al., 2009; Orvo et al., 2009; Chen et al., 2011, 2012). In addition, the mechanisms that mediate the tubulation of
Arf1-GEFs (Hsu and Yang, 2009). Brefeldin A (BFA), a fungal metabolite, has been found to be a specific inhibitor that interacts with the interaction between Sec7-domain Arf-GEF and Arf proteins in the cytoplasm (Boevink et al., 1998). As such, the morphology of the protein transport in the ER–Golgi interface appears very different between mammalian and plant cells.

Transport of proteins from the ER to the Golgi apparatus in both animal and plant cells starts from a transitional ER domain called ER export site (ERES). In mammalian cells, the ERESs are relatively stationary (Hammond and Glick, 2004), which are linked by the sec7 domain of a GEF, a Golgi intermediate compartment made by the fusion of COPI vesicles formed at the ERES (will be discussed below). In plant cells, individual ERESs are believed to be motile and tightly associated with Golgi stacks (da Silva et al., 2004). The ERES works together with individual Golgi stacks as a single functional unit (da Silva et al., 2004); no intermediate ERGIC has been revealed in plant cells. Yet it appears that the molecular mechanisms underlying protein transport in the ER–Golgi interface are very conserved between animal and plant kingdoms (Lee and Miller, 2007; Hsu and Yang, 2009; Hwang and Robinson, 2009; Marti et al., 2010; Chen et al., 2012).

In animal cells, transport from the ER to the Golgi apparatus is mediated by COPII vesicles (Lee and Miller, 2007). The proteins required for the formation of COPII vesicles from the ER include small Sar1 GTPase, membrane anchored guanine exchange factor (GEF) Sec12, Sec23, Sec24 heterodimers for cargo selection and initiation of ER membrane curving (Bickford et al., 2004), and Sec13/Sec33 for the final formation of cage-like vesicles (Lee and Miller, 2007). The fusion of COPII vesicles to generate ERGIC, which will further mature into cis-Golgi cisternae, requires small Rab1 GTPase and ER-localized SNAP receptors (soluble N-ethylmaleimide-sensitive-factor adaptor-protein receptors) proteins (Bonifacino and Glick, 2004). In plant cells, despite the morphological difference in the ER–Golgi interface, all these key proteins exist (Basham et al., 2008; Marti et al., 2010). It has been experimentally demonstrated that Sec24 as well as Rab-D proteins, homologs of Rab1, are required for ER-to-Golgi transport in plant cells (Nebenfuhr et al., 2001; Qian and Zheng, 2011). In mammalian cells, the retrograde Golgi transport, essential for recycling of proteins and lipids back to the ER in order to maintain equilibrium with the COPII-dependent transport, occurs mainly through COPI vesicles (Hsu and Yang, 2009). The COPI coat is composed by two protein complexes, F- and B-COP. The formation of COPI vesicles in Golgi cisternae requires small Arf1 GTPase, whose activity is regulated by Sec7-domain Arf1-GEFs (Hsu and Yang, 2009). Brefeldin A (BFA), a fungal metabolite, has been found to be a specific inhibitor that interferes with the interaction between Sec7-domain Arf1-GEFs and Arf proteins (Nebenfuhr et al., 2002). In Arabidopsis thaliana, electron tomography analysis have shown the existence of two types of COPI coated vesicles, the COPIa vesicles derived from cis-Golgi and the COPIb vesicles derived from trans-Golgi (Hwang and Robinson, 2009).

