Potato mop-top virus: Variability, Movement, and Suppression of Host Defence

Pruthvi Balachandra Kalyandurg
Faculty of Natural Resources and Agricultural Sciences
Department of Plant Biology
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2019

Acta Universitatis agriculturae Sueciae
2019:59

Cover: Illustration depicting the primary objectives of thesis: understanding the PMTV genetic variability and interactions with the host during intercellular movement and suppression of host defence system.

ISSN 1652-6880
ISBN (print version) 978-91-7760-436-5
ISBN (electronic version) 978-91-7760-437-2

Print: SLU Service/Repro, Uppsala 2019

Potato mop-top virus: Variability, Movement and Suppression of Host Defence

Abstract

Potato mop-top virus (PMTV) causes an economically damaging disease called potato spraing. Despite being reported across the potato growing regions in the world, very little genetic variability has been reported for the virus. Also, the knowledge on how PMTV suppresses host defence mechanism, and how it interacts with the host during the cell-to-cell and long-distance movement is still insufficient to develop successful preventive measures against the PMTV infection.

This thesis work identified high diversity of the PMTV in the Andean region of Peru compared to the rest of the world. Among the PMTV genome, *CP-RT* and *8K* genomic regions accumulated the largest number of mutations. Through phylogenetic analysis of the RNA-CP segment we identified two prevailing genotypes around the world. Based on the pathobiological differences, we named these lineages as S (severe), and M (mild) types. The phylogenetic relationship determined in this study helped us to propose a novel classification of PMTV isolates.

Our analysis to address the selection pressure on the PMTV genome revealed that the ORF encoding the 8K protein, a viral suppressor of RNA silencing (VSR) is under strong positive selection. Characterization of the RNA silencing suppression activity of the 8K protein from seven highly diverse isolates revealed that the 8K encoded by a Peruvian isolate, P1 exhibits stronger RNA silencing suppression activity compared to that of other isolates. Through mutational analysis, we identified that Ser-50 is necessary for these differences. Through deep sequencing for sRNAs, we identified that VSRs reduce the sRNA accumulation. We observed lower amount of siRNAs with U residue at the 5'-terminus suggesting that P1 8K might affect AGO1-mediated RNA silencing.

The present work also identified key host factors necessary for the cell-to-cell and long distance movement of the virus. We showed that the actin network and certain class VIII myosins motors are important for the cell-to-cell movement of PMTV. The dependency on the acto-myosin network for PMTV movement was further demonstrated by the fluorescence recovery after photo bleaching experiments that resulted in compromised delivery of the TGB1 at the plasmodesmata upon disrupting actin and inhibiting two class VIII myosins. In contrast, class XI myosins did not have a significant effect on the cell-to-cell movement of the PMTV, although they appear to be important viral long-distance movement.

Analysis of PMTV TGB1 interactions the with host proteins revealed that TGB1 interacts with *Nicotiana benthamiana* HIPP26 protein, a vascular expressed, metallochaperone that acts as a plasma membrane to nucleus stress signalling relay. PMTV infection upregulated the expression of *HIPP26* and altered its subcellular localization from plasmodesmata to the nucleus. Knockdown of *NbHIPP26* expression resulted in inhibition of virus long-distance movement, but not the cell-to-cell movement. Together, this data suggests that PMTV hijacks NbHIPP26 to facilitate the long-distance movement of the virus.

Keywords: Potato mop-top virus, variability, genotype constellation, RNA silencing suppression, positive selection, cell-to-cell movement, long-distance movement, myosins, actin, HIPP26.

Author's address: Pruthvi Balachandra Kalyandurg, SLU, Department of Plant Biology, P.O. Box 7080, 750 07 Uppsala, Sweden

Potatismopptoppvirus:Variabilitet, förflyttning och undertryckande av värdens försvar

Vetenskaplig sammanfattning

Potatismopptoppvirus (PTMV) orsakar sjukdomen rostringar i potatis vilket kan ha stora ekonomiska konsekvenser. Trots att viruset har rapporterats från områden runt om hela världen där potatis odlas har endast en mycket liten genetisk variation rapporterats för viruset. Dessutom är kunskapen om hur PMTV undertrycker värdens försvarsmekanismer och hur det interagerar med värden under förflyttning mellan celler och över längre avstånd inom växten fortfarande inte tillräcklig för att kunna utveckla framgångsrika förebyggande åtgärder mot PMTV-infektion.

I detta avhandlingsarbete identifierades en hög diversitet för PMTV i peruanska Anderna jämfört med övriga delar av världen. Regionerna *CP-RT* och *8K* accumulerade det största antalet mutationer i PMTV-genomet. Med fylogenetisk analys av segmentet RNA-CP identifierade vi två genotyper som var allmänt utbredda runt om i världen. Baserat på patobiologiska skillnader benämnde vi dessa linjer som typerna S (allvarlig) och M (mild). Baserat på de fylogenetiska släktskap som bestämts i denna studie föreslår vi en ny klassificering av PMTV-isolat.

Vår analys för att studera selektionstrycket på PMTV-genomet visade att den öppna läsram (ORF) som kodar för 8K-proteinet, vilket är ett virusprotein som undertrycker RNA-interferens (VSR), är under stark positiv selektion. Karaktäriseringen av 8K-proteinets förmåga att undertrycka RNA-interferens för sju vitt skilda isolat visade att 8K som kodas av ett peruanskt isolat, P1, visade starkare förmåga att undertrycka RNA-interferens jämfört med det från andra isolat. Med mutationsanalys kunde vi identifiera Ser-50 som nödvändigt för dessa skillnader. Genom djup sekvensering av sRNA fann vi att VSR-proteiner minskar ackumuleringen av sRNA. Vi såg en lägre mängd av siRNA med kvävebasen U vid 5’-änden vilket tyder på att P1 8K skulle kunna påverka AGO1-medierad RNA-interferens.

Det föreliggande arbetet identifierade också nyckelfaktorer hos värden för virusförflyttning från cell till cell eller över längre avstånd inom växten. Vi visade att nätverket av aktin och vissa myosinmotorer av klass VIII är viktiga för PMTVs förflyttning från cell till cell. Beroendet av aktomyosin-nätverket för förflyttning av PMTV demonstrerades vidare genom experiment med metoden fluorescens efter ljusblekning vilka resulterade i störd transport av TGB1 till plasmodesmata efter upplösning av aktin och inhibering av två klass VIII-myosiner. Däremot hade klass XI-myosiner ingen signifikant effekt på förflyttning av PMTV från cell till cell även om de verkade vara viktiga för förflyttning av virus över längre avstånd inom växten.

Analys av interaktionerna mellan PMTVs TGB1 och värdproteiner visade att TGB1 interagerar med proteinet HIPP26 från *Nicotiana benthamiana*, vilket är ett

metallchaperon som uttrycks i ledningsvävnad och verkar vid överföringen av stress-signaler från cellmembranet till cellkärnan. Vid PMTV-infektion uppreglerades uttrycket av *HIPP26* och ändrade dess lokalisering i cellen från plasmodesmata till cellkärnan. Nedreglering av uttrycket av NbHIPP26 med virusinducerad genavstängning resulterade i inhibering av virusförflyttning över längre avstånd i växten, men inte förflyttningen från cell till cell. Sammantaget tyder dessa data på att PMTV kapar NbHIPP26 för att möjliggöra virusets förflyttning över längre avstånd i växten.

Författarens adress: Pruthvi Balachandra Kalyandurg, SLU, Department of Plant Biology, P.O. Box 7080, 750 07 Uppsala, Sweden

పొటాటొ మొప్-టాప్ వైరస్ – జన్యు వైవిధ్యము, కదలికలు, మరియు స్వీయ రక్షణ

సంక్షిప్తముగా:
బంగాళాదుంప పంట ప్రపంచములోనే మూడవ ప్రధాన పంటగా పేర్కొనబడినది. అయితె ఎన్నో సూక్ష్మ క్రిములు ఈ పంట దిగుబడిని మరియు నాణ్యతనూ నష్టపరుస్తూ ఉంటాయి. అందులో ప్రధానముగా వైరస్ మరియు వైరస్ వంటి వ్యాధి కారకమైనటువంటి క్రిములు చాలా నష్టము కలిగించగలవు. అటువంటి కోవకు చెందిన ఒక వైరస్ పొటాటొ మొప్-టాప్ వైరస్. ఈ వైరస్ పొటాటో స్పిండ్ అను వ్యాధిని కలుగచేస్తుంది. ఈ వైరస్ విర్గావిరిడె అను కుటుంబమునకు మరియు పొమొవైరస్ అను ప్రజాతి కి చెందినది. ఈ వ్యాధి వల్ల బంగాళాదుంపలో నల్లటి చారలు ఎర్పడి అమ్మటానికి పనికిరాకుండా, చాలా నష్టమును కలిగిస్తాయి. ప్రస్తుతానికి ఈ వైరస్ జాతులను ఐరోపా, ఉత్తర మరియు దక్షిన అమెరికా, ఆసియా (చైనా, జపాన్, పాకిస్తాన్, ద. కొరియా) లో కనుగొనబడినది. అయినప్పటికీ రసాయన పద్ధతులతో వైరస్ లను నాశనము చేయి పద్ధతులు లేకపోవటచేత ఇటువంటి వైరస్ల ప్రభావము పంట పైన ఎక్కువ అవుతోంది.

ప్రపంచంలోని దాదాపు బంగాళాదుంప సాగు చేయబడుతున్న అన్ని ప్రాంతాలలో నివేదించబడినప్పటికీ, ఈ వైరస్ జాతుల యొక్క జన్యు వైవిధ్యం చాలా తక్కువగా నివేదించబడింది. అంతేకాకుండా వైరస్ ఏ విధంగా మొక్క యొక్క రక్షణ వ్యవస్థతో పోరాడుతుందన్న విషయము మరియు ఏ విధంగా మొక్క యొక్క కణాలలోని ప్రోటీన్లను ప్రభావితం చేస్తున్నది అన్న విషయపరిజ్ఞానం వైరస్లకు వ్యతిరేకంగా నివారణ చర్యలను ప్రస్తుతం అభివృద్ధి చేయడానికి సరిపోదు.

ఈ పరిశోధన ద్వారా ప్రపంచంలోని ఇతర ప్రాంతాలతో పోలిస్తే పెరూదేశంలోని ఆండియన్ పర్వత ప్రాంతంలో పిఎమ్టివి జాతుల జన్యువులలో అధిక మొత్తంలో వైవిధ్యాన్ని గుర్తించింది. అంతేకాకుండా ఫైలోజెనెటిక్ విశ్లేషణ ద్వారా ప్రపంచవ్యాప్తంగా రెండు జన్యురూపాల ఉనికిని గుర్తించింది. అవి కలిగించే వ్యాధి తీవ్రత ఆధారంగా, S (తీవ్రమైన) మరియు M (తేలికపాటి) అను రెండు సమూహాలు వున్నట్టుగా గుర్తించడం జరిగింది. ఆసక్తికరంగా, గతంలో ప్రపంచంలోని ఇతర ప్రాంతాలతో గుర్తింపబడిన అన్ని పిఎమ్టివి జాతులు, మరియు పెరూలోని కొన్ని పిఎమ్టివి జాతులు S-రకానికి చెందినవి కాగా, పెరూదేశానికి చెందిన పిఎమ్టివి జాతులు ఎక్కువ భాగం M- సమూహం లోనికి వస్తాయి. ఈ పరిశోధన ఆధారంగా పిఎమ్టివి జాతులుయొక్క కొత్త వర్గీకరణ ప్రతిపాదించబడినది. అది మాత్రమె కాక ఆండియన్ పర్వత ప్రాంతంలోనే ఈ వైరస్ పుట్టింది అన్న ప్రతిపాదనకు ఈ పరిశోధన మరింత బలం చేకూర్చింది. మరింత సమాచారం కోసం దయచేసి మొదటి ప్రచురణ కథనాన్ని చదవండి.

