Diversity of Plant Virus Movement Proteins: What Do They Have in Common?

Authors:

Yuri L. Dorokhov, Ekaterina V. Sheshukova, Tatiana E. Byalik, Tatiana V. Komarova

Date Submitted: 2021-06-21

Keywords: self-movement, MP/vRNA complex, cell-to-cell movement, intercellular transport, plasmodesmata, tobacco mosaic virus, movement protein (MP)

Abstract:

The modern view of the mechanism of intercellular movement of viruses is based largely on data from the study of the tobacco mosaic virus (TMV) 30-kDa movement protein (MP). The discovered properties and abilities of TMV MP, namely, (a) in vitro binding of single-stranded RNA in a non-sequence-specific manner, (b) participation in the intracellular trafficking of genomic RNA to the plasmodesmata (Pd), and (c) localization in Pd and enhancement of Pd permeability, have been used as a reference in the search and analysis of candidate proteins from other plant viruses. Nevertheless, although almost four decades have passed since the introduction of the term “movement protein” into scientific circulation, the mechanism underlying its function remains unclear. It is unclear why, despite the absence of homology, different MPs are able to functionally replace each other in trans-complementation tests. Here, we consider the complexity and contradictions of the approaches for assessment of the ability of plant viral proteins to perform their movement function. We discuss different aspects of the participation of MP and MP/vRNA complexes in intra- and intercellular transport. In addition, we summarize the essential MP properties for their functioning as “conditioners”, creating a favorable environment for viral reproduction.

Record Type: Published Article

Submitted To: LAPSE (Living Archive for Process Systems Engineering)

Citation (overall record, always the latest version):	LAPSE:2021.0549
Citation (this specific file, latest version):	LAPSE:2021.0549-1
Citation (this specific file, this version):	LAPSE:2021.0549-1v1

DOI of Published Version: https://doi.org/10.3390/pr8121547

processes

MDPI

Review

Diversity of Plant Virus Movement Proteins: What Do They Have in Common?

Yuri L. Dorokhov [1,2,*], Ekaterina V. Sheshukova [1], Tatiana E. Byalik [3] and Tatiana V. Komarova [1,2]

1 Vavilov Institute of General Genetics Russian Academy of Sciences, 119991 Moscow, Russia; ekaterina.sheshukova@gmail.com (E.V.S.); t.komarova@belozersky.msu.ru (T.V.K.)
2 Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, 119991 Moscow, Russia
3 Department of Oncology, I.M. Sechenov First Moscow State Medical University, 119991 Moscow, Russia; bialik@bk.ru
* Correspondence: dorokhov@belozersky.msu.ru

Received: 11 November 2020; Accepted: 24 November 2020; Published: 26 November 2020

Abstract: The modern view of the mechanism of intercellular movement of viruses is based largely on data from the study of the tobacco mosaic virus (TMV) 30-kDa movement protein (MP). The discovered properties and abilities of TMV MP, namely, (a) in vitro binding of single-stranded RNA in a non-sequence-specific manner, (b) participation in the intracellular trafficking of genomic RNA to the plasmodesmata (Pd), and (c) localization in Pd and enhancement of Pd permeability, have been used as a reference in the search and analysis of candidate proteins from other plant viruses. Nevertheless, although almost four decades have passed since the introduction of the term "movement protein" into scientific circulation, the mechanism underlying its function remains unclear. It is unclear why, despite the absence of homology, different MPs are able to functionally replace each other in trans-complementation tests. Here, we consider the complexity and contradictions of the approaches for assessment of the ability of plant viral proteins to perform their movement function. We discuss different aspects of the participation of MP and MP/vRNA complexes in intra- and intercellular transport. In addition, we summarize the essential MP properties for their functioning as "conditioners", creating a favorable environment for viral reproduction.

Keywords: movement protein (MP); tobacco mosaic virus; plasmodesmata; intercellular transport; cell-to-cell movement; MP/vRNA complex; self-movement

1. Introduction

Plasmodesmata (Pd) are channels that provide cell-to-cell flux as they pierce the cell walls and connect the cytoplasm of neighboring cells. Plant pathogens such as viruses exploit the pre-existing systems and mechanisms of intra- and intercellular trafficking of susceptible plants. To invade the distal parts of the plant, viruses follow the pathway of photoassimilate translocation. However, to reach the vascular system, the virus has to spread between cells, overcoming the restricted natural permeability of Pd for macromolecules. According to the function, the first described protein facilitating viral intercellular spread was designated viral "transport protein" [1] or "translocation protein" [2]. However, subsequently, another term became more common, namely, "movement protein" (MP) [1,3], which allows some ambiguity regarding the mechanism underlying its function. Studies performed in the last four decades have resulted in the discovery of several types of viral MP-encoding genetic arrangements. The transport function could be performed by one MP or shared between two or more proteins. However, in all cases, these proteins have the same purpose - facilitation of intercellular virus

spread by exploiting host plant pathways of intracellular trafficking and secretion and affecting the system of Pd permeability regulation and control [4]. Viral MPs are divided into two types: (a) MPs that increase the Pd size exclusion limit (SEL) without modifying the Pd structure and (b) MPs that interact with the components of Pd and modify the Pd channel with multi-subunit tubular structures consisting of tightly packed MP molecules such that intercellular viral transfer is mediated by these MP-formed "tubules".

Notably, the concept underlying the function of MPs was formulated based on studies of tobacco mosaic virus (TMV). During decades of research on the TMV 30-kDa MP, the following features of this viral protein were revealed [4–9]. TMV MP (a) binds in vitro to viral single-stranded RNA/DNA in a sequence-independent manner; (b) participates in the formation of a stable viral ribonucleoprotein (vRNP) complex that moves through Pd; (c) is targeted to Pd and docks there via the Pd localization signal; (d) increases the Pd SEL by interacting with the host factors and Pd-associated proteins; and (e) moves independently of the viral RNA into the neighboring cells, creating favorable conditions for viral infection (serving as a cell "conditioner") [10], including the spread of RNA silencing to produce a wave of small RNA-mediated gene expression changes ahead of infection to increase host susceptibility [11].

Thus, studies on TMV MP have resulted in the identification of the main properties of MP, which could serve as a guide for the identification of other viral proteins facilitating the intercellular spread of viral genetic material. Nevertheless, it appears that not all proteins designated as MPs possess the full set of capabilities identified for TMV 30-kDa MP.

We aimed to identify the common features characteristic of plant virus MPs, consider the complications and contradictions in the approaches for MP identification and define the term "movement protein". We assessed the applicability of different methods that are usually applied for the study of potential MPs.

2. Brief Description of Methods for Evaluation of the Transport Function of Viral Proteins

2.1. Use of Transmission Electron Microscopy

The use of traditional transmission electron microscopy (TEM) to study structural features of symplastic transport in plants began long before the discovery and emergence of the concept of "movement proteins". The use of TEM made it possible to acquire high-resolution electron microscopic data on the structure of Pd, which led to the creation of the first model of a primary plasmodesma [12] and the determination of its hydrodynamic radius [13]. Development of this technique later allowed electron tomography to be used to obtain unprecedented insights into the 3D ultrastructure of Pd [14]. However, TEM made it possible to detect TMV MP in Pd of transgenic tobacco, for the first time [15]. Findings demonstrated that secondary Pd are a specific site where this protein localizes [16]. Subsequently, immunolabeling fluorescence and electron microscopy showed that viral replicative complexes (VRC) containing proteins involved in TMV genome replication [17,18] and large amounts of MP [19] are localized at the orifice of Pd. TEM also permitted the detection of potato virus X (PVX) coat protein (CP) [20], potato leaf roll virus (PLRV) MP17 [21], and other MPs of plant viruses in the Pd cavity. TEM analysis is highly tedious since small and rare structures, including Pd, must be searched for. Therefore, an approach using a correlative light and electron microscopy (CLEM) technique that combines fluorescent imaging and TEM has been developed for analyzing cells infected with a virus [22]. By combining both microscopy platforms in analysis of the same sample, remarkable results can be achieved, as occurred for GFP-tagged Pd-located protein 5 (PDLP5), for instance [23]. Another example is the localization of remorin interacting physically with the PVX TGB1 protein, which was detected not only in plant plasma membrane domains but also in the Pd cavity [24]. Moreover, TEM enabled impressive results to be achieved in the study of MP and the intercellular transport of tubule-forming viruses such as cowpea mosaic virus (CPMV), for which viral RNA is transported from the cell to cell in the form of virions [4,25]. This method of intercellular movement of viral RNA was also typical for representatives of Bromoviridae, such as *Alfalfa mosaic virus* (AMV) and *Brome mosaic virus*

(BMV). TEM in combination with immunogold labeling revealed long tubular structures containing both MP and virus particles at the surface of infected protoplasts, indicating the functioning of the tubule-guided mechanism [26]. The use of TEM in combination with double-immunogold assays also enabled the discovery that similar to AMV, *Prune dwarf virus* (PDV), another member of the genus *Ilarvirus* that also belongs to the Bromoviridae family, moves in the form of virions from cell to cell via MP-generated tubular structures [27]. Interestingly, the authors performed computer-run 3D modeling and found structural resemblance between PDV and AMV MPs. Of course, as a tool for studying viral transport proteins, TEM has significant limitations [22,28]. For TEM, material of interest, such as plant leaf tissue, must be prepared to withstand observation under an electron beam in a vacuum. Therefore, it is necessary to chemically cross-link proteins to lock them in place. Subsequent treatments include dehydration in solvent to enable penetration of water-immiscible resins, polymerization of resins, ultrathin sectioning, and staining with electron-dense heavy metals. This process not only is labor intensive but also can distort cellular structures, such as Pd [29]. Regarding the topic under consideration, another drawback of TEM is more important. In particular, this approach hardly allows for observation of minor modifications of intercellular transport at the early stages of infection; thus, only late events and the most vivid structural changes in Pd are registered, such as characteristic occurrences for tubule-forming viruses, for example [30]. Therefore, study of the functioning of MPs should include the other methods described below.

2.2. Complementation and Reverse Genetics Experiments

During the study of the TMV MP transport function, a set of particular methods and approaches was developed and established. Furthermore, these methods were used in the study of other viruses. Among these techniques is the trans-complementation test, based on the phenomenon in which a viral protein synthesized in trans (often from a transgene integrated into the plant genome) can maintain or intensify the infection of the "dependent" virus that is temporarily or constantly defective in movement. Studies of the functions of genes encoding MPs have also led to the development of several trans-complementation techniques [31,32] (Tables 1 and 2). At the first stages of the study of TMV transport function, two temperature-sensitive (ts) mutants, namely, the Ni 2519 mutant of TMV A14 strain and Ls1 mutant of tomato strain TMV L, play an important role. These mutant variants can spread in plants at a low permissive (22–25 °C) temperature but not at a high non-permissive (32–33 °C) temperature. Ni2519 mutant, studied by Harald H Jockusch [33], was obtained after TMV A14 treatment with nitrous acid, resulting in the mutation leading to the substitution of arginine144 with glycine in the 30-kDa protein [34,35]. Ls1 is a temperature-sensitive mutant due to a single amino acid substitution in MP: proline154 for serine [2,36,37].

The first experimental setup for the trans-complementation was transgenic tobacco expressing gene encoding the TMV U1 30-kDa protein, which demonstrated the efficient intercellular transport of TMV Ls1 mutant at nonpermissive temperatures [3]. The second setup was the use of infectious cDNA copies of TMV [38,39], through which the phenotype of the Ls1 mutant was reproduced using nucleotide substitutions. The third approach involves simultaneous delivery into cells of the studied viral *MP* gene with the movement-defective viral genome during joint bombardment [40,41]. The fourth approach is the use of transient expression methods by which a movement-defective infectious plant virus (for example, TMV or PVX) and an investigated putative viral movement protein gene are introduced during joint agroinfection [42].