**THE ORGANIZATION AND DYNAMICS OF POST-GOLGI TRAFFIC NETWORK IN PLANT CELLS**

Proteins and lipids, after being transported and modified in the Golgi apparatus, are further transported to either the plasma membrane, or to the lysosome/vacuole (in plant cells). In mammalian cells, cargo molecules modified in the Golgi apparatus are usually delivered to the TGN where they are sorted into either secretory vesicles that move to the plasma membrane, or clathrin-coated vesicles to deliver cargoes to the endosomes and then the lysosome (Teub and Kornfeld, 1997). This anterograde protein transport to the plasma membrane is usually balanced by various clathrin-coated vesicle mediated endocytic pathways (Grunenberg and Maxfield, 1995; Clague and Ulev, 2001). After being formed in the plasma membrane, clathrin-coated endocytic vesicles are first delivered to the early endosome (EE), a compartment that can be marked by Rab5, a small GTPase required for the fusion of clathrin-coated vesicles to the EE (Goebl et al., 1991). Rab5 is believed to be localized at the plasma membrane and to the lysosome. It is thought that the protein recycling at the EE via the RE is mediated by Rab11 (Grant and Donaldson, 2009). The LE is believed to mature by the fusion of different EEs mediated by small Rab7 GTPase and LE-specific SNAP receptors (Bonifacino and Glick, 2004; Rink et al., 2005). At the LE, ESCRT (the endosomal sorting complexes required for transport) at the surface can selectively pick ubiquitinated membrane proteins to be directed to the lysosomes for further degradation (Katzmann et al., 2001).

In plant cells, initially the TGN was thought to be absent, but recent evidence suggests that the TGN may be a motile organelle derived from the Golgi apparatus (Dettmer et al., 2006; Kang et al., 2011; Qian et al., 2011). It has been demonstrated that the plant TGN could act as a sorting station and simultaneously release two types of vesicles: secretory vesicles heading to the plasma membrane and the cell wall (Preuss et al., 2006; Szulmalski and Nielsen, 2009; Qian et al., 2011), and clathrin-coated vesicles mediating transport to vacuoles (Kang et al., 2011). The organization of the dynamics of the post-Golgi traffic network in plant cells is, however, not well studied. It appears that protein transport in the plant post-Golgi traffic network is quite distinct from that in mammalian cells. There are several lines of evidence suggesting that the TGN in plants also serves as an EE (Dettmer et al., 2006; Qian et al., 2011). At the TGN/EE, proteins can be either recycled back to the plasma membrane (Ueda et al., 2001), or further transported to multivesicular bodies (MVB) or prevacuolar compartments (PVC), a compartment equivalent to the LE in animal cells (Spitzer et al., 2009). In plant cells, no RE has been identified. Different from mammalian cells, in plant cells the TGN/EE is highlighted by the presence of different Rab-A proteins (Szulmalski and Nielsen, 2009; Kang et al., 2011; Qian et al., 2011), homologs of the animal Rab5 protein, the MVB/PVC is marked by Rab-F proteins (Ueda et al., 2001), homologs of the animal Rab5 in animal cells. MVB/PVC...
Viruses are obligate intracellular parasites that depend on cellular materials for their replication and spread. As mentioned in the introduction, replication and assembly of many membrane enveloped animal viruses occur in an intracellular compartment termed virus factory. Virus inclusion, viroplasm, or viroosome whose generation requires the modification of the host secretory pathway (Novoa et al., 2005; Netherton et al., 2007; Miller and Krijnse-Locker, 2008). Despite the fact that most plant viruses are not membrane enveloped, recent research revealed that plant viruses also use the host secretory pathway for viral replication (Laliberte and Sanfacon, 2010; Verchot, 2011).

Studies with TMV, an extensively studied member of tobamoviruses, showed that TMV infection causes severe modifications of the ER, converting it into large irregular aggregates early in infection (Reichel and Beachy, 1998). This ER-derived structure contains virus particles, the virus-encoded 183 and 126 kDa replicases, movement protein (MP), vRNA, ribosomes, and host translation elongation factor EF1-a (Beachy and Zaitlin, 1975; Henlein et al., 1998; Reichel and Beachy, 1998; Mas and Beachy, 1999, Figuereira et al., 2002). Therefore it is believed that this ER-derived structure is the site for TMV replication, which is often termed viral replication complex (VRC; Henlein et al., 1998). However, how exactly these TMV replication complexes are formed is not yet clear. The MP and the replicase components 126 and 183-kDa proteins are associated with the ER (Reichel and Beachy, 1998; Hagwara et al., 2003). The MP, when expressed ectopically, can induce modifications in the ER (Reichel and Beachy, 1998) and the 126-kDa replicase, when expressed ectopically, can also induce the formation of cytoplasmic inclusion bodies (Ding et al., 2004). Therefore it is possible that these viral proteins are involved in the formation of TMV replication complexes. Furthermore, the TMV replication complexes are found to be associated with the host transmembrane proteins TOM1 (Yamana et al., 2000; Hagwara et al., 2003) and ARL8, a small GTP-binding protein (Nishikiori et al., 2011). Both proteins are required for efficient TMV RNA replication (Nishikiori et al., 2011). This suggests that host proteins also play important roles in the formation of TMV replication complexes in the secretory pathway. It will be interesting to study how exactly these viral and host proteins are involved in the process.