పిఎమ్టివి జన్యువుపై పరిణామ ఒత్తిడి పరిష్కరించడానికి చేసిన మా విశ్లేషణలో మొక్క యొక్క రక్షణ వ్యవస్థతో పోరాడగలిగిన ఒక ప్రోటీన్ బలమైన పరిణామ ఒత్తిడి లో ఉందని తెలిసింది. అందులో భాగంగా ఏడు వైవిధ్యమైన పిఎమ్టివి జాతులు నుండి ఈ ప్రోటీన్ యొక్క పోరాట లక్షణాన్ని పరీక్షించటం జరిగింది. వీటిలో ఒక జాతి బలముగా మొక్క యొక్క రక్షణ చర్యను నిరోధించగలుగుతోందనిమేము కనుగొన్నాము. ఆసక్తికరంగా, దీని జన్యుక్రమము లో మార్పు వల్ల కేవలం రెండు అమైనో ఆమ్లాల వ్యత్యాసం ఈ బలమైన రక్షణ నిరోధక చర్యకు గల కారణము అని తెలిసింది. ఈ రెంటిలో ఏ అమైనో ఆమ్లము చాలా ముఖ్యమైనదో కూడా జన్యుమార్పిడి పరిశోధనల ద్వారా కనుగొనటం జరిగింది. మరింత సమాచారం కోసం దయచేసి రెండవ ప్రచురణ కథనాన్ని చదవండి.

మూడవ పరిశోధనలో భాగంగా, వైరస్ ఒక మొక్క కణం నుండి మరొక కణానికి, మరియు ఆ కణజాలమును వీడి మొక్కలోని వేరే భాగమునకు ఎలా రవాణా అవుతుందో మేము అధ్యయనం చేసాము. ఈ పరిశోధన ఫలితాలు మూడవ మరియు నాల్గవ ప్రచురణలుగా అందించబడ్డాయి. ఈ ప్రచురణలలో వైరస్లు మైయోసిన్ వంటి కొన్ని మొక్క ప్రోటీన్లను ఎలా హైజాక్ చేసి మొక్క యొక్క మరొక భాగాలకు రవాణా అవుతాయో వివరణాత్మక సమాచారం యివ్వబడినది. మరీ ముఖ్యంగా, మొక్కలలో కరువును తట్టుకోవటానికి ఈ వైరస్ కారణమవుతుందని మేము కనుక్కున్నాము. ఈ ఫలితాలు వ్యాధి తీవ్రతనే కాకుండా, భవిష్యత్తులో కరువు ప్రభావాన్ని తగ్గించడానికి కొత్త మార్గాలను అన్వేషించడానికి కూడా దోహదపడుతాయి.

రచయిత చిరునామా: పృథ్వీ బాలచంద్ర కళ్యాణదుర్గం, వృక్ష శాస్త్ర విభాగము, స్వీడిష్ వ్యవసాయ విశ్వవిద్యాలయము, పోస్ట్ బాక్స్ సంఖ్య **7080**, **750 07**, ఉప్ప్సాల, స్వీడన్.

అంకితము

**మా తాతగారు, మొదటి గురువు కీ.శే. శ్రీ మామిళ్ళపల్లి సుబ్రహ్మణ్యం శర్మ గారు
మరియు నా తల్లిదండ్రులు శ్రీ నాగభూషణం శ్రీమతి మీనాక్షిదేవి గార్లకు.**

Dedication

To my grandfather, *Sri* Mamillapalli Subrahmanyam, for teaching me to *question*, and for being a dearest friend.

To my parents, *Sri* Nagabushanam Kalyandurg and *Smt.* Meenakshi Devi Kalyandurg.

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Kalyandurg, P.** , Gil, J. F., Lukhovitskaya, N. I., Flores, B. , Müller, G. , Chuquillanqui, C. , Palomino, L. , Monjane, A. , Barker, I. , Kreuze, J. and Savenkov, E. I. (2017). Molecular and pathobiological characterization of 61 Potato mop-top virus full-length cDNAs reveals great variability of the virus in the centre of potato domestication, novel genotypes and evidence for recombination. *Molecular Plant Pathology*, 18: 864-877.

II **Kalyandurg, P. B.**[§]**,** Tahmasebi, A.[§], Vetukuri, R. R., Kushwaha, S., Lezzhov, A. A., Solovyev, A. G., Grenville-Briggs, L. J., Savenkov, E. I. Efficient RNA silencing suppression activity of Potato Mop-Top Virus 8K protein is driven by variability and positive selection. *Virology*, 535 (2019) 111-121.

III **Kalyandurg, P. B.**, Savenkov, E. I. TGB1-mediated cell-to-cell movement of Potato mop-top virus requires class VIII myosins (manuscript)

IV Cowan, G. H., Roberts, A. G., Jones, S., Kumar, P., **Kalyandurg, P. B.**, Gil, J. F., Savenkov, E. I., Hemsley, P. A., Torrance, L. (2018). Potato Mop-Top Virus Co-Opts the Stress Sensor HIPP26 for Long-Distance Movement. *Plant Physiology* 176:2052-2070.

[§] indicates shared first authorship

My contribution to the papers included in this thesis was as follows:

I I did the phylogenetic and variability analyses, and I was involved in pathobiological characterization. Wrote the first draft.

II I participated in planning the project and performed major part of experiments and analyses. I wrote the first draft of the manuscript and took part in final editing and correspondence of revision.

III I was involved in planning of project and performed all the experiments, and wrote the manuscript.

IV I conducted experiments that revealed the role of HIPP26 in long-distance movement of PMTV.

Abbreviations

AGO	Argonaute protein
BYV	*Beet yellow virus*
CaMV	*Cauliflower mosaic virus*
CMV	*Cucumber mosaic virus*
CP	Coat protein
CP-RT	Coat protein read-through
CPMV	*Cowpea mosaic virus*
DCL	Dicer-like protein
DNA	Deoxyribonucleic acid
DRB	Double-stranded RNA-binding protein
dsRNA	Double-stranded ribonucleic acid
ELISA	Enzyme linked immunosorbent assay
ER	Endoplasmic reticulum
FRAP	Fluorescence recovery after photo bleaching
GFLV	*Grapevine fanleaf virus*
GFP	Green fluorescent protein
HcPro	Helper-component proteinase
HIPP	Heavymetal-associated isoprenylated plant protein
HR	Hypersensitive reaction
LatB	Latrunculin B
MetT	Methyltransferase
MP	Movement protein
ORF	Open reading frame
PAMP	Pathogen-associated molecular patterns
PDLP	Plasmodesmata-located Protein

PMTV	*Potato mop-top virus*
PVX	*Potato virus X*
RDR	RNA-directed RNA polymerase
RDRP	RNA-dependent RNA polymerase
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
siRNA	Small interfering RNA
SLAC	Single-likelihood ancestor counting
SNP	Single nucleotide polymorphism
TGB	Triple gene block
TMV	*Tobacco mosaic virus*
TRV	*Tobacco rattle virus*
VIGS	Virus-induced gene silencing
VSR	Viral suppressor of RNA silencing

1 Introduction

"An inefficient virus kills its host. A clever virus stays with it"

-James Lovelock

Viruses are unique molecular biological entities that can infect any form of life from bacteria to humans. Plant viruses are known to cause significant agricultural losses around the world. However, the effect of viral infections in many wild plants is minimal because of natural selection and co-evolution of the viruses and their hosts (Bos, 1999). Viruses that kill their host are less likely to survive over evolutionary time than the ones that co-evolve with their hosts or the ones that cause moderate symptoms (Bos, 1999; Roossinck, 2015). However, intensive agricultural practices such as moving crop species to new countries lead to the spread of plant viruses to new regions and to indigenous, and/or cultivated plants (Matthews and Hull, 2002). This might result in the emergence of new genetic variants of the viruses with increased pathogenicity.

As viruses have no metabolism of their own, they depend on the host for their replication and movement. Thus, the pathogenicity of the virus depends on the ability to replicate and spread in the hosts (Holt et al., 1990; Moreno et al., 1997; Roossinck and Palukaitis, 1990; Watanabe et al., 1987). The complex molecular interactions between the hosts and the viruses during the process of infection lead to metabolic and cytological abnormalities in the host, which leads to symptom development.

Potato mop-top virus (PMTV) is reported to infect potato crop across the majority of the potato growing regions in the world. The symptom development by PMTV varies depending on the potato cultivars, and the environmental conditions, also causing symptomless infections (Latvala-Kilby et al., 2009; Sandgren, 1995). However, when causing symptoms on the tubers, PMTV causes significant economic losses. In fact, PMTV is considered as one of the most harmful pathogens of potatoes (*Solanum tuberosum*) and is one of the most

important plant viruses in Scandinavia (Beuch, 2013; Latvala-Kilby et al., 2009).

1.1 Disease, symptoms and transmission of PMTV

PMTV was first discovered as a causal agent of the disease called potato 'spraing' by Calvert and Harrison in 1966 in the United Kingdom (Calvert and Harrison, 1966). The 'spraing' disease is characterized by slightly raised necrotic arcs and rings on the potato surface and flecks in the tuber flesh (Figure 1A) (Calvert and Harrison, 1966). As a result of the severe quality problems, the tubers are rejected both for the chips production industry and the fresh potato market.

