Experimental Virus Evolution Reveals a Role of Plant Microtubule Dynamics and TORTIFOLIA1/SPIRAL2 in RNA Trafficking

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Abstract

The cytoskeleton is a dynamic network composed of filamentous polymers and regulatory proteins that provide a flexible structural scaffold to the cell and plays a fundamental role in developmental processes. Mutations that alter the spatial orientation of the cortical microtubule (MT) array of plants are known to cause important changes in the pattern of cell wall synthesis and developmental phenotypes; however, the consequences of such alterations on other MT-network-associated functions in the cytoplasm are not known. In vivo observations suggested a role of cortical MTs in the formation and movement of Tobacco mosaic virus (TMV) RNA complexes along the endoplasmic reticulum (ER). Thus, to probe the significance of dynamic MT behavior in the coordination of MT-network-associated functions related to TMV infection and, thus, in the formation and transport of RNA complexes in the cytoplasm, we performed an evolution experiment with TMV in Arabidopsis thaliana tor1/spr2 and tor2 mutants with specific defects in MT dynamics and asked whether TMV is sensitive to these changes. We show that the altered cytoskeleton induced genetic changes in TMV that were correlated with efficient spread of infection in the mutant hosts. These observations demonstrate a role of dynamic MT rearrangements and of the MT-associated protein TORTIFOLIA1/SPIRAL2 in cellular functions related to virus spread and indicate that MT dynamics and MT-associated proteins represent constraints for virus evolution and adaptation. The results highlight the importance of the dynamic plasticity of the MT network in directing cytoplasmic functions in macromolecular assembly and trafficking and illustrate the value of experimental virus evolution for addressing the cellular functions of dynamic, long-range order systems in multicellular organisms.

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files. Nucleotide sequences were deposited at GenBank under accession numbers KF972427-KF972436.

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Introduction

Microtubules (MTs) are involved in a multitude of cellular processes such as intracellular transport and localization of organelles, determination of cell shape, or the perception and response to mechanical stimulus [1]. The plant MT network is highly dynamic and continuously remodeled into new arrangements in response to environmental and developmental information. In contrast to animal cells, where MTs are attached to the centrosome and extend with their polymerizing plus ends towards the cell periphery, cortical plant MTs are localized underneath the plasma membrane (PM) and form a barrel-shaped interphase array of dispersed MTs that do not share a common nucleation site. New MTs nucleate from mobile γ-tubulin-containing complexes that are, in most cases, recruited to existing MTs. The new MTs emerge either at a 40° angle or in parallel to the associated MTs and thus form either branched/crossover or interbundle arrangements, respectively. Upon nucleation, the new MTs may be severed away from their minus ends, thus creating free minus ends. The liberated minus ends of the severed MTs are now free to depolymerize which, if balanced by polymerization at the plus end, results in treadmilling and the translocation of the...
MTs along the PM [2–4]. MT severing also creates new plus ends that can regrow to elongated MTs at the crossover site [5]. Recent studies indicate that the severing activity of katanin at MT crossover sites is inhibited by the presence of TORTIFOLIA1/SPIRAL2 (TOR1), a MT-associated protein that promotes MT growth and stabilizes MT crossovers [6,7]. By controlling MT severing, TOR1 appears to play a central role in regulating local MT patterning within the cortical array. Consistently, katanin and tor1 mutants show alterations in the MT array. These and other mutations that affect the dynamic plasticity of the MT cytoskeleton also cause a wide range of developmental phenotypes thus illustrating the important role of MT array patterning during plant development [8]. However, although even slight changes in plant MT alignment caused by such mutations are known to affect growth, barely anything is known about the global consequences of such mutations on localized MT network-associated functions in the cytoplasm. It appears likely that the local patterning of MTs within the cortical array directs the local scaffolding for localized cellular functions and thus the functional and spatial organization of the cellular cortex. A role of MT patterning in directing localized functions of the cell is supported by specific local MT arrangements directing the patterns of cell wall synthesis in xylem and pavement cells [9]. Moreover, recent observations indicate that MTs are associated with endosomes and therefore could influence the abundance of membrane proteins such as PIN2 [10]. MTs linked to an endosomal pathway may also play a role in the targeting of non-cell-autonomous proteins to plasmodesmata (PD) [11]. MTs may also provide a framework for localized protein turnover processes such as ERAD (ER-associated degradation) [12,13] and autophagy [14]. These and other observations suggest that locally organized MTs may facilitate the localized formation, maintenance, and turnover of PM domains and also of membrane-associated macromolecular complexes that are destined for transport to PD [15].

