Plant Viruses. Invaders of Cells and Pirates of Cellular Pathways[1]

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Plant viruses, discovered over a century ago when the science of virology was born (for review, see Creager, 2002), are obligate parasites on their hosts. Through their life cycle, from virus accumulation to intracellular, local, and systemic movement, viruses utilize plant proteins, normally involved in host-specific activities, for their own purposes. Although the first identification of a host protein interacting with plant viral RNA took place more than 25 years ago (for review, see Buck, 1999; Waigmann et al., 2004), the true complexity of this interaction between plant viruses and their hosts to allow virus accumulation and spread is just now becoming understood.

In addition, the ability of the host to defend itself against virus replication and spread is now known to be much more complex than was thought not long ago. During the early 1990s, the first findings were published suggesting that a plant host defense system targeting viral RNA with extreme sequence specificity existed (e.g. de Haan et al., 1992; Lindbo and Dougherty, 1992). Initially, these observations were not fully understood to represent an RNA-mediated host defense system now referred to as the RNA interference (RNAi), but with time were well differentiated from the better studied host and transgene defense systems mediated through proteins (e.g. the hypersensitive reaction of *Nicotiana tabacum* cv Xanthi NN against tobacco mosaic virus [TMV] and coat protein-mediated resistance; Beachy, 1999; Marathe et al., 2002). In the last few years, plant molecular virologists and biologists have moved with increasing speed to document the incredibly complex interactions between virus and host factors necessary to allow or defeat virus infections in the presence of RNAi (e.g. Baulcombe, 2004). Thus, plant viruses, besides their traditional role as causative agents of numerous plant diseases, represent molecular tools to examine and dissect diverse basic cellular processes in plants, ranging from intracellular transport and nucleocytoplasmic shuttling (Lazarowitz and Beachy, 1999; Oparka, 2004) to intercellular transport (Waigmann et al., 2004) to gene silencing (Moissiard and Voinnet, 2004).

This focus issue reports new insights into how viruses may utilize host factors to accumulate and move intracellularly to position for intercellular movement (Chen et al., 2005; Ju et al., 2005; Liu et al., 2005). Also, information further illuminating the ''give and take'' between virus and host factors battling for control during RNAi is presented (Chellappan et al., 2005; Liu et al., 2005; Schwach et al., 2005). *Update* articles on virus-host interactions during virus replication and movement in this issue review recent information in these areas to provide clues for productive future research (Boevink and Oparka, 2005; Thivierge et al., 2005). In this *State of the Field* editorial, we introduce the research and *Update* articles in this issue and review recent literature on virus-host interactions not addressed in the *Updates*.

VIRUS ACCUMULATION

For both DNA and RNA plant viruses, the accumulation of progeny virus involves translation and replication of viral sequences (Buck, 1999; Ahlquist et al., 2003; Noueiry and Ahlquist, 2003; Hanley-Bowdoin et al., 2004; Ishikawa and Okada, 2004; Räjamaki et al., 2004, and refs. therein). These plant viruses rely on the host to provide factors to aid their accumulation. The *Update* article by Thivierge et al. (2005) presents a summary of recent insights into the mechanisms by which positive-sense single-stranded RNA viruses take advantage of the host cell mRNA processing and translation machinery.

Research on virus-host interactions during DNA virus accumulation has also moved forward. For example, an NAC domain protein, SINAC1, from tomato (*Solanum lycopersicum*) that interacts with a geminivirus replication enhancer (REn) protein was identified and suggested to participate in viral replication (Selth et al., 2005). NAC family members, which function in plant development and defense responses (e.g. Xie et al., 2000; Hegedus et al., 2003), are known to interact with other geminivirus proteins, such as RepA, but in those instances they inhibited rather than promoted viral replication (Xie et al., 1999). Furthermore, a NAC protein interaction with an RNA virus coat protein is

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necessary during a resistance response in Arabidopsis (*Arabidopsis thaliana*; Ren et al., 2000). That host proteins from a single family display different functions during DNA and RNA virus infection illustrates the complexity of the virus-host interaction process.