For an adequate interpretation of the results of complementation, the following aspects should be kept in mind. (a) The complementation can occur between unrelated viruses demonstrating the nonspecificity of viral transport systems [31,43]. This means that a trans-complementation approach could be used for RNA-containing viruses belonging to different taxonomic groups and having significant differences in genome structure. (b) The use of complementation methods revealed a close relationship between cell-to-cell movement and host range of plant viruses [1,31], i.e., for plant species to be a host for a particular virus, the indispensable feature is to maintain the ability of that virus spread

within plant and to be "compatible" with viral transport protein(s). (c) It is necessary to take into account and differentiate the phenomena of complementation of nonfunctional MP and synergism [32] mediated by other viral proteins, such as viral silencing suppressors, e.g., PVX TGB1 protein [43] and PVX Hc-Pro [44].

When assessing modern methods of candidate MP evaluation, it should be noted that, in addition to *Arabidopsis thaliana*, *Nicotiana benthamiana* is widely used as a model plant (Tables 1 and 2). Widespread in the field of host-pathogen research, this plant has become so popular because it is susceptible to a variety of pathogens, including a wide range of plant viruses [45]. However, the results obtained should be analyzed with some caution, as the characteristics of the candidate MPs obtained on *N. benthamiana* are not always reproducible when using other plants. For example, PVX TGB1 protein induces Pd gating and moves between cells in several host species, whereas its CP is able to move only in *N. benthamiana* leaves [46].

Table 1. Movement proteins (MPs) of the "30K" superfamily: involvement in plasmodesmata (Pd) permeability control.

Viral Genome	Selected Viruses	SSEG	MP Properties and Experimental Approach Used to Study Its Ability to Increase Pd Permeability						References
			MW	Bind.	Model/Tested Plants	Identified/Confirmed	Tub.	Mov.	
RNA positive sense	*Tobacco mosaic virus* (TMV) (genus *Tobamovirus*)	126 kDa	30 kDa	Yes	*Nicotiana tabacum (Nt)*	Trans-complementation test: complementation of TMV ts-mutant Ls1 in MP transgenic tobacco	NR	Yes	[3]
						Microinjection: increased movement of F-dextran in *MP* transgenic tobacco plants			[47]
						Microinjection *E. coli*-synthesized MP increased Pd SEL to permit passage of 20-kDa dextrans			[48]
					Nicotiana clevelandii (Nc)	Microinjection: *E. coli*-synthesized MP specifically mediated its own movement (demonstrated self-movement ability) between trichome cells as well as GUS:MP 90-kDa fusion protein			[49]
					Nt, *Nc*, *Nicotiana benthamiana (Nb)*	Particle bombardment: cell-to-cell self-movement of TMV MP:GFP or MP:2xGFP encoded by 35S-promoter-based constructs			[50–53]
	Red clover necrotic mosaic virus (RCNMV) (genus *Dianthovirus*)	p27 and p88 replicase proteins and 35 kDa MP	35 kDa	Yes	*Nb*, *Vigna unguiculata*	Microinjection: *E. coli*-synthesized RCNMV 35 kDa MP increased Pd SEL to permit passage of F-dextran. *E. coli*-synthesized and FITC-labeled 35 kDa MP moved into neighboring cell (self-movement)	NR	Yes	[54]
					Nb	Trans-complementation test: complementation of cell-to-cell movement of TMV with defective MP (TMV-MPfs) in the transgenic plant expressing the RCNMV 35 kDa MP gene			[55,56]
						Particle bombardment: RCNMV 35 kDa MP increased the SEL of Pd			[57]

Table 1. *Cont.*

Viral Genome	Selected Viruses	SSEG	MP Properties and Experimental Approach Used to Study Its Ability to Increase Pd Permeability						References
			MW	Bind.	Model/Tested Plants	Identified/Confirmed	Tub.	Mov.	
	Cucumber mosaic virus (CMV) (genus *Cucumovirus*)	CMV 2b	30 kDa (3a)	Yes	*Nt*	Trans-complementation test: increased movement of microinjected F-dextran (10 kDa) in trichome cells of 3a MP transgenic tobacco	NR	Yes	[58]
						Microinjection: *E. coli*-synthesized and FITC-labeled 3a MP moved into neighboring cell (self-movement). Unlabeled 3a MP increased Pd SEL to permit passage of F-dextran			[59]
						Particle bombardment: cell-to-cell self-movement of 3a MP:GFP or 3a MP:GUS encoded by 35S-promoter-based constructs.			[60]
	Alfalfa mosaic virus (AMV) (genus *Alfamovirus*)	NR	32 kDa (3a)	Yes	*Nt*, *Nb*	Microinjection: increased movement of F-dextran in 3a transgenic tobacco plants. Genetic analysis indicated that 3a gene of AMV is functionally interchangeable with different MPs assigned to the 30K superfamily	Yes	NR	[61–63]
	Brome mosaic virus (BMV) (genus *Bromovirus*)	NR	32 kDa (3a)	Yes	*Nt*, *Nb*	Agroinfection: cell-to-cell self-movement of BMV MP:GFP encoded by 35S-based constructs	Yes	NR	[26,64]
	Cowpea mosaic virus (CPMV) (genus *Comovirus*)	Small CP	48 kDa	Yes	*V. unguiculata*, *Nb*	Tubule-guided virus transport	Yes	NR	[65–67]
	Tobacco rattle virus (TRV) (genus *Tobravirus*)	16 kDa, 29 kDa MP	29 kDa	Yes	*Nc*, *Nt*, *Nb*	Microinjection: TRV-mediated increased movement of F-dextran in trichome cells. Trans-complementation test: TMV MP can substitute for TRV 29 kDa MP	NR	Yes(?)	[68–70]
	Potato leaf roll virus (PLRV) (genus *Polerovirus*)		17 kDa (MP17)	Yes	*Nt*	Microinjection: increased movement of F-dextran in MP17 and MP17:GFP transgenic tobacco plants	NR	NR	[71]
	Tomato bushy stunt virus (TBSV) (genus *Tombusvirus*)	P19	22 kDa	Yes	*Nt*, *Nb*	PVX-expressed 22 kDa MP complemented TBSV cell-to-cell movement defective mutants	NR	NR	[72]

Table 1. *Cont.*

Viral Genome	Selected Viruses	SSEG	MP Properties and Experimental Approach Used to Study Its Ability to Increase Pd Permeability						References
			MW	Bind.	Model/Tested Plants	Identified/Confirmed	Tub.	Mov.	
RNA negative sense	*Tomato spotted wilt virus* (TSWV) (genus *Orthotospovirus*)	NSs	30 kDa (NSm)	Yes	*Arabidopsis thaliana*	Cell-to-cell self-movement of NSm:GFP encoded by 35S-promoter-based constructs after particle bombardment	NR	Yes	[73]
	Rice yellow stunt virus (RYSV) (genus *Nucleorhabdovirus*)	P6	30 kDa (P3)	Yes	*Nb*	Trans-complementation test: P3 complemented the movement of MP-defective mutants of TMV and PVX	NR	NR	[42]
	Citrus psorosis virus (CPsV) and *Mirafiori lettuce big-vein virus* (MiLBVV) (genus *Ophioviridae*)	NR	30 kDa	Yes	*Nb*	Agroinjection: cell-to-cell self-movement of CPsV MP:mRFP or MiLBVV MP:mRFP encoded by 35S-promoter-based constructs	NR	Yes	[74]
	Raspberry leaf blotch virus (genus *Emaravirus*)	NR	30 kDa (P4)	NR	*Nb*	Trans-complementation test: P4 MP complemented the movement of MP-defective mutant of PVX	NR	NR	[75]
DNA	*Cauliflower mosaic virus* (CaMV) (genus *Caulimovirus*)	P6	40 kDa (P1)	Yes	*A. thaliana*	CaMV P1 MP is responsible for the formation of tubules through which CaMV virions move Particle bombardment: P1 MP forms tubules and does not pass from cell to cell by self-movement	Yes	NR	[76,77]
	Bean dwarf mosaic virus (BDMV) (genus *Begomovirus*)	TrAP	33 kDa (BC1)	Yes	*Phaseolus vulgaris, Nt*	Microinjection: *E. coli*-synthesized BC1 MP increased Pd SEL to permit passage of F-dextran	NR	NR	[78]
	Abutilon mosaic virus (AbMV) (genus *Begomovirus*)	AC2	33 kDa (BC1)	Yes	*Allium cepa, Nb, Nt*	Particle bombardment: AbMV BC1encoded by 35S-promoter-based constructs was only detected in single cells and never in neighboring cells	NR	NR	[79,80]

SSEG, silencing suppressor-encoding gene; MW, molecular weight; Bind., RNA/DNA binding in vitro; Tub., tubule-forming, Mov., self-movement; NR, not reported.

Table 2. MPs encoded by two or more genes: the role in intercellular viral spread.

Plant Virus MP Groups	Selected Viruses	Phl.	SSEG	MP Properties and Experimental Approach Used for Study of Its Involvement in Viral Trafficking						References
				MP comp.	Bind.	Model/Tested Plants	Identified/Confirmed	Tub.	Mov.	
Double gene block (DGB)	*Hibiscus green spot virus* (HGSV) (genus *Higrevirus*)	NR	NR	BMB1 and BMB2	Yes (BMB1)	*Nb*	The trans-complementation of cell-to-cell movement of transport-deficient potato virus X (PVX) in leaves agroinfected with BMB1 and BMB2. BMB2 directed transport of BMB1 to Pd and neighboring cells (self-movement). BMB2 increases the Pd SEL in a GFP diffusion test	NR	Yes	[81,82]
	Melon necrotic spot virus (MNSV) (genus *Carmovirus*)	NR	CP	p7A and p7B	Yes (p7A)	*Cucumis melo* cotyledons	The trans-complementation of p7A- or p7B-deficient GFP-encoding MNSV-ΔCP infectious copy by transient expression of p7A or p7B, respectively, resulted in formation of fluorescent cell clusters	NR	NR	[83]
	Turnip crinkle virus (TCV) (genus *Carmovirus*)	NR	CP	p8 and p9	Yes (p8)	*A. thaliana*	The trans-complementation of movement defective TCV mutants in transgenic *A. thaliana* plants	NR	NR	[84]
	Pelargonium flower break virus (PFBV) (genus *Carmovirus*)	NR	CP	p7 and p12	Yes (p12)	*Chenopodium quinoa* leaves and protoplasts	Site-directed mutagenesis using the PFBV infectious clone	NR	NR	[85]
Triple gene block (TGB)	*Potato virus X* (genus *Potexvirus*)	NR	TGB1	TGB1, TGB2, TGB3	Yes (TGB1)	*Nt, Nb*	Microinjection: *E.coli*-synthesized 25 kDa TGB1 increased Pd SEL to permit passage of F-dextrans	NR	Yes	[86,87]
						Nt, Nc, Nb, Lycopersicon esculentum	The trans-complementation of cell-to-cell movement of TGB1-defective GUS-encoding PVX with TGB1 fused to GFP after joint particle bombardment Cell-to-cell self-movement of PVX TGB1 fused to GFP encoded by 35S-promoter-based constructs after particle bombardment.	NR		[46,88]
						Nt	The particle bombardment: the GFP:TGB1 fusion protein moved from cell to cell in tobacco without presence of other PVX-encoded proteins (self-movement)	NR		[89]

