Impact of RNA Virus Infection on Plant Cell Function and Evolution

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Viruses are obligate symbionts that tightly interact with their hosts to complete their life cycle. Each infected cell is confronted with the accumulation of viral products and activities that have evolved to support the replication and spread of the virus in the context of host cell functions and defense responses. *Tobacco mosaic virus* encodes replicase proteins and coat protein, to replicate and protect the RNA genome, and a movement protein (MP) that binds viral RNA and manipulates the size exclusion limit of plasmodesmata to facilitate the spread of the viral genomic RNA (vRNA). The MP and replicase also interfere with the cellular RNA silencing machinery that influences plant gene expression and development. Moreover, virus-infected cells stimulate the production of a systemic signal ahead of the virus front that triggers genomic recombination leading to heritable genetic changes. Thus viruses can interact with their hosts through diverse molecular interactions. Given the high mutation rate of viruses, these interactions have implications for evolutionary processes and adaptations at the virus-host interface that may contribute to eukaryotic evolution.

Key words: Tobacco mosaic virus; **movement protein; RNA transport; plasmodesmata; microtubules; endoplasmic reticulum; virus infection; RNA silencing; silencing suppressor; small RNA; non-cell-autonomous proteins; cyanobacteria; FtsZ; virus-triggered recombination**

Introduction

Although viruses represent the most abundant biological entity on our planet,[1] little is known about their role in eukaryotic evolution. Traditionally, viruses are viewed as selfish parasites that, due to their short generation times and error-prone replication, are able to establish large population diversities that as a swarm of mutant genotypes[2] can easily adapt to changes in their host environment. This view about viruses is not surprising given that viruses play a prominent role as pathogens. However, although this perception created the widely held belief that viruses are harmful to their hosts, most viruses may rather be commensals or even be mutualists (e.g., Ref. 3). The biased view that viruses are harmful also applies to plant viruses. Plant viruses are usually seen as pathogens that are studied for the benefit of agriculture. As a consequence, research on plant viruses is usually conducted with the aim of understanding the interactions with economically important and symptomatic hosts and restricted to cultivated laboratory model or monocultured crop species in combination with standard, laboratory-strain, phenotype-producing viruses. Unfortunately, there is only little information about interactions of viruses with plants in the wild, which creates a huge gap in our overall understanding of viral diversity, evolution, and ecology in natural settings.[4] Indeed, given their biodiversity and abundance, viruses likely play an underestimated role in our ecosystems. In reality, any field plant may

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be commonly infected by one or more viruses. Some known cases of co-infecting viruses that have been addressed experimentally provided insight into the interactions and evolution of plant viruses in association with their hosts.[5]

Thus, if several virus species coexist in one host, they either compete or cooperate. Competition may occur if the infecting virus species compete for important host factors or if the viruses share sequence similarity. The latter has potential to lead to host-mediated exclusion through silencing in which siRNAs derived from infection by the first infecting virus degrade the genome of the second infecting virus. Thus, infection with a mild strain of a virus can "cross-protect" plants against a later infection by a virulent strain of the same virus.[6] Cooperation of viruses in mixed infections results if the viruses undergo symbiosis, thus share genetic information or gene products. When this interaction is mutualistic and in balance, the viral species may coevolve and increase fitness together. The interaction can also be parasitic and, thus, leading to an increase in fitness of one viral species at the expense of the other. In the extreme case of symbiosis, one virus is totally dependent on the other, and thus is an obligate symbiont. Facultative or obligate symbiotic relationships between viruses may be favorable through synergy in which one virus supports the virulence of the other virus, for example, through provision of a strong silencing suppressor.[7] Importantly, irrespective of the type of symbiosis that has evolved, the underlying tight interactions between co-infecting viruses and between viruses and their hosts are manifestations of specific and highly specialized molecular interactions between viral and cellular proteins and nucleic acids. Given the intimacy of the interactions, viruses could potentially be prime drivers of evolutionary change.[4] In fact, the high mutation rate of viruses may continuously provide new protein and nucleic acid variants with potential capacity to drive the further evolution of the corresponding cellular protein and nucleic acid counterparts. With respect to the plant:pathogen interaction, such molecular coevolution contributes to specialization of viruses and hosts and thus may contribute to host associations observed in nature.[8] Recent research on the interaction of plant viruses with their hosts has revealed some striking new details about molecular interactions at the host:virus interface, which may act as a yet unexplored creator of novel evolutionary patterns. Here, examples of such interactions, with emphasis on interactions of the plant cell with the tobamovirus *tobacco mosaic virus* (TMV), are described. Viruses are proposed to have played an important role in various evolutionary scenarios, including the origin of DNA and mammals.[9,10] They may continue to act as potent drivers in evolutive processes at the small scale,that is, at molecular interfaces with interacting host proteins and nucleic acids.