Large-scale screening for host factors that affect RNA virus accumulation has been undertaken using yeast as an alternative host distinguished by a wealth of well-characterized mutants (e.g. Kushner et al., 2003; Panavas et al., 2005). These experiments showed that host genes involved in viral accumulation may differ between viruses. For example, while brome mosaic virus and tomato bushy stunt virus each are affected in their accumulation by approximately 100 host genes, only 14 of these genes overlap between viruses. The overlapping genes encoded proteins belonging mainly to three functional groups: protein biosynthesis, protein metabolism, and transcription/DNA remodeling (Panavas et al., 2005). Interestingly, no overlap existed between tomato bushy stunt virus and brome mosaic virus for genes involved in protein targeting, membrane association, vesicle transport, or lipid metabolism (Panavas et al., 2005), suggesting that there are important differences between these viruses for host membrane targeting and intracellular transport. Although analysis in yeast allows a high-throughput analysis of yeast host factors that affect plant virus accumulation, it is important to supplement these data with information obtained in plant cells, for example, using a recently developed technology to study virus replication in a cell-free system of membrane-containing extract from uninfected evacuolated plant protoplasts (Komoda et al., 2004). The potential to utilize protoplasts from mutant plants silenced for expression of specific plant genes identified through the yeast-based selection is very exciting.

INTRACELLULAR AND INTERCELLULAR MOVEMENT

To spread between cells, viruses must first move from their replication sites to plasmodesmata at the cell periphery and then traverse these intercellular channels to enter the neighboring cell. Cell-to-cell transport of most plant viruses is mediated by specific virally encoded factors termed movement proteins (MPs), the function of which may be augmented by other viral proteins (for review, see Morozov and Solovyev, 2003; Räjamaki et al., 2004; Waigmann et al., 2004). The majority of the cell-to-cell transport machinery, however, is presumed to be provided by the host cell. One such host transport apparatus is the cytoskeleton. Although plant cytoskeletal elements were implicated in viral cell-to-cell transport a decade ago (Heinlein et al., 1995; McLean et al., 1995), the relative roles of microtubules and microfilaments in the transport process are just emerging. Recent data suggest that, for TMV, microfilaments participate in the cell-to-cell movement of the virus, whereas microtubules and microtubule-associated proteins may be involved in degradation of the viral MPs (Gillespie et al., 2002; Kragler et al., 2003). In this issue, Liu et al. (2005) demonstrate the role of microfilaments in cell-to-cell movement of TMV. Disruption of microfilaments by pharmacological agents or by virus-induced gene silencing compromised TMV spread from cell to cell, but it did not significantly affect viral accumulation within the infected cells (Liu et al., 2005). Furthermore, this study demonstrated the potential involvement of another TMV factor, the 126-kD protein, in viral transport along microfilaments; the 126-kD protein was shown to associate with viral replication complexes, modulate their size, and potentially mediate their interaction with and movement along the microfilament network (Liu et al., 2005).

Increasing evidence suggests that the cytoskeletal network does not function alone in viral transport to and through plasmodesmata. Instead, it may act together with the endomembrane transport system of the host cell. Specifically, many viral MPs may be delivered to plasmodesmata via the endoplasmic reticulum (ER), while actin/myosin filaments may regulate the flow of proteins in the ER membrane (Boevink and Oparka, 2005). Two articles in this issue address the role of ER in viral cell-to-cell transport and plasmodesmal targeting. Ju et al. (2005) show that the potato potexvirus X (PVX) triple gene block (TGB) p2, one of the proteins required for movement of this group 2 member of the TGB-containing viruses, associates with ER-derived vesicles, which in turn colocalize with actin filaments. Intriguingly, no association of the TGBp2 with Golgi vesicles was detected (Ju et al., 2005), consistent with findings in a recent report studying the movement of TGBp2-containing structures in tissue infected with potato mop-top virus, a group 1 member of the TGB-containing viruses (Haupt et al., 2005). Thus, these viruses likely use an ER-dependent pathway for plasmodesmal targeting, which is different from the Golgi-dependent targeting to plasmodesmata recently demonstrated for some cellular proteins (Sagi et al., 2005).