Table 2. *Cont.*

Plant Virus MP Groups	Selected Viruses	Phl.	SSEG	MP Properties and Experimental Approach Used for Study of Its Involvement in Viral Trafficking						References
				MP comp.	Bind.	Model/Tested Plants	Identified/Confirmed	Tub.	Mov.	
	Barley stripe mosaic virus (genus *Hordeivirus*)	NR	γb	TGB1, TGB2, TGB3	Yes (TGB1)	*Nb*, *Chenopodium amaranticolor*	Usage of infectious cDNA copies showed (a) functions required for systemic invasion of plants and (b) TGB1 interaction with nucleolar protein fibrillarin is required for cell-to-cell movement of BSMV Trans-complementation: TMV MP was able to functionally substitute for the BSMV TGB-coded MPs.	NR	NR	[90–92]
Multiple-gene blocks	Genus *Potyvirus*: *Potato virus Y*, *Bean common mosaic necrosis virus* (BCMNV), *Lettuce mosaic virus* (LMV), *Turnip mosaic virus* (TuMV)	NR	HC-Pro	CP, HC-Pro, VPg, P3N-PIPO	Yes (HC-Pro)	*Nb*, *Lactuca sativa*	Microinjected *E. coli*-synthesized and FITC-labeled CP of BCMNV or LMV and HC-Pro moved into neighboring cell (self-movement). Microinjected CP of BCMNV or LMV and HC-Pro increased Pd SEL to permit passage of F-dextrans	NR	Yes	[93]
						A. thaliana	Cell-to-cell self-movement of TuMV P3N-PIPO:GFP encoded by 35S-promoter-based constructs after particle bombardment	NR		[94]
	Beet yellows virus (BYV) (genus *Closterovirus*)	Yes	p21	p6, Hsp70 h, p64, CPm and CP	NR	*Nb*, *Claytonia perfoliata*	Genetic analysis indicated that the BYV cell-to-cell movement requires the presence of p6, Hsp70 h, p64, CPm and CP	NR	NR	[95–97]
	Citrus tristeza virus (CTV) (genus *Closterovirus*)	Yes	CP, p23 p20	p33, p6, HSP70 h, p61, CPm, CP	NR	*Nb*,*Citrus macrophylla*	Genetic analysis indicated that the CTV cell-to-cell movement requires p33, p6, HSP70 h, p61, CPm and CP	NR	NR	[98–100]

Phl., phloem-limited; SSEG, silencing suppressor-encoding gene; comp., composition; Bind., RNA binding in vitro; Tub., tubule-forming, Mov., self-movement; NR, not reported.

2.3. Assessment of MP Ability to Increase Pd Permeability

Pd permeability is determined by the size of molecules that can move through it and is estimated by a criterion termed SEL [101]. The SEL value was determined using microinjection of fluorescent probes into plant cells, and it was shown that relatively small molecules (<1 kDa) freely diffuse through the Pd of cells of an intact leaf. Moreover, it is generally accepted that the hydrodynamic Stokes radius, rather than molecular weight, is the key factor in the passage of small molecules through Pd [13]. Thus, evaluating the intercellular movement of GFP, which has a Stokes radius of 2.82 nm, the coefficient of epidermal cell Pd conductivity was calculated, which strongly depends on leaf developmental state (sink/source) and the effect of abiotic factors such as temperature (16/25°C) and illumination (light/dark) [102]. It is indisputable that the most important function of viral MPs is their ability to increase the Pd SEL [101]. This property was first identified in TMV MPs when transgenic plants expressing the 30-kD MP of TMV were studied [47]. The Pd of the leaves of these transgenic plants exhibited the ability to permit transfer of microinjected dextrans of up to 10 kDa in size, which was significantly larger than the size limit for the control leaves (<1 kDa). These experiments used a pressure injection system, exploiting air pressure as a physical force to deliver the probe into the target cell. Although it is believed that sudden changes in pressure are less harmful to plant cells than changes caused by iontophoresis [103,104], surprisingly, TMV MP could mediate the movement of coinjected fluorescent dextrans within minutes to not only neighboring cells but also cells farther away from the primary injected cell [48].

Subsequently, the particle bombardment method was developed and applied: the primary inoculated so-called "0" cell [102] received not the bacterially synthesized "candidate" MP (as during the microinjection procedure) but the plasmid encoding MP:GFP translational fusions [51]. The fusion of TMV MP with GFP did not significantly affect the functional activity of the MP (see below). This method enabled identification of a "0" biolistically transformed cell surrounded by cells with decreasing GFP fluorescence. The detected gradient of GFP fluorescence was interpreted as evidence of MP (as MP:GFP) transport from "0" cells to neighboring cells. This phenomenon seems to be based on the ability of MPs to move to neighboring healthy cells in the absence of replicating viral RNA, i.e., as a self-movement phenomenon [10], characteristic not only for TMV MP but for MPs of other viruses as well (Tables 1 and 2).

Genes encoding candidate GFP-fused MPs can also be delivered to the cell by agroinfection [53,105]. Although diluted bacterial suspensions are used for agroinfection, unlike microinjections and particle bombardment, it is sometimes very difficult to identify the "0" cell. Therefore, during agrobacterial delivery of plasmids, it is necessary to create a genetic construct containing, in addition to the expression cassette encoding a putative MP, another transcribed unit—a promoter and a terminator flanking a fluorescent protein gene for marking the "0" cell, as was carried out for example using mRFP as a marker [81].

2.4. Use of Viral Vectors Encoding MP Tagged with GFP

Viral vectors encoding MP tagged with GFP now play an important role in understanding the participation of MPs in the intracellular and intercellular trafficking of viral RNA. This approach opened up new insights regarding the intracellular distribution of MPs and their association with host components [106–111]. Thus, fluorescence microscopy of protoplasts and leaf cells infected with TMV that encodes the MP:GFP fusion protein confirmed the fact that the MP:GFP is capable of being targeted to plasmodesmata and punctate sites near the plasma membrane [112,113]. Moreover, MP:GFP supported the infectivity of the viral copy and caused the expansion of necrosis on the leaves of the indicator plant, characteristic of wild-type TMV [114]. However, the presence of the GFP sequence fused to MP of an infectious TMV copy dramatically reduces the level of protein synthesis. Thus, a significantly smaller amount of MP:GFP was synthesized from the viral vector in both tobacco leaf cells and protoplasts compared with unmodified MP [19].

The decrease in the synthesis of MP:GFP directed by the infectious copy of the TMV is explained by the peculiarity of the functioning of the TMV subgenomic promoters; specifically, their "strength" decrease when the distance between the subgenomic promoter and the 3'-end of the TMV genomic RNA increases [115–117]. However, decreased MP:GFP synthesis did not significantly affect the development of infection at fluorescent infection sites on *N. tabacum* and *N. benthamiana* leaves [19,118]. This fact is in good agreement with the data of Arce-Johnson et al. [119], obtained in studies of transgenic plants accumulating different amounts of MP. It was found that the amount of MP required for local spread of TMV is much less than the amount of MP produced during TMV infection.

3. Diversity and Heterogeneity of Viral MPs: General Characteristics and Classification

Viral MPs can be divided into several groups [4,9,25,120,121]. The largest group (Table 1) is the so-called '30K' superfamily, represented by a single gene product, as was first shown for TMV 30-kDa MP [120]. In other viruses, the transport function is performed by more than one protein (Table 2) and is distributed between two (encoded by double gene block, DGB) [81,122], three (encoded by triple gene block, TGB) [123–126] or many proteins (multiple MPs) [100,127–129].

MPs can be classified into two types according to their ability to interact with Pd [4]. The first and largest group is represented by MPs that increase the Pd SEL without affecting the Pd structure, as shown, for example, for TMV when, as is generally accepted, vRNPs spread from cell to cell [130,131]. Another small group is represented by MPs (Table 1), such as those from CPMV and cauliflower mosaic virus (CaMV), which are capable of self-interacting and forming tubular structures that replace the desmotubule, thus restructuring Pd in a drastic manner. With the aid of tubular structures, intercellular transport of the encapsidated virus occurs [132,133]. For some viruses transported as virions, the MPs, in addition to forming tubular structures, can apparently perform other functions. For example, tomato spotted wilt virus (TSWV) NSm MP is able to move independently of other viral components from a "0" cell to neighboring healthy cells of a model plant such as *A. thaliana* [73]. It is also known that MPs of tubule-forming CPMV are capable of transporting vRNPs in the AMV model system [30], which indicates the likelihood of intercellular transport in tubule-forming viruses using both vRNPs and virions [4].

4. MP Provides Intracellular Trafficking of Viral RNA to Plasmodesmata

Early studies of the synthesis of MP TMV strain OM in protoplasts showed that both MP and its mRNA could be registered as early as 2 h after inoculation of protoplasts, and after 7 h, their synthesis ceases [36]. Subsequently, the transient synthesis of MP TMV strain U1 was confirmed, and it was shown that MP was detected in protoplasts 4–6 h after protoplast inoculation. In the following hours, MP accumulated in the cells. Its amount reached a maximum by 13–16 h and then decreased [19]. Protoplast studies also confirmed the earlier conclusion that TMV MP is not involved in TMV RNA replication [2] but influences localization and intracellular trafficking of viral RNA (vRNA) and VRC [111,134]. Subsequently, the involvement of elements of the cytoskeleton and components of the endoplasmic reticulum (ER) in the intracellular trafficking of MP:GFP from the sites of synthesis in the cytoplasm to Pd was shown [134,135]. Thus, at the early stage of infection in BY-2 protoplasts, vRNA is colocalized with MP in perinuclear ER, and at a later stage, it becomes associated with vRNA-containing hair-like protrusions on the surface of the protoplasts. For the topic under consideration, it is important that the vRNA produced from the mutant infectious copy of TMV lacking functional MP was distributed not in the same manner as wild-type vRNA. Cells infected with TMV cDNA lacking the *MP* gene did not exhibit the fluorescent vRNA-containing protrusions of the cellular surface that occurred in cells infected with wild-type vRNA [134]. Thus, MP was not required for association of vRNA with perinuclear ER but was indispensable for the formation of the large irregular bodies and hair-like vRNA-containing protrusions [111,134]. Thus, already in early studies, it became apparent that MP mediates intracellular vRNA trafficking from the site of its synthesis to the Pd. The detection of TMV MP in VRC apparently reflects its ability to bind vRNA, as it is produced in the vicinity, as well as the

commonality of the location of vRNA replication and translation. This ability to co-localize with VRC is characteristic not only for TMV MP but also for MPs of other plant viruses [4,8,136].

However, the presence of MP in the VRC is not a prerequisite for its movement and localization in Pd. Experiments with trans-complementation [8,31,32] (Tables 1 and 2), indicate that the function of MP is to mobilize and trigger cellular mechanisms of symplastic intercellular trafficking. Apparently, ER membranes contribute to mechanisms of adduction MPs and replicated viral genomes in close proximity despite even trans-complementation conditions. It is known that the TMV MP introduced into the cell is located on the cytosolic face of the ER membrane [137], interacts with microtubules [131] and, thereby, provides accelerated intercellular movement of the replicating virus, as observed by Kawakami et al. [18] in *MP*-transgenic tobacco. The quantitative assessment of the rate of VRC intercellular movement showed that after viral exit from the first infected cells, the rate of spread sharply increases, which indicates the conditioning of neighboring cells, a process in which both replicase and MP can participate. When considering the possible mechanism of MP-mediated intracellular trafficking of VRC to Pd, known studies confirm the diffusion model, in which a complex including ER-associated MP, vRNA, and other cellular and viral components diffuse along the ER membrane within the Pd [138]. It can be assumed that the driving force underlying this process is the concentration gradient between an infected cell and adjacent noninfected cells, as suggested by considering the transport of photosynthetic sugar [139–141].