Possibility of Evolutive Processes at Molecular Plant: Virus Interfaces Associated with Plant Defense and Viral Counter-Defense

Interaction of Viral Silencing Suppressors with their Cellular Targets

RNA silencing in plants is viewed as a major mechanism to "combat" virus infections.[11] In this pathway, viral siRNAs (viRNA) produced from viral dsRNA replication intermediates and intramolecular dsRNA hairpins interact with AGO-containing RISC effector complexes and direct cleavage of homologous viral RNA.[12] In response to the evolvement of antiviral silencing, viruses have evolved proteins that suppress the degradation of viral RNA by interfering with RNA silencing at various steps. More than 35 individual silencing suppressor families have been identified from virtually all plant virus types, which indicates the importance and widespread existence of this counterstrategy.[12,13]

Silencing suppressors are strikingly diverse within and across kingdoms and are often encoded by novel, out-of frame overlapping

genes contained within more ancient genes. Although their acquisition appears to be recent and to have evolved independently, suppressors can share analogous biochemical properties. This convergent evolution may have been directed by evolutionary constraints given by the host environment and the molecular interactions within the ecological niche. The activity of silencing suppressors usually depends on direct binding interactions with RNA silencing pathway molecules. It appears likely that the interacting suppressor and its specific target are under constant co-evolution since the interaction places selective pressure on the plant to produce silencing pathway components that no longer serve as target for the viral silencing suppressor and thus to increase antiviral resistance, whereas the virus will respond with the selection of compensatory mutations in suppressor variants that restore this interaction. Thus, virus infection may continuously drive the microevolution of plant proteins at this plant:virus interface.

Evolution Toward "Balanced" Virus: Host Relationships?

The occurrence of mutations at the interface between viral silencing suppressors and their targets may be supported by the existence of virus variants that differ from wild-type virus by causing only mild or attenuated disease symptoms in infected plants. Recent research on natural and artificial severe and mild tobamovirus strains has shown that their attenuation correlated with mutations in the viral silencing suppressor.[14–18] The tobamoviral silencing suppressing activity resides in the viral replicase and appears to interfere with siRNA and miRNA methylation[18] and, thus, with sRNA stability.[19] The mutations in the suppressor function of naturally occurring tobamoviral strains might have been originally selected to complement mutations in the interacting silencing effector target in the natural host. These mutations might in turn have been initially selected to circumvent the activity of the viral suppressor and thus to increase resistance against the virus. Conceivably, the mutations in the silencing suppressor could also have been autonomously selected by the virus in an attempt to weaken the effects of infection on plant host development and thus to maintain host fitness. In this latter scenario, attenuated virus strains may thus evolve in response to selective pressure towards balanced plant:virus interactions that are optimized to maintain the reproductive fitness of both the virus and its host. To gain a realistic view about the evolution of symptomatic versus asymptomatic plant:virus interactions, efforts to identify and to study virus interactions with plants grown in the wild are needed.