Consistent with the above-mentioned examples, cortical MTs are also implicated in the interaction of plants with cytoplasmic viruses and their targeting to PD. Among the plant viruses that have been reported to interact with MTs, Tobacco mosaic virus (TMV) is the best characterized [16–19]. TMV replicates its RNA genome in association with MT-associated sites of the cortical endoplasmic reticulum (ER) that together with the underlying actin network provides the structure along which the viral RNA is transported to PD and into adjacent cells. The virus moves its genome between cells in a non-encapsidated form and thus may rely on cellular mechanisms usually supporting the intra- and intercellular transport of endogenous RNA complexes. The virus-encoded movement protein (MP) that binds MTs [16,20,21] is essential for cell-to-cell movement of this virus [22–24], and the ability of the virus to spread between cells is correlated with the ability of MP to interact with MTs [17,18]. Similar to RNA transport processes in other systems such as neurons or Drosophila oocytes [25–27], the transport of TMV RNA in plants is associated with the formation of mobile MP and RNA-containing particles/granules that are transported in a stop-and-go manner [18,28]. In vivo studies with conditional mutations in MP demonstrated that the formation of the granules is functionally linked to the ability of the protein to bind MTs and to its function in viral RNA movement [18,28]. Consistent with a role of MT binding, the granules move along the cortical ER and pause their movements at MT-associated ER sites [28]. The granules observed in cells at the infection front of the virus are proposed to represent early viral replication complexes (VRCs) and either spread along the ER-actin network to reach PD or remain in the infected cell to give rise to large VRC clusters or “virus factories” that are observed during later infection stages [19]. The virus factories contain replicase, viral RNA, as well as MP, and accumulate the viral coat protein (CP) and virions on the surface [29]. They are associated with the ER-actin network as well as with numerous branching MTs suggesting that the assembly and growth of VRCs may involve local reorientations in the cortical MT array [19]. The local MT rearrangements may reflect a function of MP since this protein appears to be capable to hijack the MT nucleation machinery [17,19,28,30,31]. By inducing such local MT rearrangements, the MP may support the formation, trafficking, and further maturation of VRCs by creating a local scaffold for VRC anchorage and the recruitment of host factors and ER membranes [15,19].

However, although the in vivo observations suggest a role of dynamic plasticity in the MT cytoskeleton for virus replication and movement, additional tools are required to support this model. Here, we decided to make use of the high mutation rate and evolvability of RNA viruses [32] to determine the importance of MT dynamics and patterning in the cortical MT array for virus movement by experimental virus evolution. Indeed, if the above model is valid, the dynamic plasticity of the MT cytoskeleton should be vital for the virus and any important change in the dynamic plasticity of the MT cytoskeleton should produce a selective pressure for the virus to evolve. Such evolutionary response would demonstrate the role of MT rearrangements during infection and would highlight the value of TMV evolvability as a system for testing the role of the MT cytoskeleton and its dynamic plasticity in directing cytoplasmic functions, particularly in the formation and trafficking of RNA complexes.