5. Are MP/vRNA Complexes Responsible for the Cell-to-Cell Transport of Viruses?

The discovery of the ability of TMV MP to nonspecifically bind in vitro to single-stranded RNA and DNA [142], confirmed by electron microscopy [143] and atomic force microscopy [144], led to the hypothesis of the involvement of the vRNA/30-kDa MP complex in intercellular transport [6,142]. Subsequently, in addition to TMV MP, the ability to nonspecifically bind RNA in vitro was shown for MPs of many viruses [6] (Tables 1 and 2). Possessing this ability, TMV MP can form an RNP complex in a cell with its own template; since the MP is translated in the vicinity of the viral RNA, it is likely that the MP will be part of the viral movement complex. Indeed, the presence of the MP/vRNA RNP complex in a TMV-infected cell was confirmed by a technique combining the visualization of MPs fused with a fluorescent tag with MS2-based RNA labeling technology [145]. Although the sensitivity of MS2-technology was not high and only a small number of RNA-containing MP particles could be detected, the authors were able to show that transiently expressed TMV MP accumulated in Pd and mediated the transport of its own mRNA to the Pd pore. Recently, the technique of in planta mRNA tagging was improved based on the ability of the sequence-specific binding of the bacterial transcriptional antiterminator BglG [146]. The authors were able to show that transiently expressed *MP* mRNA is specifically associated with MP and transported between cells. Moreover, in this experimental system, *MP* mRNA could move between cells when not bound to MP [146].

To explain the results obtained, researchers have hypothesized that viral RNA is capable of moving to a neighboring cell, even in the absence of the bound MP, using the cellular mechanism of RNA transport, i.e., "MP may piggyback a ride on a normal RNA transport mechanism" [145]. Indeed, recent studies have indicated the presence in plant transposable RNAs of specific nucleotide sequences recognized by RNA-binding proteins that guide the RNAs to a neighboring cell [147]. Thus, it has been established that cellular RNA with a tRNA-like structure is capable of intercellular transport [148]. It should be borne in mind that TMV RNA, as well as RNAs of many other plant viruses, has a tRNA-like structure [149]. It is possible that, in addition to participating in viral replication, tRNA-like structures are also involved in the intercellular transport of viral RNA.

When evaluating the MS2 and BglG technologies and the ability of these methods to reflect probable events in a TMV-infected cell, it should be considered that in addition to the MP, the 126-kDa replicase is also involved in cell-to-cell movement [150–153]. It is known that the TMV RNA [134], as well as the RNA of other RNA-containing plant viruses [136], released from the virion immediately binds to the ER to form VRC. VRCs are trafficked to Pd and are believed to move through Pd to spread

infection [18,136]. Moreover, it was found that only progeny viral RNA is available for the formation of movement complexes [154]. All of these results demonstrate that the Pd-mediated cell-to-cell spread is linked to replication. Therefore, the formation of a vRNP as a complex consisting only of MP and viral RNA is not sufficient for movement [8]. As we discussed above the MP/vRNA complex, in close interaction with the VRC via lateral diffusion along the ER membrane [138], moves into adjacent cells [8,131,155].

Moreover, the point of view that MP/vRNA complexes are the only structures responsible for the intercellular transport of viruses is questionable for the following additional reasons. MP mutant lacking amino acids 3, 4 and 5 (MPΔ3-5) in transgenic plants was not functional and was unable to move to Pd; therefore, the intercellular movement of a fluorescently labeled dextran of 9.4-kDa molecules was blocked [156]. Deletion of amino acids 9 to 11 (TAD 1 mutant) also prevented the localization of MP in the Pd, although it retained its ability to bind to microtubules [157,158]. Regarding the topic under discussion, it is important to note that these deletion mutations were located in the MP N-terminal 50-amino-acid region designated as the plasmodesmal localization signal (PLS) and responsible for interaction with Pd [159–162]. These small deletions in the N-terminal region of MP were outside of its RNA-binding domains [143] and, therefore, did not affect the potential ability to bind both its own and cellular mRNA. Thus, the potential ability to form MP/vRNA complexes does not guarantee MP functionality.

6. Cell Conditioning: Is It a Universal Feature of All Plant Virus MPs?

The study of the properties of TMV MP revealed its ability to move into neighboring cells (self-movement) independently of other viral components [51–53]. MP self-movement is the basis for the mechanism of cell conditioning [10] or cell "predisposing" [18] that results in creating a favorable environment for the accelerated intercellular movement of genomic vRNA in the leading edge of the viral infection focus [118]. We believe that the cell conditioning occurs as follows: first, early synthesis of MP is likely mediated by an internal ribosome entry site ($IRES_{MP,75}$) that allows translation of the MP gene directly from genomic RNA even before the synthesis of subgenomic RNA [117,163–166]. Moreover, direct experiments to restore movement function using the KK6 TMV mutant [167] containing $IRES^{CR}_{MP,75}$, confirmed the possibility of early synthesis of MP from the genomic TMV RNA [168]. Second, the MP has the ability to interact with cellular factors, performing the function of a positive regulator of Pd, ensuring MP movement into the neighboring cell [169]. Third, TMV MP is believed to function as a viral enhancer of RNA silencing (VER) by stimulating the spread of RNA silencing between cells [11,170]. This conclusion deserves a separate comment and was made based on experiments using a sophisticated system for testing the intercellular transport of the silencing signal in the *GFP*-transgenic *N. benthamiana* 16c line [171]. The essence of the system is that 10 days after the introduction of additional ectopic gene encoding GFP into the leaf by agroinfiltration, *GFP* mRNA-specific silencing occurs, in which 21 nt siRNAs move outside the ectopic *GFP* gene introduction locus for a distance of 13 cells on average (± 2 cells) [171]. To assess whether TMV MP may influence the spread of the silencing signal, the gene encoding TMV MP or its different non-functional mutants was introduced into the system [157,172,173]. Surprisingly, both MP and its deletion mutants (MPΔ3-5, aaD49-51 and MPP81S) mediated movement of 21-nt siRNAs through Pd [170]. Thus, it is undeniable that TMV MP and its tested mutants are involved in the spread of the silencing signal; however, apparently, they do this through a cellular mediator, interaction with which is not affected by mutations.

Finally, the ability of the MP to increase Pd SEL is transient and limited only by the leading edge of infection. Phosphorylation, as a posttranslational modification of TMV MP, leads to MP inactivation and thereby ends the period of permitted intercellular virus transport [174,175]. Thus, the transient cell conditioning function of TMV MP may include the mobilization of cellular mechanisms that open the Pd and the stimulation of RNA silencing movement between cells.

To what extent are the cell conditioning properties of TMV MP extended to MPs of other viruses (Tables 1 and 2)? If we exclude phloem-limited and tubule-forming viruses in which MP-formed tubular structures drastically modifying Pd, the MPs of the "30K" superfamily have characteristic features largely similar to those of TMV MP (Table 1). Among plant RNA viruses encoding more than one MP, there are very few examples of MPs with confirmed self-movement ability (Table 2). This property has been identified only for potyviruses, potexviruses and higreviruses. We believe that this is most likely due to either insufficient research on this feature in viruses or the lack of adequate model plant systems, as observed for phloem-limited viruses [128].

7. Conclusions

1. Genetic methods, including trans-complementation and techniques for Pd SEL assessment, allow identification of genes encoding proteins that are involved in the intercellular transport of plant viruses. It remains unclear how viruses belonging to different taxonomic groups and having significant differences in genome structure and host range can complement each other in the manifestation of the movement function.
2. Analysis of the properties of MPs of various viruses has been carried out in model plants, among which *N. benthamiana* has recently become the leading model due to its susceptibility to many viruses, although the use of this plant as a host could yield misleading results that are not reproducible when using the natural host of the virus.
3. TMV 30-kDa MP for a long time remained a standard in the search for candidate MPs; however, the characteristic properties of TMV MPs are rarely found in their entirety for other viral MPs.
4. The obvious ambiguity of the term "movement protein" will remain until we decipher the mechanisms of mobilization and exploitation by the virus of cellular factors that control intracellular and intercellular transport of macromolecules.

Author Contributions: Y.L.D., E.V.S., T.E.B. and T.V.K. analyzed the data, drafted the outline of the manuscript and wrote the manuscript. Y.L.D., E.V.S., T.E.B. and T.V.K. revised and finalized the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The work was funded by the Russian Science Foundation (project No. 19-74-20031).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Atabekov, J.G.; Dorokhov, Y.L. Plant virus-specific transport function and resistance of plants to viruses. *Adv. Virus Res.* **1984**, *29*, 313–364. [PubMed]
2. Leonard, D.A.; Zaitlin, M. A temperature-sensitive strain of tobacco mosaic virus defective in cell-to-cell movement generates an altered viral-coded protein. *Virology* **1982**, *117*, 416–424. [CrossRef]
3. Deom, C.M.; Oliver, M.J.; Beachy, R.N. The 30-kilodalton gene product of tobacco mosaic virus potentiates virus movement. *Science* **1987**, *237*, 389–394. [CrossRef] [PubMed]
4. Navarro, J.A.; Sanchez-Navarro, J.A.; Pallas, V. Chapter One—Key checkpoints in the movement of plant viruses through the host. In *Advances in Virus Research*; Kielian, M., Mettenleiter, T.C., Roossinck, M.J., Eds.; Virus Entry; Academic Press: Cambridge, MA, USA, 2019; Volume 104, pp. 1–64.
5. Tilsner, J.; Taliansky, M.E.; Torrance, L. Plant Virus Movement. In *ELS*; John Wiley & Sons: Hoboken, NJ, USA, 2014; ISBN 978-0-470-01590-2.
6. Waigmann, E.; Ueki, S.; Trutnyeva, K.; Citovsky, V. The Ins and Outs of Nondestructive Cell-to-Cell and Systemic Movement of Plant Viruses. *Crit. Rev. Plant Sci.* **2004**, *23*, 195–250. [CrossRef]
7. Ueki, S.; Citovsky, V. Plasmodesmata-associated proteins: Can we see the whole elephant? *Plant Signal. Behav.* **2014**, *9*, e27899. [CrossRef] [PubMed]
8. Heinlein, M. Plant virus replication and movement. *Virology* **2015**, *479–480*, 657–671. [CrossRef] [PubMed]
9. Reagan, B.C.; Burch-Smith, T.M. Viruses Reveal the Secrets of Plasmodesmal Cell Biology. *Mol. Plant Microbe Interact.* **2020**, *33*, 26–39. [CrossRef]