In the case of potyviruses, TuMV infection induces the formation of small vesicular compartments that move rapidly along the microfilaments and the ER (Beauchemin et al., 2007; Cotton et al., 2007; Grangeon et al., 2012). The polyprotein 6K2-VPg-Pro, through its hydrophobic 6K2 domain, is responsible for the formation of these motile structures (Beauchemin et al., 2007). The presence of viral dsRNA, an obligate intermediate in the replication of the ssRNA viruses, as well as the host eukaryotic initiation factor 2 isoforms (eIF(iso)4E), poly-A binding protein, heat shock cognate 70-3 protein (Hsc70-3), and the eukaryotic elongation factor 1A (eEF1A; Beauchemin and Laliberte, 2007; Beauchemin et al., 2007; Dufresne et al., 2008; Thivierge et al., 2008; Cotton et al., 2009) in the 6K2-induced vesicles suggests that the viral genome replication takes place in these structures (Cotton et al., 2009). Interestingly, in cells infected by the potyvirus tobacco etch virus (TEV), the motile 6K2 vesicular compartments are ER-derived and are associated with ER-related vesicles. The formation of the structure is COPII- and COPII-dependent (Wei and Wang, 2008). The motile 6K2 vesicles in TuMV-infected cells are associated but not perfectly with COPII vesicles as well as Golgi stacks (Grangeon et al., 2012). BFA can enhance the association of 6K2 vesicles with COPII and Golgi stacks (Grangeon et al., 2012). The interpretation of these facts is that the formation as well as the transport (will be discussed further) of the motile 6K2 structures requires an interaction between 6K2 and host proteins involved in the formation and transport of COPII and COPI vesicles.

Recently, we reported that TuMV induces the formation of a perinuclear globular structure with an amagulation of viral 6K2, ER, Golgi, and COPII membranes as well as the presence of chloroplasts within the structure (Grangeon et al., 2012). This globular structure maintains a dynamic connection with the cortical ER and the Golgi apparatus; additionally the motile 6K2 vesicles may originate from the globular structure (Grangeon et al., 2012). Indeed, the formation of large perinuclear ER-derived structures has also been observed in several other plant viruses. For example, cowpea mosaic virus (CPMV) induces the proliferation of ER membranes (de Zoeten et al., 1974; Carrette et al., 2000, 2002a) that are often next to the nuclei. By the incorporation of H3-uridine in the infected leaves, newly synthesized viral RNA can be found in the perinuclear membranous structures induced by CPMV (de Zoeten et al., 1974). Grapevine fanleaf virus (GFv) induces the formation of a punctate-spongy perinuclear structure composed of reorganized ER membranes and active synthesis of RNA is found in these ER-derived structures (Ritzenthaler et al., 2002). Potato virus X (PVX) also forms an ER-contained perinuclear structure called X-body (Tilnner et al., 2012) where the viral replicase and TGB1 are located. The exact mechanism by which these globular structures are formed remains unclear. It is known in plant cells that disruption of the formation of COPII vesicles also induces the formation of perinuclear clusters of the ER and Golgi membranes (Fuso et al., 2009). The 6K2 helicase of CPMV interacts with the ER-localized v-SNARE-like protein VAP27 in the proliferation of ER membranes (Carrette et al., 2002b), suggesting that an interaction of viral and host ER proteins may be required in the process. It is interesting, however, in TuMV infected cells, the BFA treatment does not inhibit the formation of the perinuclear globular structure nor viral replication (Grangeon et al., 2012). Although the perinuclear structure in TuMV-infected cells contains the ER and Golgi membranes, it seems that the formation of the structure does not require a functional early secretory pathway. Similarly, BFA seems not affect replication of melon necrotic spot virus but it may have a negative impact on viral cell-to-cell movement (Genoves et al., 2010). On the other hand, in GFLV-infected cells, although it is not known if the BFA treatment inhibits the formation of the perinuclear structure, such treatment results in a significant reduction of the replication efficiency of the virus (Ritzenthaler et al., 2002).