To test the functional intimacy between TMV and the MT cytoskeleton and particularly the role of the dynamic MT cytoskeleton in directing cytoplasmic functions related to the development and transport of viral RNA complexes, we challenged TMV with tortifolia 1/spiral 2 (tor1) and tortifolia 2 (tor2) mutants with specific defects in the dynamic behavior of MTs within the cortical MT array. Whereas the tor1 mutant is a knock-out of the MT-associated protein TOR1 and is inhibited in efficient MT polymerization and in the formation and maintenance of MT crossovers [6], tor2 carries a conservative arginine to lysine change at position 2 of the primary amino acid sequence (R2K mutation) of the β-tubulin 4 protein that is proposed to interfere with contacts between R2 of β-tubulin and the GTPase domain of β-tubulin and thus to reduce the rate of MT polymerization [33]. In this work, we demonstrate that the virus is rather well adapted to the A. thaliana wild type. However, the virus is highly sensitive to tor1 and tor2 mutations and responds to the respective changes in the dynamic plasticity of the MT cytoskeleton with mutations that are correlated with efficient spread within the mutant hosts without significantly increasing the efficiency of virus replication. The results demonstrate the importance of dynamic MT network plasticity for the establishment of cytoplasmic functions required for efficient TMV movement and that MT dynamics and MT-associated proteins represent constraints for virus evolution and adaptation. The results suggest an important role of TOR1, which may stabilize MT crossovers to support the formation and intercellular spread of the viral RNA complexes. We propose that these findings reflect the importance of the dynamic MT cytoskeleton in the formation and transport of viral as well as endogenous RNA complexes.

Results

To test the response of TMV to alterations in MT network dynamics, we used a TMV isolate obtained after replication of a...
TMV cDNA clone [24] in *Nicotiana benthamiana* plants (Figure 1). Using this isolate as the ancestral virus in our evolution experiment, we inoculated three sets (replicates) of five wild type, five *tor1*, and five *tor2* *A. thaliana* plants. At 15 days post inoculation (dpi), the non-inoculated, systemically infected, young leaves of all plants of a given set were pooled and used for virus extraction. The respective virus extracts were then used to inoculate new sets of plants. This passaging from one plant generation to the next was performed eight times. The number of infectious virion particles in each inoculum was estimated by quantifying the number of local cell death lesions following inoculation of hypersensitive *N. tabacum* NN plants (Figure S1). These tests demonstrated that the inoculum carried 150 to 400 infectious virion particles in all passages thus indicating the absence of strong bottlenecks during passages that could favor the accumulation of mutations by genetic drift and hinder fixation of adaptive mutations. After the eighth passage, nine final viral lineages were created (Figure 1): three lineages from *tor1* plants (Tor1-1, Tor1-2 and Tor1-3), three lineages from *tor2* plants (Tor2-1, Tor2-2 and Tor2-3), and three lineages from wild type plants (WT-1, WT-2 and WT-3).

To evaluate adaptation of TMV to each host genotype, the fitness of each experimentally evolved viral lineage was compared with that of the ancestral virus. Absolute fitness, $W$, was determined by measuring the over-time accumulation of viral RNA in the upper, non-inoculated leaves as described in the Materials and Methods section. Whereas the level of viral accumulation in plants, and thus $W$, results from intracellular replication and the number of new cells infected by intercellular movement, viral accumulation in protoplasts is determined only by intracellular replication. Thus, to distinguish the contribution of intercellular RNA movement from that of RNA replication, viral

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**Figure 1. Layout of the virus evolution experiment.** The initial TMV inoculum (ancestor) was obtained from *N. benthamiana* plants inoculated with a cDNA clone of TMV and was used for inoculation of *tor1*/*spr2*, *tor2*, and wild type (WT) *A. thaliana* plants (three sets of five plants each). Following eight passages, nine independent TMV lineages were obtained. The number of infectious particles used for each passage was controlled by local lesion assays with hypersensitive *N. tabacum* NN plants.

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accumulation was measured in three sets (replicates) of the wild type and mutant plants as well as in protoplasts upon inoculation with the respective viral lineage or the ancestral virus. The results indicate that relative to the ancestral virus the tor1-derived lineages developed a significant decrease in W in wild type plants (13%; P = 0.035) and a slight, yet statistically significant reduction of W in BY-2 protoplasts (0.53%; P = 0.031). Nevertheless, the same tor1-derived lineages showed a trend of higher W in tor1/spr2 plants (8.4%) (Figure 2). These results indicate that the mutant cytoskeleton in tor1/spr2 plants triggered changes in TMV that allowed TMV to adapt and thus to maintain or even increase fitness, i.e. viral RNA movement efficiency, in this environment. However, as shown by the decrease of W in BY-2 protoplasts, the adaptive changes induced by the mutant cytoskeleton in tor1 plants caused a fitness trade-off for replication. The reduced RNA replication efficiency of the tor1-derived lineages likely contributed to the decrease of W in wild type plants and may have prevented a more significant increase of W in tor1 plants. The ability of tor1 to trigger adaptation in TMV indicates a strong impact of dynamic MT behaviour and of TOR1 on virus replication and movement.