The association of the potexviral TGBp2 MP with microfilaments and ER resembles similar associations of the tobamoviral MP and the 126-kD protein (McLean et al., 1995; Heinlein et al., 1998; Hagiwara et al., 2003; Liu et al., 2005). This resemblance indicates physical and functional similarities between MPs and movement-associated proteins of potexviruses and tobamoviruses, suggesting that both viral groups utilize a similar method of intracellular movement, at least through a portion of this passage (Nelson, 2005).

For TMV, the role of the ER translocation and plasmodesmal targeting was explored by Chen et al. (2005) using calreticulin, a cellular protein that localizes to plasmodesmata (Baluska et al., 1999; Michalak et al., 1999; Chen et al., 2005). This study showed that the N-terminal signal peptide was critical for the ability of calreticulin to accumulate within plasmodesmata (Chen et al., 2005). Based on these observations, it is tempting to speculate that plasmodesmal targeting involves two dis-

tinct signals, a signal to enter the ER network and a putative plasmodesmata localization signal. Consistent with this idea, several types of viral MPs that "gate" plasmodesmata (e.g. Waigmann et al., 1994; Tamai and Meshi, 2001) have been shown also to associate with the ER (e.g. Heinlein et al., 1998; Haupt et al., 2005; Ju et al., 2005).

Chen et al. (2005) also showed that calreticulin interacts with TMV MP and that overexpression of calreticulin in transgenic plants redirects TMV from plasmodesmata to microtubules and compromises cell-to-cell transport of the virus. A potential, albeit indirect, functional link between viral MPs and calreticulin also may be inferred from the observations that one of the two MPs of the turnip crinkle virus (TCV) interacts with an Arabidopsis protein containing two RGD cell-attachment sequences (Lin and Heaton, 2001) that are recognized by integrins (Campbell et al., 2000), which in turn interact with calreticulin (Dedhar, 1994).

Possible roles of the calreticulin-MP interaction in regulation of plasmodesmal permeability are discussed in the *Update* article by Boevink and Oparka (2005) in this issue. These authors present a review of the latest trends and discoveries regarding the role of the ER/actin network in intracellular transport, recognition of adhesion sites at the cell periphery, modification of plasmodesmata by alteration of the cell wall structure, Hsp70 chaperones as potential translocation factors, and regulation of viral cell-to-cell movement (Boevink and Oparka, 2005).

Recently, a potential link between virus accumulation and cell-to-cell movement was identified when the eukaryotic translation factors eIF4E and eIF(iso)4E, which are required for accumulation of potyviruses (Duprat et al., 2002; Lellis et al., 2002; Ruffel et al., 2002; Nicaise et al., 2003), were also shown to aid in virus cell-to-cell movement (Gao et al., 2004). These observations supported earlier findings where plant mutants with altered eIF4E activity exhibit limited virus spread (Arroyo et al., 1996). It has been speculated that potyvirus intracellular movement may occur via an interaction of eIF4E with eIF4G, which then binds microtubules (Lellis et al., 2002). Regardless of the mechanism of eIF4E-mediated virus movement, it is important to realize that host proteins may function in several steps of the virus infection process, e.g. in the case of eIF4E, both in virus translation and/or replication and in viral cell-to-cell movement.