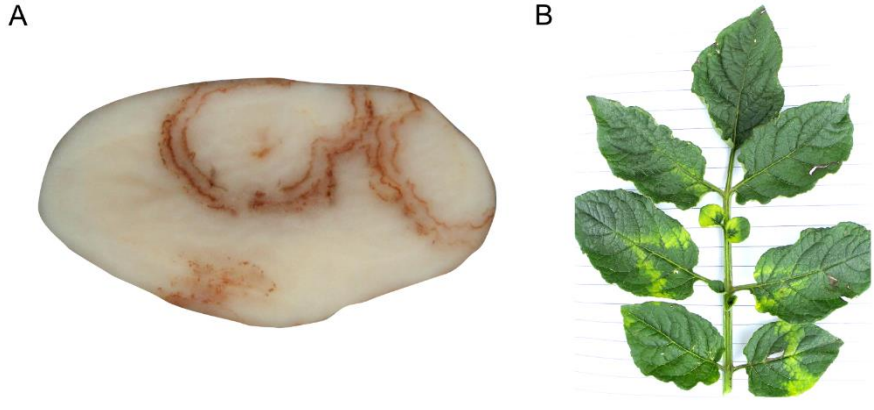


Figure 1. Symptoms caused by *Potato mop-top virus* (A) inside potato tubers, and (B) on the leaves of potato. (Pictures: A, Sutton Bridge crop storage research; B, an extract from figure published in Kalyandurg et al., 2017)

PMTV causes shortening of the internodes ('mop-top') in the infected plants. The primary infection of PMTV, i.e., when the virus infects the healthy tubers, results in the appearance of black coloured lines, arcs, rings on the surface of tubers, or internal brown arcs or flecks in the tubers (Figure 1A) (Kurppa, 1989a). The secondary infection, i.e., when new plants are grown from the infected tubers, sometimes leads to cracking and deformation of the tubers (Calvert and Harrison, 1966; Kurppa, 1989a; Tenorio et al., 2006), and may result in yield losses up to 63% depending on the cultivar (Kurppa, 1989b; Carnegie et al., 2010). The appearance of 'spraing' symptoms during the time

of harvest can be enhanced as a result of fluctuating temperatures during storage (Harrison and Jones, 1971; Kurppa, 1989a; Sandgren, 1995).

Additional symptoms appear on the upper parts of the plant include V-shaped chlorotic patterns on the leaves (Figure 1B) (Calvert and Harrison, 1966). However, the occurrence of these foliar symptoms are often associated with cold climates (Calvert, 1968), and affected by the environmental conditions such as temperature and moisture (Carnegie et al., 2010).

PMTV is transmitted by a plasmodiophorid vector called *Spongospora subterranea* (Jones and Harrison, 1969). *S. subterranea* causes powdery scab disease on potato tubers and was found to occur in potato-growing regions worldwide (Gau et al., 2013). PMTV virions enter the developing zoospores of the *S. subterranea* in an infected plant. These zoospores spread the PMTV to new host plants by penetrating into the root tissues or tubers (Jones and Harrison, 1969). The PMTV particles can survive in the resting spores of the vector for more than 15 years (Calvert, 1968).

1.2 Global distribution of PMTV

Since the first discovery of the virus in Scotland and Northern Ireland (Calvert and Harrison, 1966), PMTV was reported in many potato growing regions around the world. In Europe, PMTV was found in the Netherlands in 1969, which is the largest exporter of seed potatoes (Rabobank, 2019; van Hoof and Rozendaal, 1969). The virus was also found in Ireland (Foxe, 1980), Czech Republic (Novak et al., 1983), Switzerland (Schwärzel, 2002), Latvia (Latvala-Kilby et al., 2009), and Poland (Budziszewska et al., 2010). In the Scandinavian region, PMTV was first detected in Norway (Björnstad, 1969), and later found in Sweden (Ryden et al., 1986; Sandgren, 1995), Finland (Kurppa, 1989b), and Denmark (Mølgaard and Nielsen, 1996).

In the Andes region of South America, which is considered as the centre of domestication of the potato (Spooner et al., 2005), the virus was first reported in 1972 in Peru (Hinostroza and French, 1972; Salazar and Jones, 1975), followed by Bolivia (Jones, 1975), Venezuela (Ortega and Leopardi, 1989), and Colombia (Gil et al., 2011). The virus was also detected in Costa Rica in Central America (Montero-Astúa et al., 2008), and the USA (Lambert et al., 2003) and Canada in North America (Xu et al., 2004). In Asia, PMTV was first identified in Japan (Imoto et al., 1986), followed by the reports in China (Hu et al., 2016) and Pakistan (Arif et al., 2014). Recently PMTV was also detected in New Zealand, making it a first report from Oceania (Government of NZ, 2018).

1.3 Genome organization and properties of the genomic components of PMTV

Potato mop-top virus is the type member of genus *Pomovirus* in the family *Virgaviridae* (Adams et al., 2012). Other members in the genus *Pomovirus* include *Beet soil-borne virus* (BSBV), *Beet virus Q* (BVQ), *Broad bean necrosis virus* (BBNV), and *Colombian potato soil-borne virus* (CPSbV) (Adams et al., 2017). The PMTV genome consists of three single-stranded positive-sense RNA segments, namely RNA-rep, RNA-CP and RNA-TGB, with a length of 6 kb, 3.1 kb and 2.9 kb, respectively (Savenkov et al., 1999; Kashiwazaki et al., 1995; Scott et al., 1994; Sandgren et al., 2001). These three segments together harbour six open reading frames (ORFs) encoding eight proteins. The 3´-untranslated region (UTR) of all three segments contain identical tRNA-like structures having an anticodon for valine (Savenkov et al., 1999) (Figure 2).

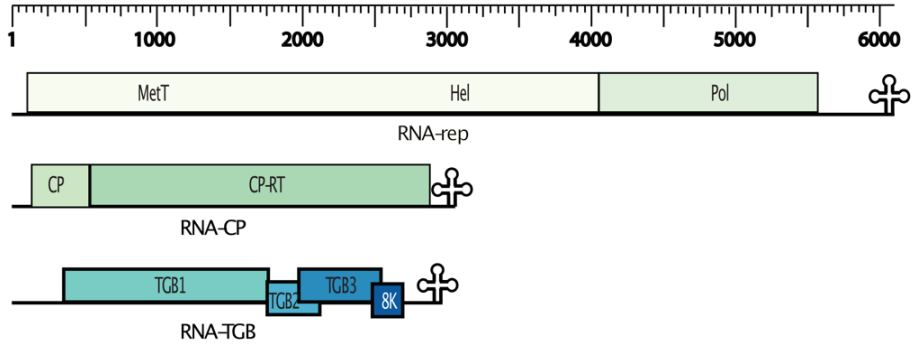


Figure 2. Genomic segments of PMTV

The RNA-rep segment contains an ORF that codes for a 148 kDa protein, and a 206 kDa protein that is produced through translational read through at the opal (UGA) stop codon of the ORF encoding 148 kDa protein. The 206 kDa protein contains a methyltransferase (MetT), a helicase and an RNA-dependent RNA polymerase (RdRp) motif, respectively (Savenkov et al., 1999). The RdRp domain catalyzes the synthesis of RNA using an RNA template by forming phosphodiester bonds between the ribonucleotides (Venkataraman et al., 2018), while helicase domain takes part in the displacement of complementary strands in the RNA duplex (Jankowsky, 2011). The MetT domain encoded by RNA-rep suggests that the viral RNAs are capped to facilitate translation (Byszewska et al., 2014).

The RNA-CP segment codes for a coat protein (CP, 20 kDa) which encapsidates the viral RNAs (Kashiwazaki et al., 1995). Through translational read-through at the amber (UAG) stop codon of the *CP* ORF, the RNA-CP also encodes a longer polypeptide, CP-RT (91 kDa) (Sandgren et al., 2001), a common feature among other soil-borne fungal transmitted viruses (Bouzoubaa et al., 1986; Koenig et al., 1997; Shirako and Wilson, 1993). CP-RT associates with one extremity of the virus particles (Cowan et al., 1997), supporting the transmission of the virus by its vector (Reavy et al., 1998). Inoculation of the virus through mechanical means under experimental conditions often results in internal deletions in the CP-RT region, causing the virus to lose the ability to be acquired and transmitted by its vector, as noticed for PMTV-T laboratory isolate that was maintained for 30 years through mechanical inoculations (Reavy et al., 1998; Sandgren et al., 2001). CP-RT is also an important factor in the long-distance movement of the virus particles, and RNA-CP (Torrance et al., 2009).

The third segment RNA-TGB contains triple gene block, which encodes TGB1 (51 kDa), TGB2 (13 kDa) and TGB3 (21 kDa) proteins, and an additional ORF coding for the 8K protein (Scott et al., 1994). The TGB proteins function in a coordinated manner and facilitate virus movement. The presence of triple gene block is a conserved feature among various other genera of viruses including *Potexvirus*, *Mandarivirus*, *Allexivirus*, *Carlavirus*, *Foveavirus*, *Hordeivirus*, *Pecluvirus* and *Benyvirus* (reviewed in Verchot-Lubicz et al., 2010).

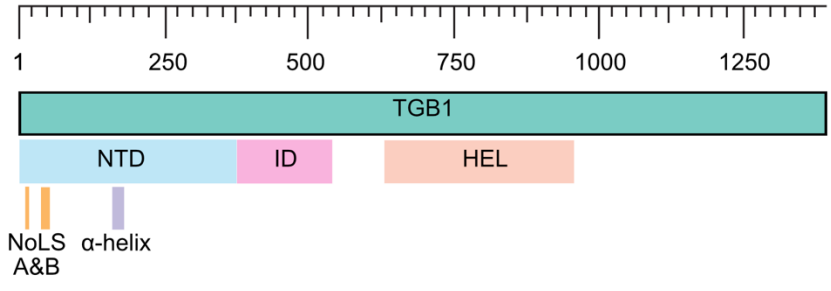


Figure 3. Domain organization of the TGB1 protein of PMTV. NTD, N-terminal domain, ID, Internal domain, Hel, Helicase domain, NoLS, predicted nucleolar localization sites.

The TGB1 protein plays a major role in the intercellular and long-distance movement of the virus. The TGB1 protein binds to the viral RNA to form a viral ribonucleoprotein complex (vRNP), that moves cell-to-cell and systemically. TGB1 protein contains three structurally distinct domains, namely, N-terminal

domain (NTD), internal domain (ID), and the helicase domain (Figure 3). The NTD domain, containing two nucleolar localization signals, binds RNA in a non-cooperative manner, whereas the other two domains bind RNA in a cooperative manner (Makarov et al., 2009). The NTD of TGB1 interacts with Importin-α, a nuclear transport receptor, and accumulates in the nucleolus. The nucleolar association of TGB1 is necessary for the viral long-distance movement. The ID of TGB1 is predicted to form an α-helix, which might mediate self-interaction of TGB1 protein to e.g. form dimers. The self-interaction of TGB1 is important for the cell-to-cell movement of PMTV (Lukhovitskaya et al., 2015). The TGB1 also interacts with CP-RT at one extremity of the virions assisting in the long-distance movement of the virus particles (Torrance et al., 2009). At the subcellular level, TGB1 localizes to plasmodesmata, cytoplasm around the nucleus, nucleolus and nucleoplasm, ER network, and occasionally decorates microtubules at later stages of virus infection (Wright et al., 2010).

The TGB2 protein is also an RNA binding protein that binds RNA in a sequence non-specific manner. TGB2 is suggested to play a role in targeting the viral RNAs to the chloroplasts for replication (Cowan et al., 2012), and is required for the vRNP intracellular movement (Zamyatnin et al., 2004). The TGB2 is an integral membrane protein that associates with the endoplasmic reticulum (ER), mobile granules in the cytoplasm, and the outer membrane of chloroplasts (Cowan et al., 2012).

The third protein encoded by TGB module, TGB3 protein is also a membrane protein that associates with the ER, motile granules and plasmodesmata at the subcellular level (Tilsner et al., 2010). The TGB3 protein contains two transmembrane domains that integrate into the membranes in a U-shaped orientation with its central loop protruding into the ER lumen. The TGB3 protein contains a conserved tyrosine-based motif that mediates the plasmodesmatal targeting (Tilsner et al., 2010), through which the TGB3 assists the intracellular movement of the vRNP-TGB2 complex towards the plasmodesmata. Although, both TGB2 and TGB3 proteins can increase the permeability of the plasmodesmata (Haupt et al., 2005), they do not move intercellularly (Haupt et al., 2005).