In addition to acquiring recessive mutations to reduce virulence, viruses may also utilize *trans*-acting functions to control their accumulation. We recently found that the silencing suppressing function of the TMV replicase is indirectly counterbalanced by a silencing supporting activity provided by the viral movement protein (MP). This protein is required for the cell-to-cell movement of viral RNA through plasmodesmata (PD) and apparently enhances the non-cell-autonomous spread of the RNA-based RNA silencing signal.[20] It may be possible that the protein facilitates the spread of the silencing signal as a side effect of its ability to gate PD and to mediate the transport of RNA. However, this activity may also have been selected during evolution to enhance the spread of viRNA into cells ahead of infection and thus to allow the host to control virus accumulation through silencing in cells immediately upon invasion rather than only later, when the cells are fully infected and viral RNA is replicated.

Interaction of viRNAs and their Targets

The ability of MP to facilitate the spread of silencing[20] may reflect the capacity of this protein to support the spread of diverse small RNA molecules (sRNAs). Thus, in addition to viRNAs that would initiate the degradation of

vRNA and thus control viral accumulation, the sRNAs could include "beneficial" viRNAs and host siRNAs by which the virus manipulates host gene expression in order to create an optimal environment in cells about to be invaded. Some animal viruses encode miRNAs that have been shown to target host genes[21–23] and plant viruses may have evolved the same capacity. Indications in this direction come from studies on *Cauliflower mosaic virus*. The 35S leader of this virus produces several viRNAs that exhibit near-perfect complementarity to *Arabidopsis* transcripts that are effectively targeted for sequence-specific downregulation during infection.[24] An ability of plant viruses to spread beneficial viRNAs and host siRNAs would account for the reported downregulation of gene expression and other physiological changes in cells at the leading front of spreading infection sites.[25,26] One has to note, however, that a role of spreading viRNAs in supporting the spread of the virus would require that the virus-encoded silencing suppressors are tightly regulated since they would otherwise block small RNAs from acting that are beneficial for the virus as well as those that act in antiviral defense. Thus, the interplay between the virus and the host silencing mechanisms can be expected to represent a highly evolved and strictly orchestrated phenomenon. In addition, as is discussed above for the interaction between silencing suppressors and their host protein targets, the interplay between viRNAs and their host mRNA targets has implications for concurrent evolvement processes at the molecular level. Thus, transcripts targeted by viRNAs should be under strong selective pressure for mutations that prevent viRNA-mediated cleavage or translational repression. In turn, such mutations should lead to the selection of compensary mutations in the corresponding viRNA. Thus, the viral suppressor:plant target protein as well as the viRNA:plant mRNA interactions may represent potent interfaces at which viral mutations may continuously provide momentum for corresponding evolutive processes by the host.

Potential Interaction of Viral Effectors with Surveilling "Guards"

The evolvement processes proposed here to occur between viRNAs and suppressor proteins on the viral side and of viRNA suppressor targets at the host side are reminiscent of the evolvement processes between microbial elicitors and host defense proteins.[27] In bacteria:plant interactions, bacterial elicitors called PAMPs (pathogen-associated molecular patterns) trigger plant immunity through recognition by plant receptor proteins. As a response to PAMP-induced immunity, bacteria have evolved effector proteins that interfere with various steps of the PAMP-immunity pathway and thus were able to reestablish virulence. In turn, plants have evolved a second layer of defense known as effector-mediated immunity, which involves resistance (R) genes. According to recent models, R-gene products monitor or "guard" the integrity of specific host defense components termed "guardees," which are the primary targets of the pathogen's virulence factors. Thus, pathogen factors that interact with guardees are under selection pressure to evolve mutations to avoid recognition by the guard and thus to break resistance. As the pathogen now remains unrecognized and again can cause disease, the plant is under selection pressure to adapt its guards or evolve new resistance genes.[27] In analogy to the guard hypothesis applied to bacterial effector proteins, silencing suppressors may function as viral effectors, that similar to bacterial effectors, act as pathogenicity factors that are monitored by resistance gene proteins. Indeed, for example in the case of tobamoviruses, one of the triggers of R-gene mediated resistance is the viral replicase,[28,29] which, as a viral effector, supports viral pathogenicity through the suppression of silencing. Consistently, at least one resistance breaking tobamovirus with a mutation in the replicase protein has been described.[30] Another reported example of a viral silencing suppressor apparently being a target of a resistance gene is the *Tomato aspermy virus* 2b protein.[31] Thus,

interactions between viral silencing suppressors and R-gene products may represent yet another important plant:virus interface for evolutive processes.