Vps24, Snf7p, and Vps4p, components of ESCRT complexes, the protoplasts (Ribeiro et al., 2008). The Gn glycoprotein localizes derived membranous structures upon ectopic expression in plant glycoproteins Gn and Gc induce the formation of ER- and Golgi-ER and Golgi (Ribeiro et al., 2009). Therefore it seems that viral co-expression (Ribeiro et al., 2009). In addition, both Gn and Gc glycoprotein Gc to Golgi in a COPII-dependent manner upon 1992; Kikkert et al., 1999). It was recently shown that the TSWV particles are retained until uptake by its insect vector (Kitajima et al., 2004; Yamanaka et al., 2000; Hirashima et al., 2003). However, it is not clear if this is also the case for TSWV. TSWV is replicated in tubular viral factories that are infecting counterpart of the animal infecting viruses within the secretory vesicles in which they migrate toward the plasma membrane (Walter and Barr, 2011). After the final virion assembly in the lumen of swollen Golgi stacks, viruses bud into and the rough ER (Walter and Barr, 2011). After the final virion assembly in the lumen of swollen Golgi stacks, viruses bud into secretory vesicles in which they migrate toward the plasma membrane and are then secreted (Walter and Barr, 2011). However, it is not clear if this is also the case for TSWV. TSWV is replicated in infected plant cells in association with a membrane modification of the ER and Golgi (Ribeiro et al., 2008). The viral particles are assembled within the infected plant cells when ribonucleoprotein complexes (RNP’s) are enveloped by Golgi cisternae. The Golgi membrane wrapped RNP’s then fuse with each other and with ER membranes, producing a singly enveloped vesicle where virus particles are retained until uptake by its insect vector (Kitajima et al., 1992; Kikkert et al., 1999). It was recently shown that the TSVV glycoproteins Gn and Gc induce the formation of ER- and Golgi-derived membranous structures upon ectopic expression in plant proplasts (Ribeiro et al., 2008). The Gn glycoprotein localizes to both the ER and Golgi and is able to redirect the ER-arested glycoprotein Gc to Golgi in a COPII-dependent manner upon co-expression (Ribeiro et al., 2009). In addition, both Gn and Gc interact with the TSVV nuclease protein (N) and RNPs in ER and Golgi (Ribeiro et al., 2009). Therefore it seems that viral Gn and Gc glycoproteins, transported within the ER and Golgi, play an important role in the generation of the hybrid ER–Golgi structure where the virus is replicated and assembled. As one of the few plant viruses that are enveloped by lipid membranes, it will be interesting to examine how TSVV migrates, once assembled in the cytoplasmatic vesicles, to the neighboring cells. Likely, TSVV moves cell-to-cell differently from its animal counterpart, because the viral NSm protein, which was shown to restore the cell-to-cell and long distance movement of a movement-defective TMV clone (Lewandowski and Adkins, 2005), forms tubules in association with plasmodesmata (PD) upon TSVV infection of N. rustica (Storms et al., 1995).

ROLE OF THE HOST SECRETORY PATHWAY IN INTRA- AND INTER-CELLULAR TRANSPORT OF VIRUSES

As described above, many plant viruses replicate in infected cells in association with membranous structures derived from the host secretory pathway (Laliberte and Sanfacon, 2010; Verchot, 2011). Consequently, virions or viral nucleic acid–protein complexes must travel from the site where they are replicated to the neighboring cells to start the replication cycle again in order to cause a systemic infection. It has been generally accepted that plant viruses move from an infected cell to its neighboring cells through PD (Schools et al., 2011; Ueki and Citovsky, 2011). However, the virions and even the naked genome of plant viruses are too large to fit through unaltered PD. To facilitate the spread of viral particles or replication complexes, most plant viruses encode at least one dedicated protein termed movement protein (Lucas, 2006) to modify the structure of PD (Lucas, 2006; Schools et al., 2011; Ueki and Citovsky, 2011).