Finally, in recent years, viral MPs have been shown to interact with numerous other cellular proteins, such as pectin methylesterases (Dorokhov et al., 1999; Chen et al., 2000; Chen and Citovsky, 2003), protein kinases (Yoshioka et al., 2004), homeodomain proteins (Desvoyes et al., 2002), DnaJ-like proteins (Soellick et al., 2000; von Bargen et al., 2001), rab acceptor-related proteins (Huang et al., 2001), β-1,3-glucanase-interacting proteins (Fridborg et al., 2003), and transcriptional coactivators (Matsushita et al., 2001, 2002). To date, only protein kinases have been shown to play a role in viral intercellular movement (Citovsky et al., 1993; Kawakami et al., 1999; Waigmann et al., 2000; Trutnyeva et al., 2005), while the functions of other MP-interacting proteins in this process remain obscure, awaiting future studies.

VIRUSES VERSUS RNAi HOST DEFENSE

Virus-host interactions during RNAi in plants are complex and understood only at a rudimentary level. In general, plants have multiple RNA silencing pathways with diverse biological roles (Baulcombe, 2004). These include the regulation of gene expression and importantly, for this short review, the control of virus accumulation. The analysis of the RNAi pathway controlling virus accumulation is complicated because some of the host genes involved in this process also function in regulating host gene expression. In addition, viruses themselves modify the final outcome by their expression of proteins that defeat the system, i.e. suppressors of RNA silencing. For a more complete understanding of this rapidly evolving area, there are many excellent recent reviews (Baulcombe, 2004; Ding et al., 2004a; Moissiard and Voinnet, 2004).

RNA silencing involves the recognition of a target RNA and its subsequent destruction. This occurs via a multistep enzymatic pathway including, in plants, an RNA-dependent RNA polymerase (RdRP; now referred to as RDR), an RNase-III-type dicer-like endonuclease (DCL), putative members of the RNA-induced silencing complex such as Argonaute, which likely binds RNA, and other proteins that may support RNA-induced silencing complex activity, such as DEAD box helicases (SDE3; for review, see Baulcombe, 2004; Meister and Tuschl, 2004). The majority of these proteins are members of gene families, and it is this multiplicity of family members that allows the plant to respond to widely varying needs (e.g. plant development and defense against virus invasion) and complicates our ability to understand each system.

One way to simplify this issue is to identify natural or created plant knockout mutants for each gene involved in RNA silencing and study their loss-of-function phenotype during virus challenge. Using this approach, Arabidopsis DCL2 was found to be required for protection against TCV (Xie et al., 2004). Arabidopsis SDE3 was required for protection against cucumber mosaic virus (CMV) but not tobacco rattle virus (TRV; Dalmay et al., 2001). For the RDRs, the tobacco RDR1 was required for protection against TMV and PVX, while its Arabidopsis homolog was required for protection against TMV-cg, a tobamovirus very closely related to turnip vein clearing virus (TVCV; Lartey et al., 1993), and TRV (Xie et al., 2001; Yu et al., 2003). Interestingly, *Nicotiana benthamiana* is a natural mutant for RDR1, and transgenic expression of an RDR1 ortholog from *Medicago truncatula* enhanced its susceptibility to TMV, TVCV, and sunn hemp mosaic virus (a tobamovirus, but only distantly related to TMV and TVCV), but not CMV or PVX (Yang et al., 2004). Similarly, RDR6 was required for protection against CMV, but not turnip mosaic virus,

TVCV, TCV, or TRV in Arabidopsis (Dalmay et al., 2000; Mourrain et al., 2000). Thus, specific RDRs likely recognize different viruses; RDR1 is required for protection against tobamoviruses and TRV, while RDR6 is required for protection against CMV.