The tor2-derived lineages showed a statistically significant average increase in W of 72.2% in tor2 plants (P<0.001) whereas W remained similar to that of the ancestral virus in the wild type plants and protoplasts (Figure 2). Thus, unlike tor1, the presence of tor2 induced TMV adaptation without causing a trade-off in viral replication efficiency. The adaptation of TMV to tor2 supports a role of MT polymerization efficiency in TMV movement. Finally, the fitness of WT-derived lineages remained similar to that of the ancestral virus in wild type plants and protoplast (Figure 2 and Table S1) indicating that the common strain of TMV (U1) is well adapted to A. thaliana.

Sequence analysis for each of the nine lineages revealed 11 nucleotide substitutions in seven lineages with respect to the ancestral virus (Table 1). The tor1/spr2- and tor2- derived lineages showed one or two nucleotide substitutions in the first open reading frame (ORF) of the viral RNA, which encodes the 126 kD and 183 kD subunits of the replicase (Table 1). Nucleotide substitutions in lineages Tor1-1, Tor1-3 and Tor2-3 caused amino acids changes whereas the Tor2-1 and Tor2-2 lineages had synonymous substitutions suggesting that in addition to changes in the protein also the secondary structure of the viral RNA or its translational efficiency may play a role in optimizing viral fitness.

**Discussion**

The cortical interphase MT array of plants is well known to drive the insertion [34,35] and trajectory of the cellulose synthase complex at the plasma membrane [36,37] and thereby direct the parallel deposition of cellulose microfibrils that mediates anisotropic growth [38,39]. However, there is only very limited information about the role of the dynamic MT array in guiding other MT-associated functions in the cytoplasm. Our previous studies established that MTs play a role during TMV infection and undergo interactions with the virus-encoded MP, and that these interactions are important for the cell-to-cell movement of the virus [17,18,21]. In vivo observations indicated that MTs are associated with VRCs functioning as replication factories during late infection as well as with early VRCs, thus with MP-associated RNA complexes functionally associated with the cell-to-cell movement of the virus [19,28]. To gain further insight into the role of the MT cytoskeleton and particularly of its dynamic plasticity in guiding cytoplasmic functions related to TMV infection, we challenged TMV with specific A. thaliana mutants affected in MT dynamics. Whereas tor1/spr2 interferes with MT orientation and growth [7,40] and the stabilization of MT crossovers [6], tor2 affects tubulin polymerization efficiency [33]. We found that TMV is sensitive to these mutations and undergoes adaptive evolution that allows it to enhance its fitness in the respective mutant hosts. The ability of the host mutations to induce adaptive changes in the virus demonstrates the importance of the dynamic plasticity of the MT cytoskeleton for guiding MT network-related functions in the cytoplasm, i.e. functions that are required to support virus infection.

Given that the mutant host environment induced viral adaption and increases in fitness with respect to systemic spread without involving increases in viral replication efficiency in any of the evolved lineages, the observations indicate a role of dynamic MT network functions in TMV RNA movement. Viral adaptation to tor1 and to tor2 correlated with the fixation of mutations clustering in the replicase-encoding ORF of the virus, thus indicating a role of the replicase subunits in these processes. Although previous
evidence supports a role of the replicase in TMV spread [41], an interaction of these proteins with MTs has not been reported. The observation that host mutations affecting the dynamic rearrangements within the MT network caused changes in the replicase is consistent with the role of the 126 k replicase protein in regulating the size of the VRCs [42] and with current models implying a role of MTs in the anchorage and assembly of these complexes [19]. Interestingly, no adaptive mutations were found in the MP, which interacts with MTs in the anchorage and assembly of these complexes [19].