10. Sheshukova, E.V.; Ershova, N.M.; Kamarova, K.A.; Dorokhov, Y.L.; Komarova, T.V. The Tobamoviral Movement Protein: A "Conditioner" to Create a Favorable Environment for Intercellular Spread of Infection. *Front. Plant Sci.* **2020**, *11*. [CrossRef]
11. Amari, K.; Vazquez, F.; Heinlein, M. Manipulation of plant host susceptibility: An emerging role for viral movement proteins? *Front. Plant Sci.* **2012**, *3*, 10. [CrossRef]
12. Robards, A.W. The ultrastructure of plasmodesmata. *Protoplasma* **1971**, *72*, 315–323. [CrossRef]
13. Terry, B.R.; Robards, A.W. Hydrodynamic radius alone governs the mobility of molecules through plasmodesmata. *Planta* **1987**, *171*, 145–157. [CrossRef] [PubMed]
14. Nicolas, W.J.; Grison, M.S.; Trépout, S.; Gaston, A.; Fouché, M.; Cordelières, F.P.; Oparka, K.; Tilsner, J.; Brocard, L.; Bayer, E.M. Architecture and permeability of post-cytokinesis plasmodesmata lacking cytoplasmic sleeves. *Nat. Plants* **2017**, *3*, 17082. [CrossRef] [PubMed]
15. Ding, B.; Haudenshield, J.S.; Hull, R.J.; Wolf, S.; Beachy, R.N.; Lucas, W.J. Secondary plasmodesmata are specific sites of localization of the tobacco mosaic virus movement protein in transgenic tobacco plants. *Plant Cell* **1992**, *4*, 915–928. [PubMed]
16. Moore, P.J.; Fenczik, C.A.; Deom, C.M.; Beachy, R.N. Developmental changes in plasmodesmata in transgenic tobacco expressing the movement protein of tobacco mosaic virus. *Protoplasma* **1992**, *170*, 115–127. [CrossRef]
17. Mas, P.; Beachy, R.N. Role of microtubules in the intracellular distribution of tobacco mosaic virus movement protein. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 12345–12349. [CrossRef]
18. Kawakami, S.; Watanabe, Y.; Beachy, R.N. Tobacco mosaic virus infection spreads cell to cell as intact replication complexes. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 6291–6296. [CrossRef]
19. Szécsi, J.; Ding, X.S.; Lim, C.O.; Bendahmane, M.; Cho, M.J.; Nelson, R.S.; Beachy, R.N. Development of Tobacco Mosaic Virus Infection Sites in *Nicotiana benthamiana*. *Mol. Plant Microbe Interact.* **1999**, *12*, 143–152. [CrossRef]
20. Oparka, K.J.; Roberts, A.G.; Boevink, P.; Santa Cruz, S.; Roberts, I.; Pradel, K.S.; Imlau, A.; Kotlizky, G.; Sauer, N.; Epel, B. Simple, but not branched, plasmodesmata allow the nonspecific trafficking of proteins in developing tobacco leaves. *Cell* **1999**, *97*, 743–754. [CrossRef]
21. Herbers, K.; Tacke, E.; Hazirezaei, M.; Krause, K.P.; Melzer, M.; Rohde, W.; Sonnewald, U. Expression of a luteoviral movement protein in transgenic plants leads to carbohydrate accumulation and reduced photosynthetic capacity in source leaves. *Plant J. Cell Mol. Biol.* **1997**, *12*, 1045–1056. [CrossRef]
22. Modla, S.; Caplan, J.L.; Czymmek, K.J.; Lee, J.-Y. Localization of fluorescently tagged protein to plasmodesmata by correlative light and electron microscopy. *Methods Mol. Biol.* **2015**, *1217*, 121–133. [CrossRef]
23. Lee, J.-Y.; Wang, X.; Cui, W.; Sager, R.; Modla, S.; Czymmek, K.; Zybaliov, B.; van Wijk, K.; Zhang, C.; Lu, H.; et al. A plasmodesmata-localized protein mediates crosstalk between cell-to-cell communication and innate immunity in Arabidopsis. *Plant Cell* **2011**, *23*, 3353–3373. [CrossRef] [PubMed]
24. Raffaele, S.; Bayer, E.; Lafarge, D.; Cluzet, S.; German Retana, S.; Boubekeur, T.; Leborgne-Castel, N.; Carde, J.-P.; Lherminier, J.; Noirot, E.; et al. Remorin, a solanaceae protein resident in membrane rafts and plasmodesmata, impairs potato virus X movement. *Plant Cell* **2009**, *21*, 1541–1555. [CrossRef] [PubMed]
25. Taliansky, M.; Torrance, L.; Kalinina, N.O. Role of plant virus movement proteins. *Methods Mol. Biol.* **2008**, *451*, 33–54. [CrossRef] [PubMed]
26. Kasteel, D.T.; van der Wel, N.N.; Jansen, K.A.; Goldbach, R.W.; van Lent, J.W. Tubule-forming capacity of the movement proteins of alfalfa mosaic virus and brome mosaic virus. *J. Gen. Virol.* **1997**, *78*, 2089–2093. [CrossRef]
27. Kozieł, E.; Otulak-Kozieł, K.; Bujarski, J.J. Ultrastructural Analysis of Prune Dwarf Virus Intercellular Transport and Pathogenesis. *Int. J. Mol. Sci.* **2018**, *19*, 2570. [CrossRef]
28. Pendle, A.; Benitez-Alfonso, Y. Immunofluorescence detection of callose deposition around plasmodesmata sites. *Methods Mol. Biol.* **2015**, *1217*, 95–104. [CrossRef] [PubMed]
29. White, R.G. Probing plasmodesmata function with biochemical inhibitors. *Methods Mol. Biol.* **2015**, *1217*, 199–227. [CrossRef]

30. Fajardo, T.V.M.; Peiró, A.; Pallás, V.; Sánchez-Navarro, J. Systemic transport of Alfalfa mosaic virus can be mediated by the movement proteins of several viruses assigned to five genera of the 30K family. *J. Gen. Virol.* **2013**, *94*, 677–681. [CrossRef]
31. Atabekov, J.G.; Malyshenko, S.I.; Morozov, S.Y.; Taliansky, M.E.; Solovyev, A.G.; Agranovsky, A.A.; Shapka, N.A. Identification and study of tobacco mosaic virus movement function by complementation tests. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **1999**, *354*, 629–635. [CrossRef]
32. Latham, J.R.; Wilson, A.K. Transcomplementation and synergism in plants: Implications for viral transgenes? *Mol. Plant Pathol.* **2008**, *9*, 85–103. [CrossRef]
33. Bosch, F.X.; Jockusch, H. Temperature-sensitive mutants of TMV: Behavior of a non-coat protein mutant in isolated tobacco cells. *Mol. Gen. Genet.* **1972**, *116*, 95–98. [CrossRef] [PubMed]
34. Zimmern, D.; Hunter, T. Point mutation in the 30-K open reading frame of TMV implicated in temperature-sensitive assembly and local lesion spreading of mutant Ni 2519. *EMBO J.* **1983**, *2*, 1893–1900. [CrossRef] [PubMed]
35. Boyko, V.; Hu, Q.; Seemanpillai, M.; Ashby, J.; Heinlein, M. Validation of microtubule-associated Tobacco mosaic virus RNA movement and involvement of microtubule-aligned particle trafficking. *Plant J. Cell Mol. Biol.* **2007**, *51*, 589–603. [CrossRef] [PubMed]
36. Watanabe, Y.; Emori, Y.; Ooshika, I.; Meshi, T.; Ohno, T.; Okada, Y. Synthesis of TMV-specific RNAs and proteins at the early stage of infection in tobacco protoplasts: Transient expression of the 30K protein and its mRNA. *Virology* **1984**, *133*, 18–24. [CrossRef]
37. Meshi, T.; Watanabe, Y.; Saito, T.; Sugimoto, A.; Maeda, T.; Okada, Y. Function of the 30 kd protein of tobacco mosaic virus: Involvement in cell-to-cell movement and dispensability for replication. *EMBO J.* **1987**, *6*, 2557–2563. [CrossRef]
38. Dawson, W.O.; Beck, D.L.; Knorr, D.A.; Grantham, G.L. cDNA cloning of the complete genome of tobacco mosaic virus and production of infectious transcripts. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 1832–1836. [CrossRef]
39. Meshi, T.; Ishikawa, M.; Motoyoshi, F.; Semba, K.; Okada, Y. In vitro transcription of infectious RNAs from full-length cDNAs of tobacco mosaic virus. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 5043–5047. [CrossRef]
40. Agranovsky, A.A.; Folimonov, A.S.; Folimonova, S.Y.; Morozov, S.Y.; Schiemann, J.; Lesemann, D.; Atabekov, J.G. Beet yellows closterovirus HSP70-like protein mediates the cell-to-cell movement of a potexvirus transport-deficient mutant and a hordeivirus-based chimeric virus. *J. Gen. Virol.* **1998**, *79*, 889–895. [CrossRef]
41. Morozov, S.Y.; Fedorkin, O.N.; Jüttner, G.; Schiemann, J.; Baulcombe, D.C.; Atabekov, J.G. Complementation of a potato virus X mutant mediated by bombardment of plant tissues with cloned viral movement protein genes. *J. Gen. Virol.* **1997**, *78*, 2077–2083. [CrossRef]
42. Zhou, X.; Lin, W.; Sun, K.; Wang, S.; Zhou, X.; Jackson, A.O.; Li, Z. Specificity of Plant Rhabdovirus Cell-to-Cell Movement. *J. Virol.* **2019**, *93*. [CrossRef]
43. Voinnet, O.; Lederer, C.; Baulcombe, D.C. A viral movement protein prevents spread of the gene silencing signal in *Nicotiana benthamiana*. *Cell* **2000**, *103*, 157–167. [CrossRef]
44. Anandalakshmi, R.; Pruss, G.J.; Ge, X.; Marathe, R.; Mallory, A.C.; Smith, T.H.; Vance, V.B. A viral suppressor of gene silencing in plants. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 13079–13084. [CrossRef] [PubMed]
45. Goodin, M.M.; Zaitlin, D.; Naidu, R.A.; Lommel, S.A. *Nicotiana benthamiana*: Its history and future as a model for plant-pathogen interactions. *Mol. Plant Microbe Interact.* **2008**, *21*, 1015–1026. [CrossRef] [PubMed]
46. Howard, A.R.; Heppler, M.L.; Ju, H.-J.; Krishnamurthy, K.; Payton, M.E.; Verchot-Lubicz, J. Potato virus X TGBp1 induces plasmodesmata gating and moves between cells in several host species whereas CP moves only in *N. benthamiana* leaves. *Virology* **2004**, *328*, 185–197. [CrossRef]
47. Wolf, S.; Deom, C.M.; Beachy, R.N.; Lucas, W.J. Movement protein of tobacco mosaic virus modifies plasmodesmatal size exclusion limit. *Science* **1989**, *246*, 377–379. [CrossRef]
48. Waigmann, E.; Lucas, W.J.; Citovsky, V.; Zambryski, P. Direct functional assay for tobacco mosaic virus cell-to-cell movement protein and identification of a domain involved in increasing plasmodesmal permeability. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 1433–1437. [CrossRef]