Possibility of Evolutive Processes at the Plant: Virus Interface Associated with Viral Movement Protein Functions

Interaction of MPs with a Macromolecular Transport Pathway through PD

MPs are multifunctional proteins that support the intercellular trafficking of the viral genome by different mechanisms. Similar to viral silencing suppressor proteins, MPs are diverse in structure and likely of polyphyletic origin. Nevertheless, structural and functional criteria led to the definition of certain superfamilies. The MP of TMV belongs to the "30k" superfamily,[32] which comprises MPs able to bind nucleic acids, to increase the size-exclusion limit of PD, to localize and to accumulate in PD, to move to neighboring cells on microinjection, to facilitate movement of RNA to neighboring cells upon microinjection, to form "tubular structures" and to interact with membranes and cytoskeletal elements. MPs like that of TMV interact with nucleic acids and modify the size exclusion limit of PD. They are thought to form an elongated complex with the viral genome and to facilitate movement through the modified PD. Other MPs of the 30k superfamily assemble into tubules within PD through which they facilitate the transport of the virus in the form of whole virions.[33,34] Despite sequence divergence, MPs share certain structural features which may be indicative of convergent evolution. Thus, members of the 30k superfamily contain a "common core" domain consisting of two α-helices separated by a series of β-sheets.[32] The MP of TMV facilitates the cell-to-cell movement of the viral RNA in a nonencapsidated form, because the viral coat protein is dispensable for movement. Thus, the MP of this virus may facilitate vRNA movement by taking advantage of plant endogenous RNA transport systems that mediate the intercellular trafficking of non-cell-autonomous plant mRNAs and siRNAs.[33,35–41] A plant paralog of a viral MP able to mediate the cell-to-cell transport of RNA across PD has been described.[42] The amino acid sequence of this *Cucurbita maxima* phloem protein CmPP16 and of related sequences shares structural similarity to the sequences of the 30k superfamily thus suggesting evolutionary homology between viral MPs and these plant non-cell-autonomous proteins (NCAPs). The hypothesis that viral MPs are derived or have convergently evolved with similar plant proteins may be supported by the growing list of plant proteins that similar to MPs can interact with PD, move between cells and have non-cell-autonomous roles during plant development. The KNOTTED homeobox protein of maize is a well-known example of a non-cell-autonomous transcription factor that similar to the TMV MP is able to modify the SEL of PD and to transport RNA between cells.[43,44] The hypothesis that MPs are derived from, or convergently evolved with, NCAPs may also be supported by findings indicating that NCAPs and MPs are subject to similar types of regulation, such as phosphorylation by a PD-associated protein kinase[45,46] and that they share interactions with proteins implicated as mediators or receptors in PD-mediated intercellular trafficking, such as NCAPP1.[47]

Interaction of TMV MP with the Cytoskeleton

Studies to address the pathway by which the MP of TMV targets viral RNA from subcellular replication sites to PD have revealed that the MP interacts with mobile ER-associated RNA particles in the cytoplasm of cells at the leading front of spreading infection sites in leaves.[48] Interestingly, similar mobile RNA particles were also seen in noninfected cells and in the