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Materials and Methods

Virus production

To generate the initial inoculum for the virus evolution experiment, a full-length cDNA clone of TMV strain U1 [24] was linearized with Acc65I and capped infectious TMV RNA was in vitro transcribed using the Ribomax Large Scale RNA production system-T7 (Promega). Three N. benthamiana plants (3 to 4 weeks old) were infected by mechanical inoculation of the second true leaf with 4 μg viral RNA per plant, in the presence of Celite 545 (Promaco). Following inoculation, the plants were maintained in a greenhouse at 16 h light/8 h dark cycles at 22/18°C. Virion particles were purified and quantified from symptomatic tissue at 7 dpi using published methods [12].
Arabidopsis plants and inoculation

Wild type, tor1 [40] and tor2 [33] A. thaliana (ecotype Landsberg erecta) plants were germinated and cultivated in growing chambers with 16 h light/8 h dark cycles at 22/18°C. The 5th and 6th leaves of 4 weeks old plants were rub-inoculated with virions in the presence of Celite 545 (Prolabo). Inoculated plants were maintained under greenhouse conditions, with 16 h light/8 h dark cycles at 22/18°C.

Viral passages in Arabidopsis

The virus evolution experiment involved eight viral passages between generations of plants, each consisting of three sets (replicates) of five plants for each plant genotype (Figure 1). For each passage at 15 dpi, three upper leaves of the systemically infected plants in each set were harvested and pooled. Viruses were extracted from these pools by addition of 3 ml of 10 mM sodium phosphate buffer (pH 7.0) per g of infected plant tissue, followed by homogenization using a Precellys tissue lyser (Bertin). The extracts were clarified by centrifugation for 10 min at 12000 x g and used for inoculation of new plants. The titers of infectious viral particles in the inoculum of each passage was determined by counting the number of local cell death lesions following inoculation of hypersensitive N. tabacum NN plants.

Analysis of viral fitness

The fitness of each viral lineage and the ancestral virus was analyzed by inoculation of A. thaliana wild type, tor1/spr2 and tor2 plants (10–13 plants each). Plants were inoculated with 1 µg of purified virions and non-inoculated upper leaves were harvested at 7, 12 and 15 dpi to evaluate systemic infection. Harvested samples were used for TMV detection by a commercial DAS-ELISA kit (DSMZ RT-0041, Germany, using the manufacturer’s instructions) and for analysis of viral RNA titer in ELISA-positive plants. RNA titer was determined by quantitative RT-PCR [44] using the TaqMan probe 6-FAM-AGACACGCCACATGT-TTTGGTCGCA and primers TMV-3093F (CCGATCTCAAAACCCTTGCA) and TMVIR (CGAACAGGTGTGCCTT-GACA). Viral RNA titers (Table S1) were used to calculate absolute fitness, W, defined as W = e^r, where r, the Malthusian growth rate, was estimated by regression of the logarithm of the number of viral RNA molecules that accumulate over time (7, 12 and 15 dpi). W was analyzed by using a Generalized Linear Model [45]. All W calculations refer to times after infection during which the virus was actively spreading into non-inoculated leaves.

To determine replication efficiency in the absence of virus movement, W was measured in infected tobacco Bright Yellow-2 (BY-2) protoplasts [46]. Protoplasts (1×10^6 cells) were inoculated with 1 µg of purified virions using electroporation [47]. Samples were collected at 0, 6, 12, 24, 48 and 72 hours post inoculation and used for total RNA extraction and subsequent analysis by quantitative RT-PCR. Three independent experiments (biological replicates) were performed for each viral lineage and the ancestor. The results of these quantifications are shown in Table S2.