The 8K protein encoded by RNA-TGB is a cysteine-rich protein that acts as a suppressor of RNA silencing (Lukhovitskaya et al., 2013). PMTV 8K is an integral membrane protein that associates with and rearranges the ER-derived membranes in the plant cells when expressed transiently by agroinfiltration (Lukhovitskaya et al. 2005). The 8K protein is dispensable for viral replication and the long-distance movement of the virus in *N. benthamiana* and *N. tabacum*

plants (Savenkov et al., 2003). However, it appears to play an important role in enhancing the virus accumulation in these plant species (Lukhovitskaya et al., 2013).

1.3.1 Genome variability of PMTV

Even though PMTV was detected in many regions around the world, the PMTV isolates were reported to share a high level of sequence identity, with only about 0% - 2% genetic variability (Beuch et al., 2015; Latvala-Kilby et al., 2009; Ramesh et al., 2014; Santala et al., 2010). Previously, phylogenetic analysis based on RNA-CP component and the 8K protein identified two distinct clades and showed no strict geographical distribution among the isolates from various parts of the world (Beuch et al., 2015; Latvala-Kilby et al., 2009; Santala et al., 2010). As mentioned earlier, the Andean region is considered as center of domestication of potato. Hence, we hypothesized that a higher genetic variation of PMTV might exist in the Andean regions of South America. A recent study on Colombian isolates identified three genetically distinct PMTV variants (Gil et al., 2016) further supporting our hypothesis. Characterization of the genetic variability of the PMTV was one of aims of this thesis and is addressed in Paper I.

1.4 Virus-host interactions

Compared to other microbial pathogens such as bacteria and fungi, most of the viruses have smaller genome that has a limited coding capacity (Wang, 2015). Over the course of infection, viruses and their hosts respond to each other through complex molecular interactions. These interactions occur at various stages of infection starting from viral genome translation to establishing a systemic infection. Deciphering these interactions not only allow us to develop successful antiviral strategies, but also provides great insight into the mechanisms of plant cellular processes. Studying the virus-host interactions with in the context of PMTV movement and 8K-mediated suppression of RNA silencing are two other aims of this thesis.

In line with the scope of this thesis, virus-host interactions during the virus movement and RNA silencing mechanism are discussed in the following sections.

1.4.1 Virus movement

Following the entry into the cells, and subsequent replication, viruses must egress from initially infected cells to other parts of the plant in order to spread through the host. Plant viruses use two distinct mechanisms to establish the systemic infection in the host, a slow cell-to-cell movement via the plasmodesmata, followed by a rapid long-distance movement via the plant vasculature (Samuel, 1934) (Figure 4). The cell-to-cell movement occurs from the initially infected cells to the adjacent cells, which are usually the mesophyll or epidermal cells to the vascular bundle (Carrington et al., 1996). The long-distance movement occurs from source (net carbon exporting) to sink (net carbon importing) tissues, through the plant vascular tissues, usually through phloem sieve elements (Leisner and Turgeon, 1993; Lemoine et al., 2013). The coordination between the virus and host-encoded proteins is paramount for successful virus movement in the host. The restriction of the movement can lead to subliminal infection, where the virus can carry out replication, but not the intercellular movement, causing confinement of the infection to the initially infected cell (Bamunusinghe et al., 2013; Hull, 2013a).

As the primary objective of the viruses is to transport the infectious material, viruses have evolved to move their genetic material as different forms to adapt to various hosts. On the basis of requirement of CP, the intercellular movement can be categorised into two types (reviewed in Rojas et al., 2016).

The first type of movement occurs in the form of a vRNP complex, where the virus movement protein (MP) binds the viral RNA. The vRNP movement can be further categorised into three types, depending on the requirement of the CP. In certain genera of viruses, the CP is dispensable for the intercellular movement of the vRNP complex. Some examples for such movement form include viruses belonging to the genus *Tobamovirus, Carmovirus, Hordeivirus, Pomovirus, Dianthovirus, Tombusvirus* (Rojas et al., 2016). However, for some viruses including the members of *Potyvirus,* CP acts as a MP and thus is required for the movement of the virus as a vRNP complex (Dolja et al., 1995, 1994). Additionally, in certain viruses, the CP takes part in the movement of the vRNP complex as a non-virion form, as typified for *Potato virus X* (PVX), where the vRNA-MP-CP complex moves intercellularly (Lough et al., 1998). Similar mechanisms have been identified in certain DNA viruses e.g. *Geminivirus*, where the vRNP includes one or two MPs and the CP for the intercellular movement.

The second type of movement occurs as virus particles. Viruses belonging to genera *Closterovirus*, *Nepovirus*, *Caulimovirus*, *Comovirus*, *Bromovirus*, *Alfamovirus* and *Cucumovirus* move as virions (Schoelz et al., 2011).

1.4.1..1 Cell-to-cell movement

The cell-to-cell movement of the virus can be differentiated into two stages, intracellular movement and intercellular movement. During the first stage, the newly replicated viral genomes are transported intracellularly from the sites of replication towards the plasma membrane. Most viruses use the host intracellular transport system that includes cytoskeletal or endomembrane system to carry out the intracellular movement (Carmen Herranz et al., 2009).

The plant viruses face a unique challenge during their intercellular movement in the hosts due to the presence of cell wall. Thus, in order to move from cell-to-cell, plant viruses must pass through the plasmodesmata cytoplasmic connections between the cells to move to the neighbouring cells (Niehl and Heinlein, 2011). Viral proteins interact with various host cellular factors in the process of the cell-to-cell movement. The following sections consider some of the important factors involved in this process.

1.4.1..1.1 Host factors involved in the cell-to-cell movement of viruses

As obligate parasites, viruses depend on host cellular factors for their movement, and encode proteins that can hijack these host factors for their own benefit. Hence, the compatibility of the virus MPs, the host proteins, and cellular components is vital for efficient virus transport (Carrington et al., 1996). Even though not all host components involved in this process are known, most viruses localize to specific cellular components, including cytoskeletal elements, ER, and interact with certain motor proteins for trafficking their infectious material from the site of replication towards the plasmodesmata, and sometimes intercellularly (Rojas et al., 2016).

1.4.1..1.1.1 Plant cytoskeleton

The plant cytoskeleton is a structure that is composed of microtubules and actin microfilaments that provides mechanical support to the cell. Microtubules are long tubular components made of alpha and beta subunits of tubulin molecules. The dimers of these subunits form linear protofilaments that wind together to form a 24 nm wide hollow cylinder (Goddard et al., 1994). Actin microfilaments

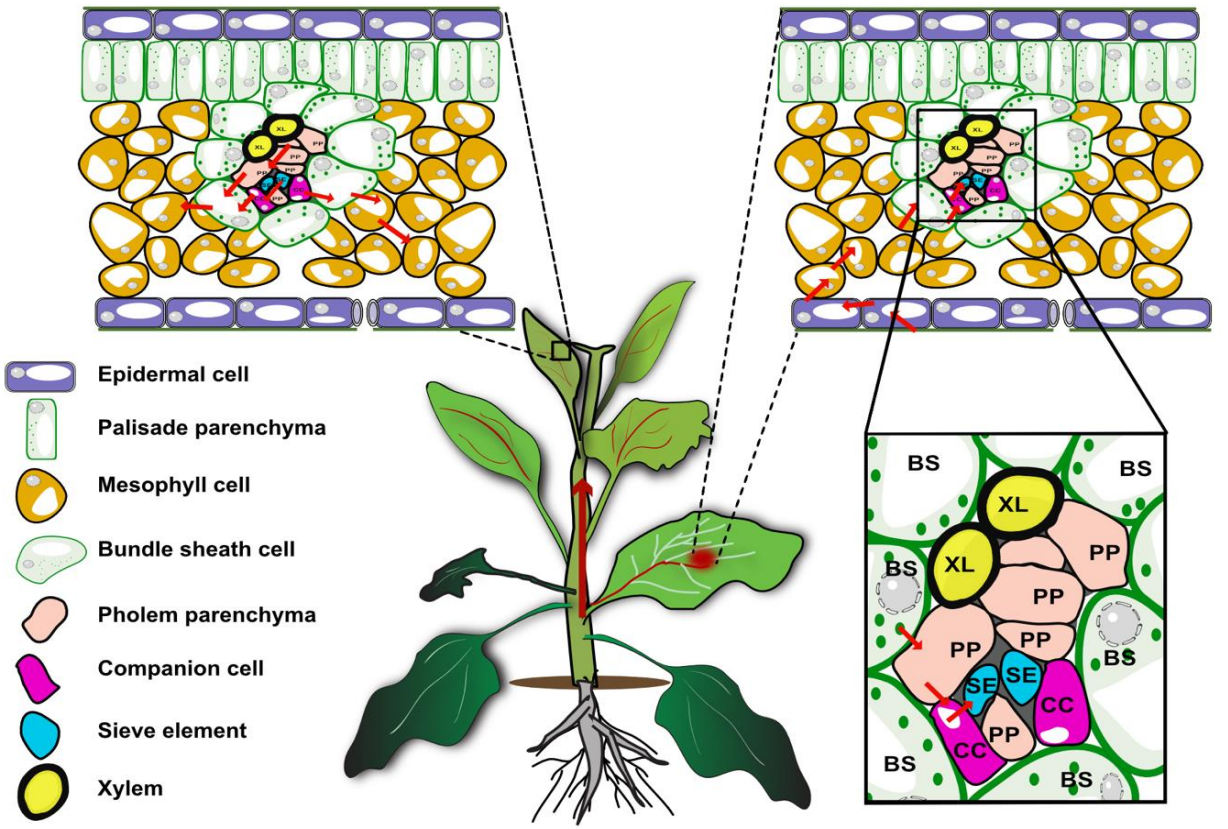


Figure 4. Movement of virus in the plant. Following the initial infection (red spot), the virus moves through the epidermal cells, mesophyll cells, bundle sheath cells, phloem parenchyma, into companion cells through which virus enters sieve elements. Upon entry into the phloem sieve elements, virus moves rapidly (red arrow) to cause systemic infection. Once reaching sink tissues, virus unloads from veins and spreads cell-to-cell to establish infection.

are made of actin monomers arranged as a long spherical chain, and are narrower than the microtubules with a diameter of about 6 nm (Perez et al., 1986). Both microtubules and actin filaments rapidly polymerize and depolymerize making the cytoskeleton structure highly dynamic and constantly changing. The dynamic nature of cytoskeleton provides necessary force for the movement of cellular components and viruses intra- and intercellularly (Burckhardt and Greber, 2009).

The plant cytoskeleton plays an important role in the growth and development of the plants including cell division, cell expansion, ER network organization, intracellular motility, and cytoplasmic streaming (Staiger, 2000). It also plays a major role in mediating the plant response to a diverse range of environmental factors such as abiotic and biotic stresses (Blume et al., 2017; Day et al., 2011; Wang et al., 2011). The plant cytoskeleton acts as a defence system against bacterial and fungal infections. However, during the plant-virus interactions, viruses hijack the cytoskeleton for their spread (Takemoto and Hardham, 2004).