49. Waigmann, E.; Zambryski, P. Tobacco mosaic virus movement protein-mediated protein transport between trichome cells. *Plant Cell* **1995**, *7*, 2069–2079. [CrossRef]
50. Crawford, K.M.; Zambryski, P.C. Subcellular localization determines the availability of non-targeted proteins to plasmodesmatal transport. *Curr. Biol.* **2000**, *10*, 1032–1040. [CrossRef]
51. Crawford, K.M.; Zambryski, P.C. Non-targeted and targeted protein movement through plasmodesmata in leaves in different developmental and physiological states. *Plant Physiol.* **2001**, *125*, 1802–1812. [CrossRef]
52. Kotlizky, G.; Katz, A.; van der Laak, J.; Boyko, V.; Lapidot, M.; Beachy, R.N.; Heinlein, M.; Epel, B.L. A Dysfunctional Movement Protein of Tobacco mosaic virus Interferes with Targeting of Wild-Type Movement Protein to Microtubules. *Mol. Plant Microbe Interact.* **2001**, *14*, 895–904. [CrossRef]
53. Burch-Smith, T.M.; Zambryski, P.C. Loss of increased size exclusion limit (ISE)1 or ISE2 increases the formation of secondary plasmodesmata. *Curr. Biol.* **2010**, *20*, 989–993. [CrossRef] [PubMed]
54. Fujiwara, T.; Giesman-Cookmeyer, D.; Ding, B.; Lommel, S.A.; Lucas, W.J. Cell-to-Cell Trafficking of Macromolecules through Plasmodesmata Potentiated by the Red Clover Necrotic Mosaic Virus Movement Protein. *Plant Cell* **1993**, *5*, 1783–1794. [CrossRef] [PubMed]
55. Giesman-Cookmeyer, D.; Silver, S.; Vaewhongs, A.A.; Lommel, S.A.; Deom, C.M. Tobamovirus and dianthovirus movement proteins are functionally homologous. *Virology* **1995**, *213*, 38–45. [CrossRef]
56. Tremblay, D.; Vaewhongs, A.A.; Turner, K.A.; Sit, T.L.; Lommel, S.A. Cell wall localization of Red clover necrotic mosaic virus movement protein is required for cell-to-cell movement. *Virology* **2005**, *333*, 10–21. [CrossRef] [PubMed]
57. Kaido, M.; Funatsu, N.; Tsuno, Y.; Mise, K.; Okuno, T. Viral cell-to-cell movement requires formation of cortical punctate structures containing Red clover necrotic mosaic virus movement protein. *Virology* **2011**, *413*, 205–215. [CrossRef] [PubMed]
58. Vaquero, C.; Turner, A.P.; Demangeat, G.; Sanz, A.; Serra, M.T.; Roberts, K.; García-Luque, I. The 3a protein from cucumber mosaic virus increases the gating capacity of plasmodesmata in transgenic tobacco plants. *J. Gen. Virol.* **1994**, *75*, 3193–3197. [CrossRef]
59. Ding, B.; Li, Q.; Nguyen, L.; Palukaitis, P.; Lucas, W.J. Cucumber mosaic virus 3a protein potentiates cell-to-cell trafficking of CMV RNA in tobacco plants. *Virology* **1995**, *207*, 345–353. [CrossRef]
60. Itaya, A.; Hickman, H.; Bao, Y.; Nelson, R.; Ding, B. Cell-to-cell trafficking of cucumber mosaic virus movement protein:green fluorescent protein fusion produced by biolistic gene bombardment in tobacco. *Plant J.* **1997**, *12*, 1223–1230. [CrossRef]
61. Poirson, A.; Turner, A.P.; Giovane, C.; Berna, A.; Roberts, K.; Godefroy-Colburn, T. Effect of the alfalfa mosaic virus movement protein expressed in transgenic plants on the permeability of plasmodesmata. *J. Gen. Virol.* **1993**, *74*, 2459–2461. [CrossRef]
62. Zheng, H.; Wang, G.; Zhang, L. Alfalfa Mosaic Virus Movement Protein Induces Tubules in Plant Protoplasts. *Mol. Plant Microbe Interact.* **1997**, *10*, 1010–1014. [CrossRef]
63. Pallas, V.; Aparicio, F.; Herranz, M.C.; Sanchez-Navarro, J.A.; Scott, S.W. The molecular biology of ilarviruses. *Adv. Virus Res.* **2013**, *87*, 139–181. [CrossRef] [PubMed]
64. Kaido, M.; Inoue, Y.; Takeda, Y.; Sugiyama, K.; Takeda, A.; Mori, M.; Tamai, A.; Meshi, T.; Okuno, T.; Mise, K. Downregulation of the NbNACa1 gene encoding a movement-protein-interacting protein reduces cell-to-cell movement of Brome mosaic virus in *Nicotiana benthamiana*. *Mol. Plant Microbe Interact. MPMI* **2007**, *20*, 671–681. [CrossRef] [PubMed]
65. Bertens, P.; Wellink, J.; Goldbach, R.; van Kammen, A. Mutational Analysis of the Cowpea Mosaic Virus Movement Protein. *Virology* **2000**, *267*, 199–208. [CrossRef] [PubMed]
66. Carvalho, C.M.; Pouwels, J.; van Lent, J.W.M.; Bisseling, T.; Goldbach, R.W.; Wellink, J. The Movement Protein of Cowpea Mosaic Virus Binds GTP and Single-Stranded Nucleic Acid In Vitro. *J. Virol.* **2004**, *78*, 1591–1594. [CrossRef]
67. Liu, L.; Grainger, J.; Cañizares, M.C.; Angell, S.M.; Lomonossoff, G.P. Cowpea mosaic virus RNA-1 acts as an amplicon whose effects can be counteracted by a RNA-2-encoded suppressor of silencing. *Virology* **2004**, *323*, 37–48. [CrossRef]
68. Ziegler-Graff, V.; Guilford, P.J.; Baulcombe, D.C. Tobacco rattle virus RNA-1 29K gene product potentiates viral movement and also affects symptom induction in tobacco. *Virology* **1991**, *182*, 145–155. [CrossRef]

69. Derrick, P.M.; Barker, H.; Oparka, K.J. Increase in Plasmodesmatal Permeability during Cell-to-Cell Spread of Tobacco Rattle Virus from Individually Inoculated Cells. *Plant Cell* **1992**, *4*, 1405–1412. [CrossRef]
70. Deng, X.; Kelloniemi, J.; Haikonen, T.; Vuorinen, A.L.; Elomaa, P.; Teeri, T.H.; Valkonen, J.P.T. Modification of Tobacco rattle virus RNA1 to serve as a VIGS vector reveals that the 29K movement protein is an RNA silencing suppressor of the virus. *Mol. Plant Microbe Interact.* **2013**, *26*, 503–514. [CrossRef]
71. Hofius, D.; Herbers, K.; Melzer, M.; Omid, A.; Tacke, E.; Wolf, S.; Sonnewald, U. Evidence for expression level-dependent modulation of carbohydrate status and viral resistance by the potato leafroll virus movement protein in transgenic tobacco plants. *Plant J. Cell Mol. Biol.* **2001**, *28*, 529–543. [CrossRef]
72. Scholthof, H.B.; Scholthof, K.B.; Jackson, A.O. Identification of tomato bushy stunt virus host-specific symptom determinants by expression of individual genes from a potato virus X vector. *Plant Cell* **1995**, *7*, 1157–1172. [CrossRef]
73. Huang, Y.; Hong, H.; Xu, M.; Yan, J.; Dai, J.; Wu, J.; Feng, Z.; Zhu, M.; Zhang, Z.; Yuan, X.; et al. Developmentally regulated *Arabidopsis thaliana* susceptibility to tomato spotted wilt virus infection. *Mol. Plant Pathol.* **2020**, *21*, 985–998. [CrossRef] [PubMed]
74. Borniego, M.B.; Karlin, D.; Peña, E.J.; Robles Luna, G.; García, M.L. Bioinformatic and mutational analysis of ophiovirus movement proteins, belonging to the 30K superfamily. *Virology* **2016**, *498*, 172–180. [CrossRef]
75. Yu, C.; Karlin, D.G.; Lu, Y.; Wright, K.; Chen, J.; MacFarlane, S. Experimental and bioinformatic evidence that raspberry leaf blotch emaravirus P4 is a movement protein of the 30K superfamily. *J. Gen. Virol.* **2013**, *94*, 2117–2128. [CrossRef] [PubMed]
76. Schoelz, J.E.; Harries, P.A.; Nelson, R.S. Intracellular transport of plant viruses: Finding the door out of the cell. *Mol. Plant* **2011**, *4*, 813–831. [CrossRef] [PubMed]
77. Uchiyama, A.; Shimada-Beltran, H.; Levy, A.; Zheng, J.Y.; Javia, P.A.; Lazarowitz, S.G. The Arabidopsis synaptotagmin SYTA regulates the cell-to-cell movement of diverse plant viruses. *Front. Plant Sci.* **2014**, *5*, 584. [CrossRef] [PubMed]
78. Noueiry, A.O.; Lucas, W.J.; Gilbertson, R.L. Two proteins of a plant DNA virus coordinate nuclear and plasmodesmal transport. *Cell* **1994**, *76*, 925–932. [CrossRef]
79. Zhang, S.C.; Wege, C.; Jeske, H. Movement proteins (BC1 and BV1) of Abutilon mosaic geminivirus are cotransported in and between cells of sink but not of source leaves as detected by green fluorescent protein tagging. *Virology* **2001**, *290*, 249–260. [CrossRef]
80. Kleinow, T.; Happle, A.; Kober, S.; Linzmeier, L.; Rehm, T.M.; Fritze, J.; Buchholz, P.C.F.; Kepp, G.; Jeske, H.; Wege, C. Phosphorylations of the Abutilon Mosaic Virus Movement Protein Affect Its Self-Interaction, Symptom Development, Viral DNA Accumulation, and Host Range. *Front. Plant Sci.* **2020**, *11*. [CrossRef]
81. Lazareva, E.A.; Lezzhov, A.A.; Komarova, T.V.; Morozov, S.Y.; Heinlein, M.; Solovyev, A.G. A novel block of plant virus movement genes. *Mol. Plant Pathol.* **2017**, *18*, 611–624. [CrossRef]
82. Lazareva, E.A.; Lezzhov, A.A.; Chergintsev, D.A.; Golyshev, S.A.; Dolja, V.V.; Morozov, S.Y.; Heinlein, M.; Solovyev, A.G. Reticulon-like properties of a plant virus-encoded movement protein. *New Phytol.* **2020**. [CrossRef]
83. Genovés, A.; Navarro, J.A.; Pallás, V. Functional analysis of the five melon necrotic spot virus genome-encoded proteins. *J. Gen. Virol.* **2006**, *87*, 2371–2380. [CrossRef] [PubMed]
84. Li, W.; Qu, F.; Morris, T.J. Cell-to-cell movement of turnip crinkle virus is controlled by two small open reading frames that function in trans. *Virology* **1998**, *244*, 405–416. [CrossRef] [PubMed]
85. Fernández-Miragall, O.; Hernández, C. An Internal Ribosome Entry Site Directs Translation of the 3′-Gene from Pelargonium Flower Break Virus Genomic RNA: Implications for Infectivity. *PLoS ONE* **2011**, *6*, e22617. [CrossRef]
86. Angell, S.M.; Davies, C.; Baulcombe, D.C. Cell-to-Cell Movement of Potato Virus X Is Associated with a Change in the Size-Exclusion Limit of Plasmodesmata in Trichome Cells of *Nicotiana clevelandii*. *Virology* **1996**, *216*, 197–201. [CrossRef] [PubMed]
87. Lough, T.J.; Shash, K.; Xoconostle-Cázares, B.; Hofstra, K.R.; Beck, D.L.; Balmori, E.; Forster, R.L.S.; Lucas, W.J. Molecular Dissection of the Mechanism by Which Potexvirus Triple Gene Block Proteins Mediate Cell-to-Cell Transport of Infectious RNA. *Mol. Plant Microbe Interact.* **1998**, *11*, 801–814. [CrossRef]