absence of MP, which might suggest that MP mediates interactions of the virus with an existing RNA transport pathway. The mobile particles undergo transient contacts with microtubules, which may provide anchorage sites for assembly and guidance. The MP exhibits features of a genuine microtubule-associated protein, both *in vivo* and *in vitro*[49] and, therefore, could provide the link between the membrane-associated particles and the cytoskeleton. The interaction of MP with microtubules appears to involve interactions with factors playing a role in the regulation of microtubule assembly since the MP was recently shown to interact *in vivo* and *in vitro* with GFP-labeled microtubule End-Binding Protein 1,[50] and also was shown to bind γ-tubulin *in vitro*.[48] An interaction with microtubule assembly factors is also indicated by the observation that in addition to microtubule binding the protein shows interference with centrosomal microtubule nucleation activity when expressed in mammalian cells.[51] An earlier report indicated interactions of MP with actin,[52] which deserves further study. These findings provide examples suggesting that MP interacts with cellular membrane- and cytoskeleton-associated factors essential for cell dynamic processes and PD-mediated macromolecular trafficking. Given the importance of these processes in development the plant may not have much freedom to interfere with MP functions by mutations without risking fitness. Thus, mutations in corresponding plant genes may be rather subtle. Nevertheless, nonlethal mutations in tobamovirus MP can affect viral host range.[53] A clearly visible case of evolutionary processes occurring at the virus:plant interface for virus movement may be exemplified by the well-described evolutionary arms race between potyviral genome-linked protein (VPg) and eIF4E/eIF4G translation initiation factors.[54] Recessive resistance against virus movement caused by mutations in elF4E is overcome by mutations in the viral VPg. Because plants encode several eIF4E isoforms, the mutations in the particular interacting eIF4E gene are not lethal. Thus, the interaction with the virus can act as a driving force for co-evolution, leading to diversification of both the avirulence gene (VPg) and the resistance gene (eIF4E).[55]

Interaction of TMV MP with Cytoskeleton and Cell Junctions in Cyanobacteria

It is unknown at which time the ability of plant viruses to move-cell-to-cell has evolved. Intriguingly, the MP has the capacity to interact with the cell junctions of the multicellular cyanobacterium *Anabaena*, which suggests a degree of functional analogy between intercellular communication mechanisms of multicellular prokaryotes and plants.[56] In *Anabaena*, MP induces the formation of MP-associated filaments traversing the intercellular septa which may be similar in nature to the fibrous MP-associated material localized to PD in MP-expressing plants.[57,58] Moreover, within the *Anabaena* cells, the MP interacts with ring-like structures reminiscent of the Z-ring, the cytoskeletal structure involved in bacterial cell division.[59] This ring-like distribution of the MP may indicate that the protein associates with the *Anabaena* homolog[60] of the essential procaryotic cell division protein FtsZ.[61,62] The molecular structure of FtsZ is congruous with that of eukaryotic tubulin[63,64] confirming the homology and probable common ancestry of these proteins. Although FtsZ exhibits only low sequence identity to tubulin (10–18% at the amino acid level), its structural homology to tubulin may be sufficient for interactions with MP, either directly or via FtsZ-associated proteins. These observations may suggest that the ability of MP to interact with cytoskeletal elements and to modify cell junctions evolved as an early adaptation to multicellularity. Thus, viral MP may have influenced the refinement of PD-mediated intercellular communication pathways during evolution.

Possibility of Evolutive Processes Associated with a Virus-induced Recombination Response