1.4.1..1.1.2 Microtubules

The role of the microtubule network in virus movement is not very well understood. Many of the studies addressing the role of microtubules in the virus movement were focused on the *Tobacco mosaic virus* (TMV), and it has been shown that the TMV MP binds with the microtubules and the viral RNA, suggesting that the microtubular network mediates the transport of TMV infectious unit from the replication sites to the plasma membrane. Association of TMV MP with microtubules was further confirmed by mutating the ORF encoding MP, which abolished the virus movement and association with microtubules (Boyko et al., 2007, 2000). Interestingly, several of tobamoviruses contain a GxxP (where x represents any amino acid) structural motif that is conserved in the tubulins, and is critical for the microtubular assembly. Mutations in this motif resulted in reduced cell-to-cell movement of TMV, suggesting that the tobamoviral MPs mimic structural motifs in the tubulins for their movement (Boyko et al., 2000). Two models have been proposed for the microtubule network mediated movement of TMV vRNP complex: active transport of vRNA-MP complex mediated by kinesin molecular motors, or the movement as a result of the force generated during microtubular dynamics (Hull, 2013a).

MPs of some other viruses including, Hsp70 of BVY and TGB1 of PMTV were reported to associate with the microtubules. However, disrupting

microtubules using chemical inhibitors such as oryzalin and colchicine did not inhibit the intercellular movement of these viruses (Prokhnevsky et al., 2005; Wright et al., 2010). Interestingly, similar experiments with TMV also did not inhibit the virus movement. It was later identified that these chemical inhibitors do not disrupt the microtubules completely (Seemanpillai et al., 2006). Collectively, these results highlight the importance of further studies in understanding the role of microtubules in the virus movement.

1.4.1..1.1.3 Actin microfilaments

Unlike the microtubules, actin microfilaments are considered to extend through the plasmodesmata (White and Barton, 2011). Actin filaments are implicated in the intercellular transport of the cellular cargo, and in regulating the permeability of the plasmodesmata (Chen et al., 2010). Transient expression of 10 kDa fluorescent dextran in *N. benthamiana* and *N. tabacum* plants treated with chemical disruptor of actin, cytochalasin D, resulted in increased cell-to-cell movement of the dextran, while treating with actin-stabilizing toxin, phalloidin prevented its movement, suggesting that the actin dynamics play a role in regulating the transport through plasmodesmata (Ding et al., 1996). However, similar studies in other species, namely, *Azolla pinnata, Hordeum vulgare, Arabidopsis* root cells, and *Tradescantia virginiana* stamen hairs indicated no significant difference in the intercellular movement (White and Barton, 2011) suggesting that the role of actin in regulating the plasmodesmata is varied among the plant species, and tissues.

Evidence suggests that many viruses hijack the actin filaments for their transport, as MPs of many viruses e.g. TMV, and *Cucumber mosaic virus* (CMV) were reported to track along the actin filaments to reach the plasmodesmata (Su et al., 2010). Latrunculin B (LatB) treatment inhibited the cell-to-cell movement of TMV, PVX and TBSV (Harries et al., 2009). In the case of PVX, LatB treatment disrupted the association of MP with the ER network which in turn inhibited the cell-to-cell movement (Mitra et al., 2003), suggesting that the actin-mediated membrane association is necessary for the cell-to-cell movement.

1.4.1..1.1.4 Motor proteins

Motor proteins are a part of the cell machinery that move along the cytoskeletal components. In plant cells, kinesins and myosins are classified as motor proteins that move on the microtubules, and actin filaments respectively. These motor

proteins are involved in regulating and organizing the cytoskeleton and drive the transport of cellular components (Neben Uhr and Dixit, 2018). As one of the aims of this thesis is on understanding the movement of the virus through the acto-myosin network, the following section briefly describes the myosin motor molecules.

Plant myosins are widely classified into two classes, class VIII and class XI based on the phylogenetic analysis (Reddy and Day, 2001). The basic structure of the myosins includes a microfilament binding motor domain, a neck domain and a cargo-binding tail domain. Myosins move on the microfilaments in an ATP dependent fashion, where hydrolysis of ATP results in a reversible conformational change in their motor domains, which is translated into larger movement by the stiff neck region at the C-terminal end of the motor domain (Preller and Manstein, 2013). Thus for every hydrolytic cycle, one ATP molecule is used, which is coordinated with binding and release of the motor domain on the actin filament, that causes one step forward towards the plus end of the actin filament (reviewed in Ryan and Nebenführ, 2018).

Among the class VIII and class XI myosins, class XI myosins are well studied and are reported to be similar to the class V myosins in the animals (Kinkema and Schiefelbein, 1994). Class XI myosins are characterized by having longer neck domain and a globular tail domain that allows for cargo binding. The presence of a longer neck domain allows the class XI myosins move with faster velocity compared to class VIII on the actin filaments (Tominaga and Nakano, 2012). While class XI myosins mostly are involved in the propelling the organelles during the cytoplasmic streaming (Wang and Pesacreta, 2004), class VIII myosins localize primarily to the plasma membrane and are involved in endocytosis and plasmodesmatal trafficking (Reichelt et al., 1999).

The viral cell-to-cell movement, along with the transport of Golgi complexes was inhibited by the overexpression of an actin-binding protein suggesting that the motor-driven transport along the actin filaments is important for the cell-to-cell movement of the virus (Hofmann et al., 2009). Recently, few studies identified that specific classes of myosins take part in the transport of viral MPs. Through dominant-negative inhibition of myosins, it was identified that several viruses use myosins for their intercellular movement (Amari et al., 2014, 2011; Avisar et al., 2008).

1.4.1..1.1.5 Plasmodesmata

Among the cellular structures, plasmodesmata are the primary barriers for the intercellular movement of the viruses (Lee and Lu, 2011). Plasmodesmata are the intercellular junctions in plant cells that allow cytoplasmic continuity (Lucas et al., 2009). Plant viruses exploit these organelles to carry out the intercellular movement of their infectious material to achieve the systemic infection in their hosts. The plasmodesmata are formed during cell division, when ER gets trapped within the cell plate, forming a desmotubule or appressed ER (Robards and Lucas, 1990). The space between the desmotubule and the plasma membrane is usually of 2.5-3.0 nm size, and its translocation capacity is tightly controlled (Ding, 1998). These nanopores serve as a channel for the exchange of nutrients and other signalling molecules such as transcription factors and small RNA molecules between the cells (Sager and Lee, 2014).

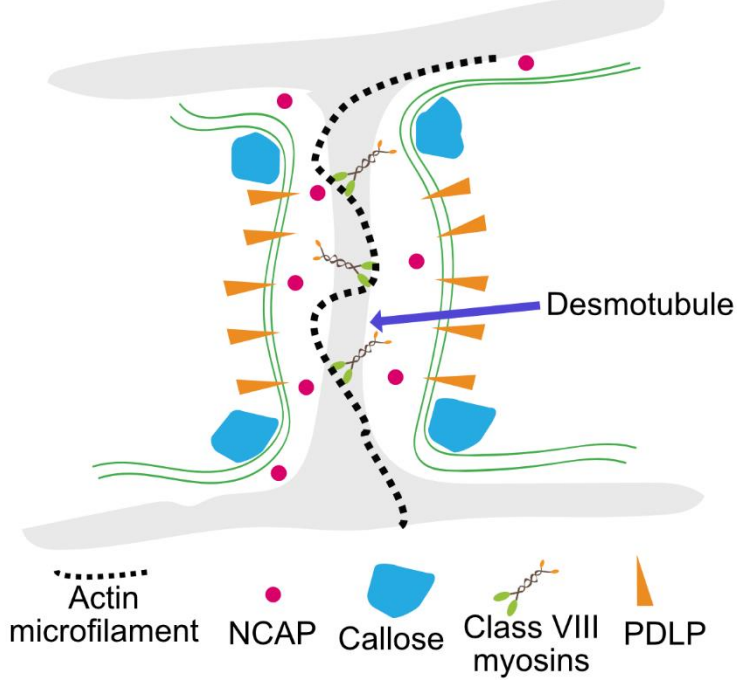


Figure 5. Structure of simple plasmodesmata and various cellular factors associated with plasmodesmata. NCAP, Non-cell-autonomous proteins. PDLP, Plasmodesmata-located protein.

Structurally, plasmodesmata have been classified into two types, primary and secondary plasmodesmata (Ehlers and Kollmann, 2001). Primary plasmodesmata are formed during the cell division and usually found in young tissues (Hepler, 1982). Primary plasmodesmata have simple structure with a

single plasma membrane-lined channel connecting the adjacent cells (Overall and Blackman, 1996). As the leaf tissues mature and progress from sink to source tissues, the primary plasmodesmata undergo branching to form secondary plasmodesmata (Faulkner et al., 2008; Oparka et al., 1999). The secondary plasmodesmata often contains a central cavity, and multiple channels linking the cells.

The upper limit of the size of the molecules that can move through the plasmodesmata, called size exclusion limit (SEL), is tightly controlled by the deposition of callose, a β-glucan polysaccharide, around the neck region (Vatén et al., 2011). The SEL of the secondary plasmodesmata is also significantly lower than the primary plasmodesmata. Both biotic and abiotic stresses have been found to influence the callose deposition. Indeed, the salicylic acid (SA) defence signalling pathway, mediated by EDS1, NPR1, and PDLP5 regulates this callose deposition by inducing callose synthase activity (Wang et al., 2013).

The SEL generally is far less than the size of macromolecules including both host and viral nucleic acids, and virus particles that are transported through plasmodesmata (Gibbs, 1976). Hence, in order to carry out the intercellular transport of non-cell-autonomous proteins, the plasmodesmata interact with specific proteins that can increase the SEL to allow their movement (Lucas, 2006). Additionally, various plasmodesmata associated proteins such as plasmodesmata-located proteins (PDLPs) that are present along the plasma membrane (Amari et al., 2010; den Hollander et al., 2016), remorin (REM), a plasma membrane protein (Perraki et al., 2014; Raffaele et al., 2009; Sasaki et al., 2018) were reported to interact with the viral MPs.

The viral MPs, like that of TMV, have been identified to interfere with the callose deposition by recruiting β-1,3 glucanases that can degrade the callose which is induced by the viral infection (Epel, 2009). These findings were further supported by a study that showed viral spread is positively correlated with the expression of β-1,3 glucanases (Elvira et al., 2008; Gorovits et al., 2007). Similar findings were reported in mutant tobacco plants deficient in β-1,3 glucanases, where the cell-to-cell movement of TMV, PVX and the MP of cucumber mosaic virus were reduced (Fridborg et al., 2003; Iglesias and Meins, 2000). The callose deposition levels were increased in the same mutant, suggesting that the virus induces expression of β-1,3 glucanases for the degradation of callose.