Nucleotide sequencing

The consensus nucleotide sequences of the TMV ancestor and the nine TMV lineages were obtained by sequencing of reverse-transcribed and PCR-amplified viral RNA extracted from virions (purified as above) belonging to the 8th passage. Viral RNA was purified from virions by a protocol involving phenol/chloroform/isoamyl alcohol extraction followed by isopropanol precipitation [48]. Nine pairs of primers mapping along the TMV genome were used (Table S3). Reverse transcription was performed at 42°C for 1 h with SuperScriptIII Reverse Transcriptase (Invitrogen), followed by PCR amplification using iProof High-Fidelity DNA Polymerase (BIO-RAD) in a GeneAmp PCR System 9700 (Applied Biosystems) and applying 2 min at 94°C, 35 cycles of 15 s at 94°C, 15 s at 55°C and 40 s at 72°C, and a final incubation for 5 min at 72°C. PCR products were purified using Illustra GFX PCR DNA and Gel Band Purification kit (GE Healthcare). Nucleotide sequences were determined with a 3130xL Genetic Analyzer (Applied Biosystems) and assembled with the program STADEN 2.0.0b6 [49]. Nucleotide sequences were deposited at GenBank under accession numbers KF972427-KF972436.

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Supporting Information

Figure S1 Local necrotic lesion assay using hypersensitive tobacco NN plants to estimate the number of infectious particles in a plant extract. The left half of the leaf was inoculated with a control extract from a healthy A. thaliana plant. The right half of the leaf was inoculated with extracts from a TMV-infected A. thaliana plant (WT-1 from the fourth passage). The number of lesions reflects the number of particles in the inoculum. (JPG)

Table S1 Accumulation of the TMV ancestor and evolved lineages in non-inoculated, systemic leaves in tor1/spr2, tor2, and WT plants at 7, 12 and 15 dpi. (DOCX)

Table S2 Accumulation of the TMV ancestor and evolved lineages in tobacco BY-2 protoplasts at 6, 12, 24, 48 and 72 hpi. (DOCX)

Table S3 Forward (F) and reverse (R) primers used for TMV genome amplification by RT-PCR and nucleotide sequencing. (DOCX)

Author Contributions

Conceived and designed the experiments: EJP AS HB AN LR MH. Performed the experiments: EJP IF. Analyzed the data: EJP IF SFE LR MH. Contributed reagents/materials/analysis tools: HB SFE MH. Contributed to the writing of the manuscript: EJP AS HB AN LR MH. Read and approved the manuscript: EJP IF AS HB AN SFE LR MH.

References

1. Fletcher DA, Mellins RD (2010) Cell mechanics and the cytoskeleton. Nature 463: 485–492.
2. Murata T, Sonobe S, Baskin TI, Hyodo S, Hasezawa S, et al. (2005) Microtubule-dependent microtubule nucleation based on recruitment of gamma-tubulin in higher plants. Nat Cell Biol 7: 961–968.
3. Shaw SL, Kamzylar R, Ehrhardt DW (2003) Sustained microtubule treadmilling in Arabidopsis cortical arrays. Science 300: 1715–1718.
4. Nakamura M, Ehrhardt DW, Hashimoto T (2010) Microtubule and katanin-dependent dynamics of microtubule nucleation complexes in the acentrosomal Arabidopsis cortical array. Nat Cell Biol 12: 1064–1070.
23. Deom CM, Schubert KR, Wolf S, Holt CA, Lucas WJ, et al. (1990) Molecular determination of plant microtubule organization by modulating microtubule severing. Curr Biol 25: 1902–1907.

24. Holt CA, Beachy RN (1991) In vivo complementation of infectious transcripts from Tobacco mosaic virus movement protein. J Virol 65: 3907–3921.

25. Dohner K, Nagel CH, Sodeik B (2005) Viral stop-and-go along microtubules: Potentiating virus movement by CELL-DIVISION-CYCLE protein 46. Plant Physiol 165: 2093–2100.

26. Ashby J, Boutant E, Seemanpillai M, Groner A, Sambade A, et al. (2006) CDC48 function during TMV infection: Regulation of virus movement and replication by degradation? Plant Signal Behav 8: e22963.

27. Ketelaar T, Voss C, Dimmock SA, Thumm M, Hussey PJ (2004) Arabidopsis homologues of the autophagy protein Atg1 are a novel family of microtubule binding proteins. FEBS Lett 567: 302–306.