Plants respond to a local infection with systemic reactions that are not restricted to exposed cells but also occur in distant organs. Well-known examples for such systemic reactions are systemic acquired resistance[65] and systemic wound signaling.[66] These phenomena involve the production of mobile signals that can evoke specific responses in tissues that are distant from the event that incited its production. Interestingly, TMV infection can trigger a systemic increase in the frequency of somatic intrachromosomal recombination events.[67] This finding suggests the existence of an activated systemic recombination signal that moves through the plant, triggering genomic change. The systemic activation of recombination results in an increased frequency of progeny plants with genetic and epigenetic changes, indicating that this phenomenon may be part of an adaptive measure to virus infection. In plant genomes, there are hundreds of R genes, each of which determines the recognition specificity for one or a few pathogenic signals.[68] Because many of the R genes are present in clusters,[68,69] virus-induced DNA rearrangement in these clusters might lead to the creation of R genes with new specificities. This example indicates that viruses, like other stresses, have the potential to drive evolutionary change.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Breitbart, M. & F. Rohwer. 2005. Here a virus, there a virus, everywhere the same virus? *Trends Microbiol.* **13:** 278–284.
2. Domingo, E. 2002. Quasispecies theory in virology. *J. Virol.* **76:** 463–465.
3. Turnbull, M. & B. Webb. 2002. Perspectives on polydnavirus origins and evolution. *Adv. Virus Res.* **58:** 203–254.
4. Wren, J.D. *et al*. 2006. Plant virus biodiversity and ecology. *PLoS Biol.* **4:** e80.
5. Roossinck, M.J. 2005. Symbiosis versus competition in plant virus evolution. *Nat. Rev. Microbiol.* **3:** 917–924.
6. Ratcliff, F.G., S.A. Macfarlane & D.C. Baulcombe. 1999. Gene silencing without DNA: RNA-mediated cross-protection between viruses. *Plant Cell* **11:** 1207–1215.
7. Pruss, G. *et al*. 1997. Plant viral synergism: The potyviral genome encodes a broad-range pathogenicity enhancer that transactivates replication of heterologous viruses. *Plant Cell* **9:** 859–868.
8. Malpica, J.M. *et al*. 2006. Association and host selectivity in multi-host pathogens. *PLoS One* **1:** e41.
9. Raoult, D. & P. Forterre. 2008. Redefining viruses: Lessons from mimivirus. *Nat. Rev. Microbiol.* **6:** 315–319.
10. Zimmer, C. 2006. Did DNA come from viruses? *Science* **312:** 870–872.
11. Voinnet, O. 2001. RNA silencing as a plant immune system against viruses. *Trends Genet.* **17:** 449–459.
12. Ding, S.W. & O. Voinnet. 2007. Antiviral immunity directed by small RNAs. *Cell* **130:** 413–426.
13. Li, F. & S.W. Ding. 2006. Virus counterdefense: Diverse strategies for evading the RNA-silencing immunity. *Annu. Rev. Microbiol.* **60:** 503–531.
14. Bao, Y., S.A. Carter & R.S. Nelson. 1996. The 126- and 183-kilodalton proteins of *tobacco mosaic virus*, and not their common nucleotide sequence, control mosaic symptom formation in tobacco. *J. Virol.* **70:** 6378–6383.
15. Shintaku, M.H. *et al*. 1996. Mapping nucleotides in the 126-kDa protein gene that control the differential symptoms induced by two strains of *tobacco mosaic virus*. *Virology* **221:** 218–225.
16. Ding, X.S. *et al*. 2004. The *tobacco mosaic virus* 126-kDa protein associated with virus replication and movement suppresses RNA silencing. *Mol. Plant Microbe Interact.* **17:** 583–592.
17. Kubota, K. *et al*. 2003. *Tomato mosaic virus* replication protein suppresses virus-targeted posttranscriptional gene silencing. *J. Virol.* **77:** 11016–11026.
18. Vogler, H. *et al*. 2007. Modification of small RNAs associated with suppression of RNA silencing by