88. Morozov, S.Y.; Solovyev, A.G.; Kalinina, N.O.; Fedorkin, O.N.; Samuilova, O.V.; Schiemann, J.; Atabekov, J.G. Evidence for Two Nonoverlapping Functional Domains in the Potato Virus X 25K Movement Protein. *Virology* **1999**, *260*, 55–63. [CrossRef]
89. Yang, Y.; Ding, B.; Baulcombe, D.C.; Verchot, J. Cell-to-Cell Movement of the 25K Protein of Potato virus X Is Regulated by Three Other Viral Proteins. *Mol. Plant Microbe Interact.* **2000**, *13*, 599–605. [CrossRef]
90. Petty, I.T.; French, R.; Jones, R.W.; Jackson, A.O. Identification of barley stripe mosaic virus genes involved in viral RNA replication and systemic movement. *EMBO J.* **1990**, *9*, 3453–3457. [CrossRef]
91. Solovyev, A.G.; Zelenina, D.A.; Savenkov, E.I.; Grdzelishvili, V.Z.; Morozov, S.Y.; Lesemann, D.E.; Maiss, E.; Casper, R.; Atabekov, J.G. Movement of a barley stripe mosaic virus chimera with a tobacco mosaic virus movement protein. *Virology* **1996**, *217*, 435–441. [CrossRef]
92. Li, Z.; Zhang, Y.; Jiang, Z.; Jin, X.; Zhang, K.; Wang, X.; Han, C.; Yu, J.; Li, D. Hijacking of the nucleolar protein fibrillarin by TGB1 is required for cell-to-cell movement of Barley stripe mosaic virus. *Mol. Plant Pathol.* **2018**, *19*, 1222–1237. [CrossRef]
93. Rojas, M.R.; Zerbini, F.M.; Allison, R.F.; Gilbertson, R.L.; Lucas, W.J. Capsid protein and helper component-proteinase function as potyvirus cell-to-cell movement proteins. *Virology* **1997**, *237*, 283–295. [CrossRef] [PubMed]
94. Vijayapalani, P.; Maeshima, M.; Nagasaki-Takekuchi, N.; Miller, W.A. Interaction of the Trans-Frame Potyvirus Protein P3N-PIPO with Host Protein PCaP1 Facilitates Potyvirus Movement. *PLoS Pathog.* **2012**, *8*. [CrossRef] [PubMed]
95. Peremyslov, V.V.; Hagiwara, Y.; Dolja, V.V. HSP70 homolog functions in cell-to-cell movement of a plant virus. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14771–14776. [CrossRef] [PubMed]
96. Alzhanova, D.V.; Hagiwara, Y.; Peremyslov, V.V.; Dolja, V.V. Genetic analysis of the cell-to-cell movement of beet yellows closterovirus. *Virology* **2000**, *268*, 192–200. [CrossRef]
97. Dolja, V.V. Beet yellows virus: The importance of being different. *Mol. Plant Pathol.* **2003**, *4*, 91–98. [CrossRef] [PubMed]
98. Tatineni, S.; Robertson, C.J.; Garnsey, S.M.; Bar-Joseph, M.; Gowda, S.; Dawson, W.O. Three genes of Citrus tristeza virus are dispensable for infection and movement throughout some varieties of citrus trees. *Virology* **2008**, *376*, 297–307. [CrossRef]
99. Bak, A.; Folimonova, S.Y. The conundrum of a unique protein encoded by citrus tristeza virus that is dispensable for infection of most hosts yet shows characteristics of a viral movement protein. *Virology* **2015**, *485*, 86–95. [CrossRef]
100. Folimonova, S.Y. Citrus tristeza virus: A large RNA virus with complex biology turned into a valuable tool for crop protection. *PLoS Pathog.* **2020**, *16*, e1008416. [CrossRef]
101. Oparka, K.J.; Roberts, A.G. Plasmodesmata. A Not So Open-and-Shut Case. *Plant Physiol.* **2001**, *125*, 123–126. [CrossRef]
102. Liarzi, O.; Epel, B.L. Development of a quantitative tool for measuring changes in the coefficient of conductivity of plasmodesmata induced by developmental, biotic, and abiotic signals. *Protoplasma* **2005**, *225*, 67–76. [CrossRef]
103. Oparka, K.J.; Murphy, R.; Derrick, P.M.; Prior, D.A.M.; Smith, J.A.C. Modification of the pressure-probe technique permits controlled intracellular microinjection of fluorescent probes. *J. Cell Sci.* **1991**, *98*, 539–544.
104. Kragler, F. Analysis of the conductivity of plasmodesmata by microinjection. *Methods Mol. Biol.* **2015**, *1217*, 173–184. [CrossRef] [PubMed]
105. Stonebloom, S.; Burch-Smith, T.; Kim, I.; Meinke, D.; Mindrinos, M.; Zambryski, P. Loss of the plant DEAD-box protein ISE1 leads to defective mitochondria and increased cell-to-cell transport via plasmodesmata. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 17229–17234. [CrossRef] [PubMed]
106. Baulcombe, D.C.; Chapman, S.; Santa Cruz, S. Jellyfish green fluorescent protein as a reporter for virus infections. *Plant J. Cell Mol. Biol.* **1995**, *7*, 1045–1053. [CrossRef] [PubMed]
107. Heinlein, M.; Epel, B.L.; Padgett, H.S.; Beachy, R.N. Interaction of tobamovirus movement proteins with the plant cytoskeleton. *Science* **1995**, *270*, 1983–1985. [CrossRef] [PubMed]
108. McLean, B.G.; Zupan, J.; Zambryski, P.C. Tobacco mosaic virus movement protein associates with the cytoskeleton in tobacco cells. *Plant Cell* **1995**, *7*, 2101–2114. [CrossRef]

109. Oparka, K.J.; Boevink, P.; Santa Cruz, S. Studying the movement of plant viruses using green fluorescent protein. *Trends Plant Sci.* **1996**, *1*, 412–418. [CrossRef]
110. Padgett, H.S.; Epel, B.L.; Kahn, T.W.; Heinlein, M.; Watanabe, Y.; Beachy, R.N. Distribution of tobamovirus movement protein in infected cells and implications for cell-to-cell spread of infection. *Plant J. Cell Mol. Biol.* **1996**, *10*, 1079–1088. [CrossRef]
111. Más, P.; Beachy, R.N. Distribution of TMV movement protein in single living protoplasts immobilized in agarose. *Plant J.* **1998**, *15*, 835–842. [CrossRef]
112. Heinlein, M.; Padgett, H.S.; Gens, J.S.; Pickard, B.G.; Casper, S.J.; Epel, B.L.; Beachy, R.N. Changing patterns of localization of the tobacco mosaic virus movement protein and replicase to the endoplasmic reticulum and microtubules during infection. *Plant Cell* **1998**, *10*, 1107–1120. [CrossRef]
113. Trutnyeva, K.; Ruggenthaler, P.; Waigmann, E. Movement Profiles: A Tool for Quantitative Analysis of Cell-to-Cell Movement of Plant Viral Movement Proteins. *Methods Mol. Biol.* **2008**, *451*, 317–329. [CrossRef] [PubMed]
114. Boyko, V.; Ferralli, J.; Ashby, J.; Schellenbaum, P.; Heinlein, M. Function of microtubules in intercellular transport of plant virus RNA. *Nat. Cell Biol.* **2000**, *2*, 826–832. [CrossRef] [PubMed]
115. Culver, J.N.; Lehto, K.; Close, S.M.; Hilf, M.E.; Dawson, W.O. Genomic position affects the expression of tobacco mosaic virus movement and coat protein genes. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 2055–2059. [CrossRef] [PubMed]
116. Dawson, W.O. A personal history of virus-based vector construction. *Curr. Top. Microbiol. Immunol.* **2014**, *375*, 1–18. [CrossRef] [PubMed]
117. Dorokhov, Y.L.; Sheshukova, E.V.; Komarova, T.V. Tobamovirus 3′-Terminal Gene Overlap May be a Mechanism for within-Host Fitness Improvement. *Front. Microbiol.* **2017**, *8*. [CrossRef]
118. Oparka, K.J.; Prior, D.A.; Santa Cruz, S.; Padgett, H.S.; Beachy, R.N. Gating of epidermal plasmodesmata is restricted to the leading edge of expanding infection sites of tobacco mosaic virus (TMV). *Plant J. Cell Mol. Biol.* **1997**, *12*, 781–789. [CrossRef]
119. Arce-Johnson, P.; Kahn, T.; Reimann-Philipp, U.; Rivera-Bustamante, R.; Beachy, R. The amount of movement protein produced in transgenic plants influences the establishment, local movement, and systemic spread of infection by movement protein-deficient tobacco mosaic virus. *Mol. Plant Microbe Interact.* **1995**, *8*, 415–423. [CrossRef]
120. Melcher, U. The "30K" superfamily of viral movement proteins. *J. Gen. Virol.* **2000**, *81*, 257–266. [CrossRef]
121. Lucas, W.J. Plant viral movement proteins: Agents for cell-to-cell trafficking of viral genomes. *Virology* **2006**, *344*, 169–184. [CrossRef]
122. Navarro, J.A.; Pallás, V. An Update on the Intracellular and Intercellular Trafficking of Carmoviruses. *Front. Plant Sci.* **2017**, *8*, 1801. [CrossRef]
123. Morozov, S.Y.; Solovyev, A.G. Triple gene block: Modular design of a multifunctional machine for plant virus movement. *J. Gen. Virol.* **2003**, *84*, 1351–1366. [CrossRef] [PubMed]
124. Verchot-Lubicz, J.; Torrance, L.; Solovyev, A.G.; Morozov, S.Y.; Jackson, A.O.; Gilmer, D. Varied Movement Strategies Employed by Triple Gene Block–Encoding Viruses. *Mol. Plant Microbe Interact.* **2010**, *23*, 1231–1247. [CrossRef] [PubMed]
125. Solovyev, A.G.; Kalinina, N.O.; Morozov, S.Y. Recent Advances in Research of Plant Virus Movement Mediated by Triple Gene Block. *Front. Plant Sci.* **2012**, *3*. [CrossRef] [PubMed]
126. Park, M.-R.; Jeong, R.-D.; Kim, K.-H. Understanding the intracellular trafficking and intercellular transport of potexviruses in their host plants. *Front. Plant Sci.* **2014**, *5*. [CrossRef] [PubMed]
127. Dolja, V.V.; Kreuze, J.F.; Valkonen, J.P.T. Comparative and functional genomics of closteroviruses. *Virus Res.* **2006**, *117*, 38–51. [CrossRef] [PubMed]
128. Dawson, W.O.; Bar-Joseph, M.; Garnsey, S.M.; Moreno, P. Citrus tristeza virus: Making an ally from an enemy. *Annu. Rev. Phytopathol.* **2015**, *53*, 137–155. [CrossRef]
129. Revers, F.; García, J.A. Molecular biology of potyviruses. *Adv. Virus Res.* **2015**, *92*, 101–199. [CrossRef]
130. Liu, C.; Nelson, R.S. The cell biology of Tobacco mosaic virus replication and movement. *Front. Plant Sci.* **2013**, *4*, 12. [CrossRef]
131. Heinlein, M. Viral Transport and Interaction with the Host Cytoskeleton. In *Plant-Virus Interactions: Molecular Biology, Intra- and Intercellular Transport*; Kleinow, T., Ed.; Springer International Publishing: Cham, Switzerland, 2016; pp. 39–66. ISBN 978-3-319-25489-0.