1.4.1..1.1.6 Viral movement proteins

The capacity of the virus to efficiently infect the host depends on the expression of one or more MPs that potentiate movement of the virus (Atabekov and Dorokhov, 1984). The first evidence for the presence of viral MPs came from the study on a temperature-sensitive mutant of TMV which showed that the spread of the virus was inhibited at restrictive temperatures (Nishiguchi et al., 1978). This failure in the movement was later mapped to the 30K protein of TMV (Ohno et al., 1983). Since then TMV 30K has been extensively studied for understanding the virus movement in general.

Functionally the MPs of different viruses share similar properties; this includes, for example, supporting the passage of their genetic material by modifying the plasmodesmata (Carrington et al., 1996; Oparka et al., 1997; Wolf et al., 1989). This is supported by experimental evidence demonstrating that the MP of one virus is able to complement the movement of a different virus. For instance, the MP of TMV complemented the movement of MP-deficient BSMV (Solovyev et al., 1996), the *Dianthovirus* MP complemented TMV movement (Xiong et al., 1993). Similar findings have been reported for the PVX, where the CMV MP complemented the PVX MP (Tamai et al., 2003).

MPs use a diverse range of strategies in order to achieve the intercellular movement, which can be broadly grouped as two major forms: (i) those that form tubules by restructuring the plasmodesmata, and (ii) those that gate the plasmodesmata and increase SEL (Figure 6).

1.4.1..1.1.6.1 Movement proteins that form tubules

The MPs of several genera including *Caulimovirus*, *Nepovirus*, *Comovirus*, and *Alfamovirus* facilitate the intercellular movement of the virus particles or nucleocapsid proteins by modifying the plasmodesmata into tubule-like structures (Kitajima and Lauritis, 1969). The MP-lined tubules extend through the plasmodesmata into the cytoplasm of adjacent cells (Figure 6) (Niehl and Heinlein, 2011). These tubules are usually seen only in the primary plasmodesmata. These MPs modify the plasmodesmata by removing the desmotubule and thereby increasing the SEL of the plasmodesmata up to 50 nm (Schoelz et al., 2011). Electron microscopy revealed that the virions lined up as a single file within the tubules in the case of *Cowpea mosaic virus* (CPMV), an RNA virus, and *Dahlia mosaic virus*, a DNA virus (Kitajima and Lauritis, 1969). The MPs of these viruses usually interact with the C-terminus of the CP, as it was observed in the case of the CPMV, *Grapevine fanleaf virus* (GFLV) and *Cauliflower mosaic virus* (CaMV) (Belin et al., 1999; Bertens et al., 2003;

Huang et al., 2001). Deletion in the C-terminus of the CP of the CPMV leads to interfering with virion uptake, resulting in the formation of the empty tubules and inhibition of the virus movement (Huang et al., 2001).

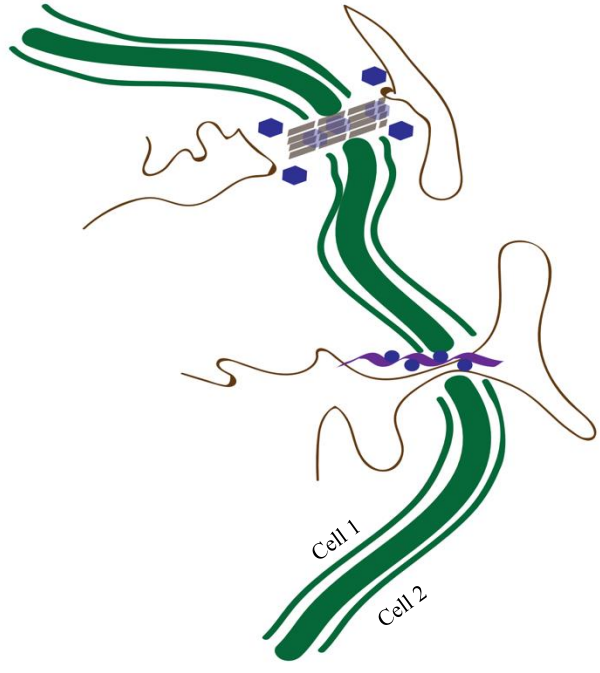


Figure 6. Movement strategies of plant viruses to cross plasmodesmata. MPs of the tubule forming viruses create tubule-like structures through which virus particles move intercellularly. On the other hand, the MPs that gate plasmodesmata increase the size exclusion limit of the plasmodesmata through which the vRNP complex moves cell-to-cell.

1.4.1..1.1.6.2 MPs that gate plasmodesmata

The MPs that gate the plasmodesmata to carry out the cell-to-cell movement can be classified into at least two groups based on the type of MP. The first group, TMV-like MPs, consists of a large, single-unit MP that transports the virus (Rojas et al., 2016). These MPs are seen in the members of *Tobamovirus*, and *Dianthovirus* genera (Schoelz et al., 2011). The ability to increase the SEL of plasmodesmata by MP of TMV was first demonstrated by injecting 10 kDa fluorescent labelled dextrans into the mesophyll cells of 30K transgenic plants, which increased the intercellular transport of dextrans (Oparka et al., 1997). As mentioned in the previous section, the MP of TMV influences the callose

deposition and increases the plasmodesmatal SEL. In addition, the TMV MP has been shown to interact with ankyrin repeat-containing protein (ANK) at the plasmodesmata that could degrade the callose, and with calreticulin, a protein involved in sequestering Ca^{2+} resulting in increased plasmodesmatal permeability (Ueki et al., 2010).

Various models have been suggested for the movement of TMV infectious unit through the plasmodesmata. The TMV MP is suggested to act as a chaperone that binds to vRNA and moves as a vRNP complex through the dilated plasmodesmata (Citovsky et al., 1992). Another model suggests an involvement of the cytoskeletal components in transporting the ER-vesicles derived from the membrane-associated vRNP complex, or the membrane-associated vRNP complex itself through the plasmodesmata (Kawakami et al., 2004). Additionally, vRNP complex of TMV has also been suggested to diffuse through the lipid matrix of the desmotubule between the cells (Kawakami et al., 2004). These results indicate that TMV MP uses more than one mode of intercellular transport through the plasmodesmata.

The second class of MPs are segmented and shorter MPs that co-ordinate the movement of the virus. The segmented MPs can be encoded by double-gene-block of the viruses belonging to genus *Carmovirus* (Marcos et al., 1999) or triple-gene-block-encoded proteins of nine genera of viruses belonging to families *Alpha-*, *Beta-flexiviridae*, *Virgaviridae,* and an unassigned genus *Benyvirus* (Verchot-Lubicz et al., 2010). Based on the properties of the MPs, these MPs are grouped as potex-, hordei-, and pomo-like TGBs. Potex-like MPs are encoded by the members of *Alpha-*, and *Beta-flexiviridae* families (Morozov and Solovyev, 2003). The TGBs encoded by the genera *Hordeivirus*, and *Pecluvirus* are classified as a hordei-like group of TGBs, while that of *Pomovirus* are classified as pomo-like TGBs (Verchot-Lubicz et al., 2010).

One of the striking differences between the potex-like TGB proteins from the hordei-, and pomo-like TGBs is the requirement of the CP for carrying out the intercellular movement of the virus (Ozeki et al., 2009). Moreover, the TGB1 of potex-like viruses is smaller compared to that of the other groups (Solovyev et al., 2012), and can increase the SEL independently of TGB2 and TGB3 (Howard et al., 2004; Verchot-Lubicz et al., 2010), while the TGB1 of hordei- and pomo-like viruses require TGB2 and TGB3 in delivering the vRNP complex at the plasmodesmata, and to increase the permeability of plasmodesmata (Haupt et al., 2005; Jackson et al., 2009).

1.4.1..1.1.7 Cell-to-cell movement of PMTV

The GFP-tagged TGB1 of PMTV, when expressed together with TGB2 and TGB3 proteins transports through the plasmodesmata and achieves cell-to-cell movement. However, when expressed alone, the GFP-TGB1 is confined to single cells (Zamyatnin et al., 2004), suggesting that the TGB2 and TGB3 proteins play an important role in the intracellular movement of the vRNP. Similar observations were made in the studies on the hordei-like TGB of BSMV that showed accumulation of GFP-TGB1 in the cytoplasmic bodies when expressed individually, but when co-expressed with TGB2 and TGB3, resulted in accumulation of fluorescence in multiple foci (Lim et al., 2009). However, TGB3 alone appears to be sufficient to assist the TGB1 in intercellular transport of BSMV, although the presence of TGB2 protein increases the efficiency of its movement (Lim et al., 2009).

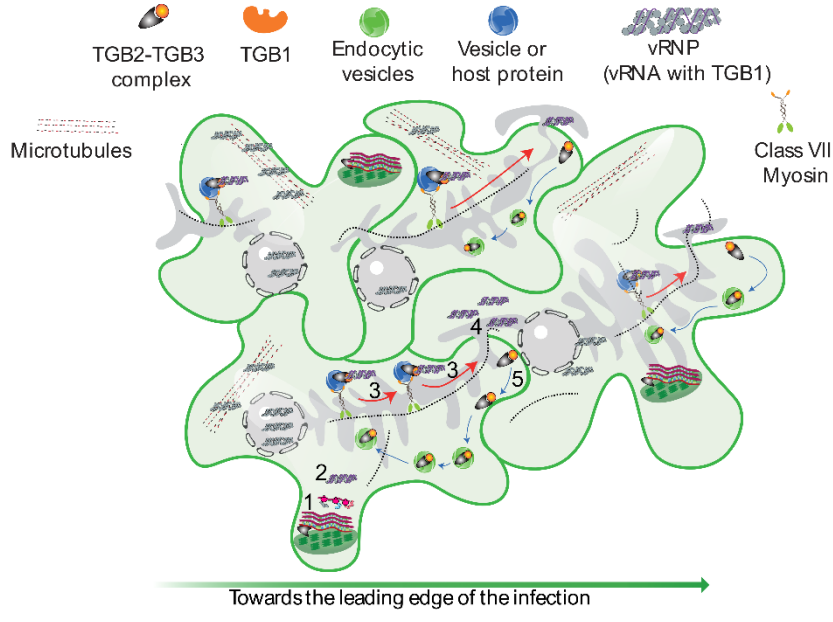


Figure 7. Current model of PMTV intracellular and cell-to-cell movement. Following the replication at the chloroplast, and subsequent translation (1), the TGB1 protein binds with the viral RNA forming a vRNP complex (2). TGB2 and TGB3 proteins assist the movement of vRNP, probably by being incorporated into vesicles or by interacting with some host protein. This complex moves towards plasmodesmata probably by hijacking acto-myosin network (3), where the vRNP complex moves to adjacent cells (4), while TGB2 and TGB3 are recycled through the endocytic vesicular pathway (5). TGB1 associates with microtubules and nucleolus at the late stages of infection.

The current model of PMTV intracellular movement suggests that, during the initial stage of infection, the vRNP complex of TGB1 and viral RNA is recruited by the TGB2 protein through its RNA interaction domain. Later the TGB2 protein recruits TGB3 protein, which directs the complex towards the plasmodesmata through the actin-ER network, where TGB2 and TGB3 increase the SEL of plasmodesmata (Haupt et al., 2005). Following the delivery at the plasmodesmata, both the TGB2 and TGB3 proteins are recycled through the endocytic recycling pathway (Haupt et al., 2005). The TGB1-RNA vRNP complex then moves intercellularly through plasmodesmata. However, the molecular mechanism of how the vRNP moves through the plasmodesmata is still unclear.