tobamovirus replicase protein. *J. Virol.* **81:** 10379–10388.
19. Ramachandran, V. & X. Chen. 2008. Degradation of microRNAs by a family of exoribonucleases in Arabidopsis. *Science* **321:** 1490–1492.
20. Vogler, H. *et al*. 2008. *Tobacco mosaic virus* movement protein enhances the spread of RNA silencing. *PLoS Pathog.* **4:** e1000038.
21. Gottwein, E. *et al*. 2007. A viral microRNA functions as an orthologue of cellular miR-155. *Nature* **450:** 1096–1099.
22. Samols, M.A. *et al*. 2007. Identification of cellular genes targeted by KSHV-encoded microRNAs. *PLoS Pathog.* **3:** e65.
23. Stern-Ginossar, N. *et al*. 2007. Host immune system gene targeting by a viral miRNA. *Science* **317:** 376–381.
24. Moissiard, G. & O. Voinnet. 2006. RNA silencing of host transcripts by *Cauliflower mosaic virus* requires coordinated action of the four Arabidopsis Dicer-like proteins. *Proc. Natl. Acad. Sci. USA* **103:** 19593–19598.
25. Maule, A., V. Leh & C. Lederer. 2002. The dialogue between viruses and hosts in compatible interactions. *Curr. Opin. Plant Biol.* **5:** 279–284.
26. Wang, D. & A.J. Maule. 1995. Inhibition of host gene expression associated with plant virus replication. *Science* **267:** 229–231.
27. Jones, J.D. & J.L. Dangl. 2006. The plant immune system. *Nature* **444:** 323–329.
28. Padgett, H.S., Y. Watanabe & R.N. Beachy. 1997. Identification of the TMV replicase sequence that activates the *N* gene-mediated hypersensitive response. *Mol. Plant Microbe Interact.* **10:** 709–715.
29. Erickson, F.L. *et al*. 1999. The helicase domain of the TMV replicase proteins induces the N-mediated defence response in tobacco. *Plant J.* **18:** 67–75.
30. Padgett, H.S. & R.N. Beachy. 1993. Analysis of a *Tobacco mosaic virus* strain capable of overcoming *N* gene-mediated resistance. *Plant Cell* **5:** 577–586.
31. Li, H.-W. *et al*. 1999. Strong host resistance targeted against a viral suppressor of the plant gene silencing defense mechanism. *EMBO J.* **18:** 2683–2691.
32. Melcher, U. 2000. The '30K' superfamily of viral movement proteins. *J. Gen. Virol.* **81:** 257–266.
33. Heinlein, M. & B.L. Epel. 2004. Macromolecular transport and signaling through plasmodesmata. *Int. Rev. Cytol.* **235:** 93–164.
34. Ritzenthaler, C. & C. Hofmann. 2007. Tubule-guided movement of plant viruses. In *Viral Transport in Plants., Plant Cell Monographs*, Vol. 7. E. Waigmann & M. Heinlein, Eds.: 63–83. Springer. Heidelberg.
35. Yoo, B.C. *et al*. 2004. A systemic small RNA signaling system in plants. *Plant Cell* **16:** 1979–2000.
36. Lucas, W.J. & J.Y. Lee. 2004. Plasmodesmata as a supracellular control network in plants. *Nat. Rev. Mol. Cell Biol.* **5:** 712–726.
37. Haywood, V., F. Kragler & W.J. Lucas. 2002. Plasmodesmata: Pathways for protein and ribonucleoprotein signaling. *Plant Cell* **14:** S303–S325.
38. Lucas, W.J., B.-C. Yoo & F. Kragler. 2001. RNA as a long-distance information macromolecule in plants. *Nat. Rev. Mol. Cell Biol.* **2:** 849–857.
40. Heinlein, M. 2002. Plasmodesmata: Dynamic regulation and role in macromolecular cell-to-cell signalling. *Curr. Opin. Plant Biol.* **5:** 543–552.
39. Heinlein, M. 2005. Systemic RNA silencing. In *Plasmodesmata, Annual Plant Reviews*, Vol. 18. K. Oparka, Ed.: 212–240. Blackwell. Oxford.
41. Kehr, J. & A. Buhtz. 2008. Long distance transport and movement of RNA through the phloem. *J. Exp. Bot.* **59:** 85–92.
42. Xoconostle-Cazares, B. *et al*. 1999. Plant paralog to viral movement protein that potentiates transport of mRNA into the phloem. *Science* **283:** 94–98.
43. Lucas, W.J. *et al*. 1995. Selective trafficking of KNOTTED1 homeodomain protein and its RNA through plasmodesmata. *Science* **270:** 1980–1983.
44. Kim, J.Y. *et al*. 2005. A novel cell-to-cell trafficking assay indicates that the KNOX homeodomain is necessary and sufficient for intercellular protein and mRNA trafficking. *Genes Dev.* **19:** 788–793.
45. Lee, J.Y. *et al*. 2005. Plasmodesmal-associated protein kinase in tobacco and Arabidopsis recognizes a subset of non-cell-autonomous proteins. *Plant Cell* **17:** 2817–2831.
46. Lee, J.Y. & W.J. Lucas. 2001. Phosphorylation of viral movement proteins–regulation of cell-to-cell trafficking. *Trends Microbiol.* **9:** 5–8.
47. Lee, J.-Y. *et al*. 2003. Selective trafficking of non-cell-autonomous proteins mediated by NtNCAPP1. *Science* **299:** 392–396.
48. Sambade, A. *et al*. 2008. Transport of TMV movement protein particles associated with the targeting of RNA to plasmodesmata. *Traffic* **9:** 2073–2088.
49. Ashby, J. *et al*. 2006. *Tobacco mosaic virus* movement protein functions as a structural microtubule-associated protein. *J. Virol.* **80:** 8329–8344.
50. Brandner, K. *et al*. 2008. TMV movement protein interacts with GFP-tagged microtubule end-binding protein 1 (EB1). *Plant Physiol.* **147:** 611–623.
51. Ferralli, J. *et al*. 2006. Disruption of microtubule organization and centrosome function by expression of *Tobacco mosaic virus* movement protein. *J. Virol.* **80:** 5807–5821.
52. Mclean, B.G., J. Zupan & P.Z. Zambryski. 1995. *Tobacco mosaic virus* movement protein associates with the cytoskeleton in tobacco plants. *Plant Cell.* **7:** 2101–2114.