How could viral particles or replication complexes generated in the virus factory move to PFD Studies of various plant viruses over the past decades indicated that host cytoskeletal elements provide viruses the means to move from their sites of replication to PD from where they travel to the neighboring cells (Schools et al., 2011; Ueki and Citovsky, 2011). However, recent studies of some plant viruses indicate that the host secretory pathway and protein transport machineries may also be hijacked by viruses for their efficient transport to PD and cell-to-cell movement. In TMV, the MP and the 126 kDa replicase proteins are required for its efficient movement (Reichel and Beachy, 1998; Hirashima and Watanabe, 2001). Both proteins as well as TMV replication complexes are found to be associated with the ER (Heinlein et al., 1998; Yanamaka et al., 2000; Hagisawa et al., 2003). However, it is interesting that lower concentrations of BFA (18 μg/ml) or overexpression of dominant negative Sar1 does not inhibit intercellular transport of neither the MP of TMV nor the TMV replication complexes to PD (Tagami and Watanabe, 2007), while a higher concentration of BFA (>50 μg/ml) did inhibit the movement of the TMV MP to PD (Heinlein et al., 1998; Wright et al., 2007). The higher concentration of BFA is known to disrupt interconnected ER network (Zheng et al., 2004). Thus the interpretation of the different effect of different BFA concentrations could be that the transport of TMV to PD requires a networked ER but does not require the COPII-COP1-dependent molecular machinery. Considering the fact that ER tubules traverse through PD to the neighboring cells, it is possible that ER tubules are used directly.
by TMV as a transport conduit for both intra- and inter-cellular transport.

In the case of the members of the genus Potyviruses, it appears that intracellular transport of virus replication complexes from the site where they replicate to PD requires the classic COPII/COPI vesicle trafficking machinery in the secretory pathway. As described in the previous section, in both TuMV- and TEV-infected cells, motile 6K2 vesicular structures are identified as a site where viral genome can be replicated (Beauchemin et al., 2003; Cotton et al., 2009; Grangeon et al., 2012). Furthermore, the movement of 6K2 and the cell-to-cell movement of TuMV can be inhibited by BFA at 10 μM (Grangeon et al., 2012). For potyviruses, the proteins VPg, CI, CP, and P3N-PIPO have been implicated in inter-cellular movement of viruses through PD (Nicolas et al., 1997; Rojas et al., 1997; Carrington et al., 1998; Wei and Wang, 2008; Wei et al., 2010). It is interesting that recently, Wei et al. (2010) demonstrated that the trafficking of P3N-PIPO and CI to PD requires a COPII-COPI-dependent protein transport machinery, but not the aconyosin system (Wei et al., 2010) as the localization of P3N-PIPO and CI to PD is abolished in infected cells treated with BFA, or in cells expressing a mutant version of Sar1 [Sar1(H74L)], but not by actin disrupting agents. It seems that the transport of the potyvirus replication complex to the cell periphery requires the classic COPII/COPI vesicle trafficking machinery in the secretory pathway and the aconyosin system, but the transport of the viral proteins to PD for the subsequent modification of PD only requires the classic COPII/COPI vesicle trafficking machinery in the secretory pathway. However, NSvc, the putative MP of rice stripe virus (RSV) and MP17, the MP of potato leaf roll virus (PLRV), when expressed alone, are targeted to PD in COPII- and actin-dependent manners (Vogel et al., 2007; Yuan et al., 2011). Similarly, the MP protein p7B of melon necrotic spot virus is found to localize to the ER, Golgi, and PD when ectopically expressed (Genoves et al., 2010). Genoves et al. (2010) show that both BFA and latrunculin B inhibit the transport of p7B to Golgi and PD at the level the ER. However, it is generally accepted that in plant cells the aconyosin system is not required for protein transport from the ER to Golgi (Brandizzi et al., 2002).