1.4.1..2 Long-distance movement

One of the first reports showing that the viruses move long-distance came from a study that suggested that the flow of metabolites in the plant influence the virus spread in the host (Bennett, 1940). The long-distance movement of the viruses mostly occurs through the plant vascular system. While most viruses move from non-vascular cells to minor veins in the leaves, some viruses move through both major and minor veins (Cheng et al., 2000; Roberts et al., 1997). The ability of the virus to move systemically depends on the capacity to enter and exit the bundle sheath cells, phloem parenchyma, companion cells and the phloem sieve elements. Once entering the sieve elements, where the sieve plate pores have larger SEL than the plasmodesmata in the leaf cells (Oparka et al., 1999), viruses move long distance rapidly and establish a systemic infection (Figure 4). Many plant species contain two structural types of phloem, external and internal phloem. The external phloem is towards the abaxial side of the leaf that transports the metabolites towards the roots in a slow manner, while the internal phloem is on the adaxial side of the leaf allows rapid transport of the metabolites towards the upper part of the plant. It has been reported that some of the potyviruses and carmoviruses first move through the external phloem towards the roots and, at or near the cotyledonary node they enter the internal phloem to move to the upper parts of the plant (Andrianifahanana et al., 1997; Gosalvez-bernal et al., 2008). However the mechanism through which they move from external to internal phloem is not known (Hull, 2013a). On reaching the upper parts of the plant, the virus exits the vascular elements and enters the mesophyll of the younger leaves.

The long-distance movement of most viruses occurs as virus particles that require the CP, however, some viruses including PMTV are capable of moving

systemically in the absence of CP, as a vRNP complex (Torrance et al., 2009). In both movement forms of PMTV, TGB1 MP plays an indispensable role. The TGB1 protein contains two nucleolar localization sites (NoLS) in the N-terminal domain (Lukhovitskaya et al., 2015), and deleting the first 84 amino acid residues resulted, not only in absence of nucleolar accumulation and microtubule labelling, but also abolished the systemic movement of the virus (Lukhovitskaya et al., 2015; Wright et al., 2010). Mutational analysis identified that the NoLSs in the N-terminal domain are important for the importin-α mediated nucleolar localization of TGB1, and for the long-distance movement of the virus, suggesting that the nucleolar passage of TGB1 is necessary for the systemic movement of PMTV (Lukhovitskaya et al., 2015).

Table 1 explains the different ways in which PMTV moves systemically. In the presence of the CP and CP-RT, PMTV moves systemically as virus particles, where the CP-RT and TGB1 protein are attached to one extremity of the virus particles (Torrance et al., 2009). In the absence of the CP-RT, virus particles are formed but failed to move systemically, suggesting that the CP-RT is not required for the virion assembly, but is necessary for the long-distance movement of the virus particles (Torrance et al., 2009).

As mentioned before, PMTV belongs to a small group of viruses where the CP is dispensable for the systemic movement as the virus can move in the form of a vRNP complex (Savenkov et al., 2003). Interestingly, in the presence of CP alone, or the CP-RT with deletions in the TGB-interacting region, the systemic movement of RNA-CP, but not the other two RNAs (as vRNP) is inhibited. The reason for this was suggested to be because of the differences in the 5' UTR sequence of RNA-CP compared to that of RNA-rep and RNA-TGB, that might prefer binding of CP over the TGB1, thereby inhibiting the formation of the vRNPs.

Table 1. Various movement forms of PMTV for carrying out systemic infection.

	+CP +CP-RT	+CP -CP-RT	-CP +CP-RT	-CP -CP-RT
RNA-rep RNA-CP RNA-TGB	All three genomic components move as vRNP and as virions.	Only RNA-rep and RNA-TGB move as vRNP.	All three genomic components move systemically as vRNPs.	All three genomic components move systemically as vRNPs.

1.4.2 Suppression of host defence system

As a result of the extensive interactions between the viruses and their hosts during the process of infection, plants go through various physiological and developmental disorders. Consequently, plants employ multiple defence strategies to restrict the viral infection, such as triggering a hypersensitive response (HR), RNA silencing, hormone-mediated defence, a defence based on pathogen-associated molecular patterns (PAMP) etc (Carr et al., 2010; Islam et al., 2019; Liu et al., 2017; Mandadi and Scholthof, 2013). The RNA silencing is one of the well-studied mechanisms and is considered as one of the common defence mechanism against plant viruses (Burgyán and Havelda, 2011).

1.4.2.1. RNA silencing

RNA silencing is a highly conserved gene silencing mechanism that degrades RNA in a nucleotide sequence-specific manner (Ding and Voinnet, 2007). This activity was first discovered in plants in an attempt to overexpress chalcone synthase (CHS) gene in petunia petals, which unexpectedly resulted in suppression of both transgene and endogenous CHS gene (Napoli et al., 2007). RNA silencing mechanism was later found out to be conserved in most of the eukaryotes. RNA silencing has a very significant role in the regulation of the plant growth and development, and takes part in DNA repair, abiotic stress response, suppression of transposons, and other foreign nucleic acids (Bajczyk et al., 2019; Chinnusamy et al., 2007; Khraiwesh et al., 2012; Manova and Gruszka, 2015).

The mechanism of RNA silencing can be divided into three stages: initiation phase that involves biogenesis of small interfering RNA (siRNAs), followed by the effector phase, where the siRNAs are loaded into the RNA induced silencing complexes (RISC), and amplification phase that causes systemic silencing.

The initiation of the RNA silencing is triggered by the presence of the double-stranded RNA (dsRNA) (Fire et al., 1998). These dsRNAs can produced be as a result of RNA-dependent RNA polymerase (RdRp) mediated dsRNA formation, as occurs in the case of RNA viruses. The presence of hairpin-like secondary structures formed by the fold-back regions of the viral ssRNA also acts as substrates for the sRNA biogenesis (Molnár et al., 2005). The dsRNAs are targeted by RNase III-type DICER-LIKE (DCL) family of proteins together with double-stranded RNA binding protein (DRB) (Hiraguri et al., 2005). Various DCL proteins process the dsRNA into siRNA duplexes (Hamilton and Baulcombe, 1999). In *Arabidopsis* four DCLs (DCL 1-4) were identified, of

which DCL4, DCL2, and DCL3 were identified to confer antiviral defence, and catalyse the production of 21-, 22-, and 24-nt vsiRNAs, respectively (Margis et al., 2006). DCL4 confers efficient defence against the RNA viruses. However, in the *dcl4* mutant background DCL2 acts as a potent antiviral defence factor (Deleris et al., 2006; Donaire et al., 2008; Garcia-Ruiz et al., 2010; Qu et al., 2008). The DCL3, although has a minor role against the RNA viruses (Qu et al., 2008; Raja et al., 2008) and may enhance antiviral defence mediated by the DCL4 and DCL2. The vsiRNAs are then stabilized at their 3' end by the HUA Enhancer 1 (HEN1) dependent methylation (Vogler et al., 2007).

During the effector phase, the siRNAs are loaded into Argonaute (AGO) containing RNA-induced silencing complex (RISC) which slices the RNA sequences with high sequence complementarity (Fagard et al., 2000). The size of the siRNAs and the 5' nucleotide of the sRNA directs preferential sorting of siRNAs into specific AGOs. For instance, AGO1 and AGO2, most important AGOs in the antiviral silencing in *Arabidopsis* (Brodersen et al., 2008), preferentially binds to sRNA with 5'-terminal U and A residues, respectively (Mi et al., 2008). Following the incorporation of siRNA duplex into the RISC complex, one strand known as guide strand is assembled with the AGO protein while the other strand, called passenger strand is discarded. It was reported that this selection based on the thermodynamic stability between the two ends of the siRNA and the strand with less stable 5' pairing is retained within the AGO protein (Khvorova et al., 2003; Schwarz et al., 2003; Takeda et al., 2008). The guide strand then binds to the mRNA or viral RNA in a sequence-specific manner which results in degradation or translational repression of the RNA by RISC (Guo et al., 2019). The siRNA along with other aberrant RNAs serve as primers to generate dsRNA via cellular RNA-directed RNA polymerase (RDR), that subsequently serves as substrates for the DCL processing, followed by RISC formation, leading to the amplification of the RNA silencing signal (Dalmay et al., 2000; Voinnet et al., 1998).

The amplified RNA silencing signal then travels intercellularly from the site of initiation to the neighbouring cells, and systemically to other parts of the plant. This movement of the RNA silencing signal was observed through grafting experiments in tobacco plants, which provided an evidence for the spread of RNA silencing signal from silenced rootstock to non- silenced scions (Palauqui et al., 1997). The short range spread of RNA silencing signal occurs in a limited area of about 10-15 cells from the site of initial silencing either through the plasmodesmata, or apoplastically through intercellular spaces or the cell walls (Mermigka et al., 2016). The short range spread of RNA silencing is predominantly mediated by the DCL4-produced 21-nt siRNAs. The systemic

silencing, on the other hand is transported to distant organs through phloem (Kalantidis et al., 2008). The silencing signal, following the movement through plasmodesmata, reaches and enters the phloem cells and follows the photo assimilate translocation route from the source to sink tissues (for review, Mermigka et al., 2016).

1.4.2.2. Suppression of RNA silencing

To counteract the RNA silencing-mediated defence, viruses evolved to encode proteins that are able to suppress the RNA silencing, called as viral suppressors of RNA silencing (VSRs) (Burgyán and Havelda, 2011). It is reported that many viruses encode at least one VSR, which in many cases is essential for the efficient virus infection (Csorba et al., 2015). Based on the diversity in their sequence and structure, it was suggested that VSRs evolved independently. Various VSRs employ different strategies to suppress host RNA silencing by blocking key steps in the RNA silencing pathway (Li and Ding, 2006). Some VSRs target multiple steps of the antiviral silencing mechanism, and thereby helping in achieving a balance between the plant defence and viral counter-defence (Iki et al., 2017; Valli et al., 2018).

1.4.2.2. 1. Binding to dsRNA

Binding to dsRNA is considered to be of the most common mechanisms the VSRs employ to suppress RNA silencing (Hull, 2013b). The VSRs are reported to bind to dsRNAs in two different ways, binding in size-independent way to various dsRNAs, and binding to specific sized dsRNAs.

1.4.2.2. 1.1. Binding to dsRNA in size-independent way

VSRs such as P14 of *Pothos latent virus*, 2b of *Tomato aspermy virus,* and P38 of TCV have been reported to bind to long dsRNA preventing processing of dsRNA into siRNAs by DCL proteins (Chen et al., 2008; Iki et al., 2017; Mérai et al., 2006, 2005). Biochemical analysis using a synthetic dsRNA revealed that the TCV P38 protein efficiently inhibits dsRNA processing into 21- and 24-nt siRNAs (Iki et al., 2017).