28. Boyko V, Ferralli J, Ashby J, Schellenbaum P, Heinlein M (2000) Function of microtubules in intercellular transport of plant virus RNA. Nat Cell Biol 2: 822–832.

29. Nelder JA, Wedderburn RW (1992) Generalized linear models. J R Stat Soc B 176: 32–37.

30. Buschmann H, Fabri CO, Hauptmann M, Hutzel P, Laux T, et al. (2004) Helical growth of the Arabidopsis mutant tortifolius does not depend on cell division patterns but involves handed twisting of isolated cells. Plant Cell 16: 2090–2106.

31. Baskin TI (2005) Anisotropic expansion of the plant cell wall. Annu Rev Cell Dev Biol 21: 203–222.

32. Ferralli J, Ashby J, Fasler M, Boyko V, Heinlein M (2006) Disruption of microtubule and centrosome function by expression of Tobacco mosaic virus movement protein 48. Plant Physiol 147: 611–623.

33. Buschmann H, Hauptmann M, Niesing D, Lloyd CW, Schaffner AR (2009) Visualization of cellular microtubules position cellulose synthase complexes in Arabidopsis. Plant J 51: 1141–1154.

34. Gutierrez R, Landbooj JJ, Parsley AR, Emons AM, Ehrhardt DW (2009) Arabidopsis cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments. Nat Cell Biol 11: 797–806.

35. Heinlein M, Epel BL, Padgett HS, Beachy RN (1995) Interaction of tobamovirus movement proteins with the plant cytoskeleton. Science 270: 1983–1985.

36. Boyko V, Ferralli J, Ashby J, Schellenbaum P, Heinlein M (2000) Function of microtubules in intercellular transport of plant virus RNA. Nat Cell Biol 2: 826–832.

37. Boyko V, Hu Q, Seemanpillai M, Ashby J, Heinlein M (2007) Validation of microtubule-associated Tobacco mosaic virus RNA movement and involvement of microtubule-associated particle trafficking. Plant J 51: 589–603.

38. Nelder JA, Wedderburn RW (1992) Generalized linear models. J R Stat Soc B 176: 32–37.

39. Lloyd C (2011) Dynamic microtubules and the texture of plant cell walls. Int Rev Cell Mol Biol 287: 287–329.

40. Buschmann H, Fabri CO, Hauptmann M, Hutzel P, Laux T, et al. (2004) Helical growth of the Arabidopsis mutant tortifolius reveals a plant-specific microtubule-associated protein. Curr Biol 14: 1515–1521.

41. Hirashima K, Watanabe Y (2001) Tobamovirus replicase coding region is involved in cell-to-cell movement. J Virol 75: 290–302.

42. Heinlein M, Padgett HS, Gens JS, Pickard EG, Casper SJ, et al. (1998) Changing patterns of localization of the Tobacco mosaic virus movement protein and replicate to the endoplasmic reticulum and microtubules during infection. Plant Cell 10: 1107–1120.

43. Asurmendi S, Berg RH, Koo JC, Beachy RN (2004) Coat protein regulates formation of replication complexes during Tobacco mosaic virus infection. Proc Natl Acad Sci USA 101: 1415–1420.

44. Crowell EF, Bischoff V, Desprez T, Rolland A, Stierhof YD, et al. (2009) Pausing of Golgi bodies via microtubules regulates secretion of cellulose synthase complexes in Arabidopsis. Plant Cell 21: 1141–1154.

45. Gutierrez R, Landbooj JJ, Parsley AR, Emons AM, Ehrhardt DW (2009) Arabidopsis cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments. Nat Cell Biol 11: 797–806.

46. Heinlein M, Padgett HS, Gens JS, Pickard EG, Casper SJ, et al. (1998) Changing patterns of localization of the Tobacco mosaic virus movement protein and replicate to the endoplasmic reticulum and microtubules during infection. Plant Cell 10: 1107–1120.

47. Heinlein M, Padgett HS, Gens JS, Pickard EG, Casper SJ, et al. (1998) Changing patterns of localization of the Tobacco mosaic virus movement protein and replicate to the endoplasmic reticulum and microtubules during infection. Plant Cell 10: 1107–1120.