As mentioned previously, in plant virus infected cells, PD has to be modified, either with an increase in SEL or formation of tubules (reviewed by Schoelz et al., 2011), to allow viral particles or replication complexes to spread. Recent data suggested that, in addition to movement-related proteins encoded by various viruses, some host proteins, for example, PDLP1, a type I membrane protein located to PD, appear to be required for successful modification of PD (Huang et al., 2000; Thomas et al., 2008; Amari et al., 2010). When Arabidopsis plants expressing mutant versions of PDLP1, PDLP2, and PDLP3 are inoculated with cauliflower mosaic virus (CaMV), a Caulimovirus that induces tubules through which CaMV virions move, significantly fewer plants present systemic infections (Amari et al., 2010). The replication of CaMV in the protoplasts carrying the triple mutation is, however, comparable to the replication in wild type Arabidopsis (Amari et al., 2010). This suggests that the PDLP1 protein plays a vital role in the movement of the virus, possibly in the modification of PD for the formation of tubules. It is interesting that the targeting of PDLP1 to PD is COPII-dependent (Thomas et al., 2008). However, in CaMV-infected cells, it appears that targeting of the MP of the virus to the cell periphery is not abolished by the treatment of BFA (Huang et al., 2000). It seems that transport of viral proteins of CaMV and host proteins to PD relies on different transport routes.

In addition to the above mentioned roles that the host secretory pathway may play in the transport of virus replication complexes and viral and host proteins required for efficient spread of viruses, it is worth noting that the TGB2 protein of potato mot top virus (PMTV), which is located to both the ER and PD and is probably involved in the modification of PD for intercellular virus movement (Cowan et al., 2002), can also be found in vesicles marked by FM4-64 (Haupt et al., 2005), a fluorescent dye that is widely used to track endocytosis in plant cells (Qi et al., 2011). TGB2 interacts with a J-domain chaperone essential for the endocytic protein transport and is also co-localized with Ara7, a Rab-F protein localized to the PVC/MVB (Haupt et al., 2005). In addition to this, the MP of TMV and a DNA virus, cabbage leaf curl virus (CaLCuV), physically interact with the clathrin-associated SNARE-interacting protein synaptotagmin (Lewis and Lazaroñiz, 2010). Synaptotagmin is a Ca$^{2+}$ sensor and down-regulation of this protein inhibits endocytosis and intercellular spread of TMV and CaLCuV (Lewis and Lazaroñiz, 2010). However, the exact role that this endocytic transport pathway may play in the virus multiplication is yet to be determined. Endocytosis is known to be a mechanism for cells to regulate the localization and/or function of some plasma membrane localized proteins (Greubsen and Maxfield, 1995), perhaps the accumulation and function of proteins required for modification of PD for efficient virus spread needs to be regulated by endocytosis.

**CONCLUDING REMARKS**

Plant cells have a sophisticated secretory pathway that is vital for their growth and survival in response for prevailing environmental conditions. In recent years, there has been an explosion of information regarding the organization of the plant secretory pathway and the molecular mechanisms that regulate protein transport in the plant secretory pathway. For successful infection of host plant cells, it seems that plant viruses have developed different yet highly host-adapted strategies to take advantage of the molecular machineries provided by the host secretory pathway to replicate and move from infected to uninfected cells. However, how plant viruses interplay with their host secretory pathway is not well understood. It is highly likely that different plant viruses interact differently with the host secretory pathway for efficient virus multiplication. To unravel the exact mechanisms by which different viruses are replicated, and transported to and through PD to neighboring cells, we need to identify more of the host factors required for the multiplication of different viruses. We need to understand how these host factors interact with different viral factors and to figure out the functional significance of these interactions in viral...
infection. We believe that a further understanding of these issues is important for those who wish to develop plant cultivars that resist virus infection. On the other hand, such research could also serve as a way to unlock the secrets of the host secretory pathway in plant cells.

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