In this issue, Schwach et al. (2005) report that RDR6 in *N. benthamiana* is required to inhibit infections by PVX, potato virus Y, and CMV, in the presence of its Y satellite RNA, but has no effect on infections by TMV, TRV, TCV, and CMV, in the absence of the Y satellite RNA. During infection with PVX, RDR6 prevented the systemic (including meristems), but not local, infection of plants (Schwach et al., 2005). Grafting experiments showed that RDR6 is required for cells to respond to a systemically moving silencing signal. The results of this study suggested that RDR6 produces double-stranded RNA precursors from the silencing signal that are used to generate short-interfering RNAs (siRNAs), which in turn allow an immediate silencing response against the target virus on its arrival (Schwach et al., 2005). This information advances our understanding of the mechanism of the host RNAi-mediated resistance pathway against virus infection. For example, as Schwach et al. (2005) suggest, exclusion of virus from the meristem is mediated by RNAi, and RDR6 is involved in this process. These results also raise issues to consider for future work in this area. For example, what are the virus and satellite RNA targets telling us about the substrate structural requirements for each RDR, or is it that factors other than substrate suitability control the ability of particular RDRs to control accumulation by specific viruses? Also, why does the Arabidopsis RDR6, but not *N. benthamiana* RDR6, protect against CMV?

It was also interesting that Schwach et al. (2005) showed that RDR6 did not control cell-to-cell movement of PVX, indicating that the silencing pathway in which this enzyme functions does not target virus intracellular or intercellular movement. In this issue, Liu et al. (2005) reported that mutant TMVs expressing 126-kD protein silencing suppressors of varying strengths were also not altered in cell-to-cell movement (for TMV suppressor characterization, see Kubota et al., 2003; Ding et al., 2004b). Earlier, such an unlinking of RNA silencing suppressor activity from cell-to-cell movement was demonstrated for the P15 suppressor from peanut clump peculovirus (Dunoyer et al., 2002). It will be interesting to determine whether or not RNA silencing ever directly targets the intracellular or cell-to-cell movement forms of the viral RNA. It may be that these forms are always protected from the host silencing machinery.

Another article in this issue reports the effect of temperature on the production of siRNAs in plants challenged with various geminiviruses, demonstrating that RNA silencing increased as the temperature was raised from 25°C to 30°C (Chellappan et al., 2005). This finding extends to DNA viruses what was found for an RNA virus, Cymbidium ringspot virus, in *N. benthamiana* (Szittya et al., 2003). Importantly, the increase in siRNA steady-state levels was most striking (3- to 6-fold) for geminiviruses not associated with a recovery phenomenon (i.e. producing fewer symptoms over time) compared with those that were associated with a recovery phenomenon. This dramatic increase in siRNAs also was correlated with the presence of one of two viral suppressors in these geminiviruses (Vanitharani et al., 2004; Chellappan et al., 2005). The critical importance of controlling temperature when studying RNAi or applying it in agriculture is also highlighted in this work (Chellappan et al., 2005).

Last, it is interesting that connections between the induction of stress in cells, which could be considered a host defense response, and virus movement may exist. For example, exposure of plants to abiotic stress, e.g. low levels of heavy metal cadmium, blocks viral systemic movement (Citovsky et al., 1998; Ghoshroy et al., 1998; Ueki and Citovsky, 2002). At the other extreme, stress may aid movement because host heat shock protein (HSP) 70 (Aoki et al., 2002) and virus-encoded HSP70-related proteins (Medina et al., 1999; Alzhanova et al., 2001; Prokhnevsky et al., 2002) likely help viral and/or host macromolecular transport through plasmodesmata. Interestingly, the induction of host HSP70s during infection by plant RNA viruses is driven by a general mechanism that senses the level of misfolded proteins in the cell, regardless of protein origin, viral or host (Aparicio et al., 2005). The study by Ju et al. (2005) in this issue touched on the role of stress in viral movement by showing that turnover of TGBp2 was greater during virus infection than when it was expressed alone in plant cells. They also determined that, in plant cells, TGBp2:green fluorescent protein had a longer half-life than free green fluorescent protein. Based on these observations, Ju et al. speculated that cell stress, represented by increased protein turnover, could aid movement of PVX between cells by translocating TGBp2 or viral movement complex out of the ER into the cytosol and making it available not only for degradation but also for transport through plasmodesmata (Ju et al., 2005). It will be interesting to see if viruses indeed have pirated the host stress response for their own purposes.

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