132. Sánchez-Navarro, J.; Fajardo, T.; Zicca, S.; Pallás, V.; Stavolone, L. Caulimoviridae Tubule-Guided Transport Is Dictated by Movement Protein Properties. *J. Virol.* **2010**, *84*, 4109–4112. [CrossRef]
133. Amari, K.; Lerich, A.; Schmitt-Keichinger, C.; Dolja, V.V.; Ritzenthaler, C. Tubule-Guided Cell-to-Cell Movement of a Plant Virus Requires Class XI Myosin Motors. *PLoS Pathog.* **2011**, *7*. [CrossRef]
134. Más, P.; Beachy, R.N. Replication of tobacco mosaic virus on endoplasmic reticulum and role of the cytoskeleton and virus movement protein in intracellular distribution of viral RNA. *J. Cell Biol.* **1999**, *147*, 945–958. [CrossRef] [PubMed]
135. Heinlein, M. The spread of Tobacco mosaic virus infection: Insights into the cellular mechanism of RNA transport. *Cell. Mol. Life Sci.* **2002**, *59*, 58–82. [CrossRef]
136. Wu, X.; Cheng, X. Intercellular movement of plant RNA viruses: Targeting replication complexes to the plasmodesma for both accuracy and efficiency. *Traffic* **2020**. [CrossRef]
137. Peiró, A.; Martínez-Gil, L.; Tamborero, S.; Pallás, V.; Sánchez-Navarro, J.A.; Mingarro, I. The Tobacco mosaic virus movement protein associates with but does not integrate into biological membranes. *J. Virol.* **2014**, *88*, 3016–3026. [CrossRef] [PubMed]
138. Guenoune-Gelbart, D.; Elbaum, M.; Sagi, G.; Levy, A.; Epel, B.L. Tobacco mosaic virus (TMV) replicase and movement protein function synergistically in facilitating TMV spread by lateral diffusion in the plasmodesmal desmotubule of *Nicotiana benthamiana*. *Mol. Plant-Microbe Interact.* **2008**, *21*, 335–345. [CrossRef]
139. Schulz, A. Diffusion or bulk flow: How plasmodesmata facilitate pre-phloem transport of assimilates. *J. Plant Res.* **2015**, *128*, 49–61. [CrossRef] [PubMed]
140. Schulz, A. Long-Distance Trafficking: Lost in Transit or Stopped at the Gate? *Plant Cell* **2017**, *29*, 426–430. [CrossRef] [PubMed]
141. Liesche, J.; Gao, C.; Binczycki, P.; Andersen, S.R.; Rademaker, H.; Schulz, A.; Martens, H.J. Direct Comparison of Leaf Plasmodesma Structure and Function in Relation to Phloem-Loading Type. *Plant Physiol.* **2019**, *179*, 1768–1778. [CrossRef]
142. Citovsky, V.; Knorr, D.; Schuster, G.; Zambryski, P. The P30 movement protein of tobacco mosaic virus is a single-strand nucleic acid binding protein. *Cell* **1990**, *60*, 637–647. [CrossRef]
143. Citovsky, V.; Wong, M.L.; Shaw, A.L.; Prasad, B.V.; Zambryski, P. Visualization and characterization of tobacco mosaic virus movement protein binding to single-stranded nucleic acids. *Plant Cell* **1992**, *4*, 397–411.
144. Kiselyova, O.I.; Yaminsky, I.V.; Karger, E.M.; Frolova, O.Y.; Dorokhov, Y.L.; Atabekov, J.G. Visualization by atomic force microscopy of tobacco mosaic virus movement protein-RNA complexes formed in vitro. *J. Gen. Virol.* **2001**, *82*, 1503–1508. [CrossRef] [PubMed]
145. Sambade, A.; Brandner, K.; Hofmann, C.; Seemanpillai, M.; Mutterer, J.; Heinlein, M. Transport of TMV movement protein particles associated with the targeting of RNA to plasmodesmata. *Traffic* **2008**, *9*, 2073–2088. [CrossRef] [PubMed]
146. Peña, E.J.; Robles Luna, G.; Heinlein, M. In vivo imaging of tagged mRNA in plant tissues using the bacterial transcriptional antiterminator BglG. *Plant J. Cell Mol. Biol.* **2020**. [CrossRef] [PubMed]
147. Luo, K.-R.; Huang, N.-C.; Yu, T.-S. Selective Targeting of Mobile mRNAs to Plasmodesmata for Cell-to-Cell Movement. *Plant Physiol.* **2018**, *177*, 604–614. [CrossRef] [PubMed]
148. Zhang, W.; Thieme, C.J.; Kollwig, G.; Apelt, F.; Yang, L.; Winter, N.; Andresen, N.; Walther, D.; Kragler, F. tRNA-Related Sequences Trigger Systemic mRNA Transport in Plants. *Plant Cell* **2016**, *28*, 1237–1249. [CrossRef]
149. Dreher, T.W. Role of tRNA-like structures in controlling plant virus replication. *Virus Res.* **2009**, *139*, 217–229. [CrossRef] [PubMed]
150. Derrick, P.M.; Carter, S.A.; Nelson, R.S. Mutation of the Tobacco Mosaic Tobamovirus 126-and 183-kDa Proteins: Effects on Phloem-Dependent Virus Accumulation and Synthesis of Viral Proteins. *Mol. Plant Microbe Interact.* **1997**, *10*, 589–596. [CrossRef]
151. Hirashima, K.; Watanabe, Y. Tobamovirus Replicase Coding Region Is Involved in Cell-to-Cell Movement. *J. Virol.* **2001**, *75*, 8831–8836. [CrossRef]
152. Knapp, E.; Dawson, W.O.; Lewandowski, D.J. Conundrum of the lack of defective RNAs (dRNAs) associated with tobamovirus Infections: dRNAs that can move are not replicated by the wild-type virus; dRNAs that are replicated by the wild-type virus do not move. *J. Virol.* **2001**, *75*, 5518–5525. [CrossRef]

153. Knapp, E.; Danyluk, G.M.; Achor, D.; Lewandowski, D.J. A bipartite Tobacco mosaic virus-defective RNA (dRNA) system to study the role of the N-terminal methyl transferase domain in cell-to-cell movement of dRNAs. *Virology* **2005**, *341*, 47–58. [CrossRef]
154. Christensen, N.; Tilsner, J.; Bell, K.; Hammann, P.; Parton, R.; Lacomme, C.; Oparka, K. The 5′ cap of tobacco mosaic virus (TMV) is required for virion attachment to the actin/endoplasmic reticulum network during early infection. *Traffic* **2009**, *10*, 536–551. [CrossRef] [PubMed]
155. Peña, E.J.; Heinlein, M. RNA transport during TMV cell-to-cell movement. *Front. Plant Sci.* **2012**, *3*. [CrossRef] [PubMed]
156. Lapidot, M.; Gafny, R.; Ding, B.; Wolf, S.; Lucas, W.J.; Beachy, R.N. A dysfunctional movement protein of tobacco mosaic virus that partially modifies the plasmodesmata and limits virus spread in transgenic plants. *Plant J.* **1993**, *4*, 959–970. [CrossRef]
157. Kahn, T.W.; Lapidot, M.; Heinlein, M.; Reichel, C.; Cooper, B.; Gafny, R.; Beachy, R.N. Domains of the TMV movement protein involved in subcellular localization. *Plant J. Cell Mol. Biol.* **1998**, *15*, 15–25. [CrossRef]
158. Fujiki, M.; Kawakami, S.; Kim, R.W.; Beachy, R.N. Domains of tobacco mosaic virus movement protein essential for its membrane association. *J. Gen. Virol.* **2006**, *87*, 2699–2707. [CrossRef]
159. Yuan, C.; Lazarowitz, S.G.; Citovsky, V. Identification of a Functional Plasmodesmal Localization Signal in a Plant Viral Cell-To-Cell-Movement Protein. *mBio* **2016**, *7*, e02052-15. [CrossRef]
160. Yuan, C.; Lazarowitz, S.G.; Citovsky, V. Identification of Plasmodesmal Localization Sequences in Proteins in Planta. *J. Vis. Exp.* **2017**, 55301. [CrossRef]
161. Yuan, C.; Lazarowitz, S.G.; Citovsky, V. The Plasmodesmal Localization Signal of TMV MP Is Recognized by Plant Synaptotagmin SYTA. *mBio* **2018**, *9*, e01314–e01318. [CrossRef]
162. Liu, Y.; Huang, C.; Zeng, J.; Yu, H.; Li, Y.; Yuan, C. Identification of two additional plasmodesmata localization domains in the tobacco mosaic virus cell-to-cell-movement protein. *Biochem. Biophys. Res. Commun.* **2020**, *521*, 145–151. [CrossRef]
163. Skulachev, M.V.; Ivanov, P.A.; Karpova, O.V.; Korpela, T.; Rodionova, N.P.; Dorokhov, Y.L.; Atabekov, J.G. Internal initiation of translation directed by the 5′-untranslated region of the tobamovirus subgenomic RNA I(2). *Virology* **1999**, *263*, 139–154. [CrossRef]
164. Dorokhov, Y.L.; Skulachev, M.V.; Ivanov, P.A.; Zvereva, S.D.; Tjulkina, L.G.; Merits, A.; Gleba, Y.Y.; Hohn, T.; Atabekov, J.G. Polypurine (A)-rich sequences promote cross-kingdom conservation of internal ribosome entry. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5301–5306. [CrossRef] [PubMed]
165. Dorokhov, Y.L.; Ivanov, P.A.; Komarova, T.V.; Skulachev, M.V.; Atabekov, J.G. An internal ribosome entry site located upstream of the crucifer-infecting tobamovirus coat protein (CP) gene can be used for CP synthesis in vivo. *J. Gen. Virol.* **2006**, *87*, 2693–2697. [CrossRef] [PubMed]
166. Komarova, T.V.; Skulachev, M.V.; Ivanov, P.A.; Klyushin, A.G.; Dorokhov, Y.L.; Atabekov, J.G. Internal ribosome entry site from crucifer tobamovirus promotes initiation of translation in *Escherichia coli*. *Dokl. Biochem. Biophys.* **2003**, *389*, 118–121. [CrossRef] [PubMed]
167. Lehto, K.; Grantham, G.L.; Dawson, W.O. Insertion of sequences containing the coat protein subgenomic RNA promoter and leader in front of the tobacco mosaic virus 30K ORF delays its expression and causes defective cell-to-cell movement. *Virology* **1990**, *174*, 145–157. [CrossRef]
168. Zvereva, S.D.; Ivanov, P.A.; Skulachev, M.V.; Klyushin, A.G.; Dorokhov, Y.L.; Atabekov, J.G. Evidence for contribution of an internal ribosome entry site to intercellular transport of a tobamovirus. *J. Gen. Virol.* **2004**, *85*, 1739–1744. [CrossRef]
169. Dorokhov, Y.L.; Ershova, N.M.; Sheshukova, E.V.; Komarova, T.V. Plasmodesmata Conductivity Regulation: A Mechanistic Model. *Plants* **2019**, *8*, 595. [CrossRef]
170. Vogler, H.; Kwon, M.-O.; Dang, V.; Sambade, A.; Fasler, M.; Ashby, J.; Heinlein, M. Tobacco mosaic virus movement protein enhances the spread of RNA silencing. *PLoS Pathog.* **2008**, *4*, e1000038. [CrossRef]
171. Himber, C.; Dunoyer, P.; Moissiard, G.; Ritzenthaler, C.; Voinnet, O. Transitivity-dependent and -independent cell-to-cell movement of RNA silencing. *EMBO J.* **2003**, *22*, 4523–4533. [CrossRef]
172. Boyko, V.; Ashby, J.A.; Suslova, E.; Ferralli, J.; Sterthaus, O.; Deom, C.M.; Heinlein, M. Intramolecular complementing mutations in tobacco mosaic virus movement protein confirm a role for microtubule association in viral RNA transport. *J. Virol.* **2002**, *76*, 3974–3980. [CrossRef]

173. Dunoyer, P.; Himber, C.; Voinnet, O. DICER-LIKE 4 is required for RNA interference and produces the 21-nucleotide small interfering RNA component of the plant cell-to-cell silencing signal. *Nat. Genet.* **2005**, *37*, 1356–1360. [CrossRef]
174. Waigmann, E.; Chen, M.-H.; Bachmaier, R.; Ghoshroy, S.; Citovsky, V. Regulation of plasmodesmal transport by phosphorylation of tobacco mosaic virus cell-to-cell movement protein. *EMBO J.* **2000**, *19*, 4875–4884. [CrossRef] [PubMed]
175. Lee, J.Y.; Lucas, W.J. Phosphorylation of viral movement proteins—Regulation of cell-to-cell trafficking. *Trends Microbiol.* **2001**, *9*, 5–8; discussion 8. [CrossRef]