53. Fenczik, C.A. *et al*. 1995. Mutational analysis of the movement protein of *Odontoglossum ringspot virus* to identify a host-range determinant. *Mol. Plant Microbe Interact.* **8:** 666–673.
54. Robaglia, C. & C. Caranta. 2006. Translation initiation factors: Aweak link in plant RNA virus infection. *Trends Plant Sci.* **11:** 40–45.
55. Charron, C. *et al*. 2008. Natural variation and functional analyses provide evidence for co-evolution between plant elF4E and potyviral VPg. *Plant J.* **54:** 56–68.
56. Heinlein, M. *et al*. 1998. Targeting and modification of prokaryotic cell-cell junctions by *Tobacco mosaic virus* cell-to-cell movement protein. *Plant J.* **14:** 345–351.
57. Moore, P. *et al*. 1992. Developmental changes in plasmodesmata in transgenic tobacco expressing the movement protein of *Tobacco mosaic virus*. *Protoplasma* **170:** 115–127.
58. Ding, B. *et al*. 1992. Secondary plasmodesmata are specific sites of localization of the *Tobacco mosaic virus* movement protein in transgenic tobacco plants. *Plant Cell* **4:** 915–928.
59. Lapidot, M. *et al*. 1993. A dysfunctional movement protein of *Tobacco mosaic virus* that partially modifies the plasmodesmata and limits spread in transgenic plants. *Plant J.* **4:** 959–970.
60. Bi, E. & J. Lutkenhaus. 1991. FtsZ ring structure associated with division in *Escherichia coli*. *Nature* **354:** 161–164.
61. Doeherty, H.M. & D.G. Adams. 1995. Cloning and sequence of *ftsZ* and flanking regions from the cyanobacterium *Anabaena* PCC 7120. *Gene* **163:** 93–96.
62. Lutkenhaus, J. 1993. FtsZ ring in bacterial cytokinesis. *Mol. Microbiol.* **9:** 403–409.
63. Löwe, J. & L.A. Amos. 1998. Crystal structure of the bacterial cell-division protein FtsZ. *Nature* **391:** 203–206.
64. Nogales, E. *et al*. 1998. Tubulin and FtsZ form a distinct family of GTPases. *Nat. Struct. Biol.* **5:** 451–458.
65. Vlot, A.C., D.F. Klessig & S.W. Park. 2008. Systemic acquired resistance: The elusive signal(s). *Curr. Opin. Plant Biol.* **11:** 436–442.
66. Schilmiller, A.L. & G.A. Howe. 2005. Systemic signaling in the wound response. *Curr. Opin. Plant Biol.* **8:** 369–377.
67. Kovalchuk, I. *et al*. 2003. Pathogen-induced systemic signal triggers genome instability. *Nature* **423:** 760–762.
68. Meyers, B.C. *et al*. 2003. Genome-wide analysis of NBS-LRR-encoding genes in Arabidopsis. *Plant Cell* **15:** 809–834.
69. Richter, T.E. & P.C. Ronald. 2000. The evolution of disease resistance genes. *Plant Mol. Biol.* **42:** 195–204.