1.4.2.2. 1.2. Binding to specifically sized dsRNA

On the other hand, several VSRs bind to specifically sized siRNAs duplexes and sequester them, and thus depleting their availability to be incorporated into the RISC. Immunoprecipitation of *Cymbidium ringspot virus* P19 protein from

infected *N. benthamiana* plants using anti-P19 antibodies and subsequent Northern blot analysis showed that P19 binds virus-specific 21-nt RNAs (Lakatos et al., 2004). In the same study, it was identified that the plants infected with a modified virus that does not express P19 resulted in high accumulation of siRNAs, suggesting that the P19 sequesters the siRNAs. Furthermore, crystallization studies of P19 protein from another tombusvirus *Carnation Italian ringspot virus* in complex with a 21-nt siRNA duplex revealed that the two molecules of P19 binds to one siRNA duplex (Vargason et al., 2003). Size-selective binding of siRNAs was identified through *in vitro* binding assays in many unrelated viruses such as the HcPro of *Tobacco etch virus*, P15 of *Peanut clump virus*, P21 of *Beet yellows virus* (BYV), and γb of *Barley stripe mosaic virus*. These VSRs efficiently bind 21-nt siRNA duplexes, but not long dsRNA (Mérai et al., 2006; Vargason et al., 2003), indicating that dsRNA binding is a widely used silencing suppression strategy and many VSRs can discriminate between short and long sRNA. However, the sites of binding among the VSRs are different. For example, the HcPro binds to 3' overhang of the 21-nt siRNA through an amino acid sequence, FRNK, conserved in its central region (Sahana et al., 2014; Shiboleth et al., 2007). On the other hand, the P19 protein binds to the duplex region of the siRNA (Vargason et al., 2003). Together, the difference in the binding properties suggest that these VSRs, even though carry out similar functions, might have evolved the siRNA-binding activities independently.

1.4.2.2. 2. Preventing the functioning of DCL proteins

Some VSRs prevent the functioning of DCL proteins by suppressing their expression, and thus preventing the accumulation of siRNAs. VSRs of some viruses like *Red clover necrotic mosaic virus* recruits the DCL proteins into viral replication complexes thus preventing processing of long dsRNA into vsiRNAs (Takeda et al., 2005). Various other VSRs such as TCV P38 (Deleris et al., 2006), CMV 2b protein (Diaz-Pendon et al., 2007) reported to interfere with the DCL functioning. Studies on the CaMV VSR, P6 protein reported that P6 interacts with and inhibits the functioning of DRB4 protein that acts as a cofactor for DCL4 (Haas et al., 2008).

1.4.2.2. 3. Interfering with RDR pathway

In plants, RDR6 mediated generation of secondary siRNAs plays an important role in the silencing based antiviral immunity (Li et al., 2014). Hence, multiple VSRs evolved to either block or downregulate the functioning of RDR, thus

preventing the siRNA biogenesis and inhibiting the signal amplification pathway. The V2 protein of *Tomato yellow leaf curl virus* binds to suppressor of gene-silencing 3 (SGS3), which is involved in the amplification of siRNA signal (Glick et al., 2008). VSRs such as P6 of *Rice yellow stunt virus*, potyvirus HC-Pro, and CMV 2b, PVX TGB1 (Fang et al., 2016; Guo et al., 2013; Okano et al., 2014; Valli et al., 2018) proteins are also reported to interfere with the RDR mediated signal amplification pathway. However, the mechanisms with which they interfere with RDR pathway is not clearly understood (Burgyán and Havelda, 2011).

1.4.2.2. 4. Inhibiting AGO proteins

As AGO proteins play an important role in the RNA silencing mechanism, several VSRs inhibit their functioning either by degrading AGO proteins, or downregulating the expression of *AGO* genes. Examples of the VSRs degrading the AGO protein includes P25 protein of PVX and P0 protein of *Polerovirus*, both of which degrade the AGO1 in two different pathways. P25 protein of PVX selectively interacts with few AGOs, and degrades the AGO1 protein through proteasome pathway (Chiu et al., 2010). On the contrary, inhibition of proteasome did not prevent P0-mediated degradation of AGO1. It has been shown that P0 protein identifies the PAZ motif and a part of its upstream sequence in AGO1 and triggers its degradation through autophagy pathway (Baumberger et al., 2007; Bortolamiol et al., 2007).

VSRs also inhibit functioning of AGO by downregulating the expression of *AGO1* gene. VSRs such as HcPro, P38, 2b and P19 proteins are reported to upregulate the expression of miR168 which inhibits the translation of the *AGO1* mRNA (Varallyay and Havelda, 2013).

1.4.2.2. 5. RNA silencing suppression activity of PMTV 8K protein

In the case of PMTV, the third genomic segment, RNA-TGB encodes an 8 kDa cysteine-rich protein, which is reported to function as a weak VSR (Lukhovitskaya et al., 2013). The 8K protein, although dispensable for the long-distance movement of the virus, appears to be an important factor for an efficient virus accumulation in *N. benthamiana* and *N. tabacum* (Lukhovitskaya et al. 2005).

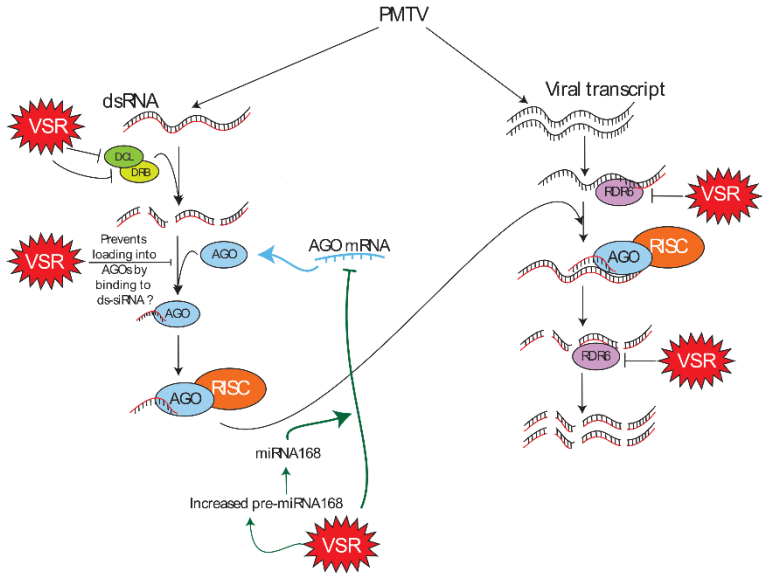


Figure 8. A model for antiviral RNA silencing mechanism and various stages where suppressor of RNA silencing interfere with the RNA silencing pathway.

2 Aims of the study

The specific objectives of the study were:

- To characterise the **variability of PMTV** in the Andean region of Peru.
- To characterize the **RNA silencing suppression activity** of the 8K protein of PMTV isolates from Peru and Sweden.
- To uncover the role of the acto-myosin network in the **cell-to-cell movement** of the virus.
- To identify TGB1-interacting partners (host protein) and assess their role in the virus cell-to-cell and systemic movement.

3 Results and discussion

3.1 Diversity of potato mop-top virus (Paper I)

3.1.1 Genetic variability and phylogenetic relationship of the PMTV isolates

Previous studies on PMTV isolates obtained from Europe, Asia, and North America reported very little genetic variability of PMTV (Beuch et al., 2015; Hu et al., 2016; Latvala-Kilby et al., 2009; Ramesh et al., 2014). We hypothesized that the reason for the low variability could be as a result of limited number of isolates sequenced so far. In this study, we characterized the diversity of PMTV by sequencing and analysing the genome of isolates from the Andean region of Peru and Sweden.

PMTV isolates were collected from 12 potato fields present in three different locations in the Andean region of Peru (Figure 1 and Table S1, Paper I). A total of 61 full-length genomic segments of PMTV were amplified using primers specific for well-conserved 5′- and 3′ termini. Between nine and 30 clones for each full-length genomic component were sequenced. To understand the rate of mutations in different cistrons, we carried out single-likelihood ancestor counting (SLAC) analysis that showed an uneven distribution of mutations with the *CP-RT* and *8K* cistrons accumulated the highest number of mutations, while the *RdRp* ORF accumulated lowest number of mutations (Figure 2, Paper I).

The phylogenetic analysis based on the sequences of PMTV isolates from Peru and the sequences of isolates available in the GenBank showed that there are two lineages of RNA-rep and RNA-TGB, and three lineages of RNA-CP (Figure 3, Paper I). In the RNA-rep phylogenetic tree, the clade I grouped isolates from Peru, Europe, Canada, USA and Colombia, clade II was

exclusively represented by Colombian isolates. The sequences in the clade I shared about 97% identity with the clade II. In the RNA-TGB bootstrap consensus tree, the Peruvian isolates were grouped in clade I together with isolates from Europe, Canada, USA and Colombia. The clade II was represented by single isolate from Peru, which shared 92-94% identity with isolates from clade I.

The phylogenetic tree of RNA-CP segment revealed two major clades and one novel genotype (genotype 3). While the clade I grouped isolates from Peru and other parts of the world, clade II and the novel genotype was exclusively represented by isolates from Peru, suggesting higher variability of RNA-CP in Peru compared to other parts of the world. Genotype 3 shared 80% identity with isolates from clade I and clade II.

3.1.2 Novel classification of PMTV isolates and Global spread of PMTV

Based on the new deduced phylogenetic relationship among the PMTV isolates, we suggested a novel classification of the PMTV isolates. In this classification, the genotype of each RNA segment is taken into consideration. Based on this classification, all the isolates described so far were catalogued into four genotype constellations (Table 2, Paper I), of which, two constellations were found exclusively in Peru, and another constellation was found in Colombia, suggesting that the Andes region has a higher diversity of PMTV.

Interestingly, one constellation was represented by isolates from Colombia, Europe, North America, Asia and Peru, suggesting that this particular genotype constellation was firstly introduced into Europe, which probably served as a source to the other parts of the world. A recent study on the global diversity of *S. subterranea*, the vector of PMTV suggested that *S. subterranea* was probably first introduced into Europe from South America, and was subsequently spread to other parts of the world (Gau et al., 2013). Considering the *S. subterranea* being the vector for PMTV, it can be hypothesized that the PMTV was first introduced into Europe, which served as a source of the virus to other potato growing regions of the world.

3.1.3 Role of CP-RT in the pathogenicity of PMTV

Existence of two different genotypes of RNA-CP as determined by the phylogenetic analysis suggests that there might be differences in their biological properties. To address that, we inoculated plants with the *in vitro* generated RNA transcripts from the infectious cDNA clones of the PMTV isolates

representing each of the lineages of the RNA-CP phylogenetic tree. Quantifying the virus accumulation using ELISA indicated that viruses containing RNA-CP belonging to clade I of phylogenetic tree accumulated in significantly lower amounts than the viruses containing RNA-CP from clade-II (Figure 6A and 6C, Paper I). Based on the differences in pathobiological properties, we termed clade I and clade II as S (severe) and M (mild) strains, respectively. Single-segment reassortant of the S-type, with the M-type RNA-CP segment resulted in decreased accumulation of virus (Figure 6D, Paper I). Notably, the amino acid differences in the S- and M-types were located in the read-through domain (Figure 5A, Paper I), suggesting that the read-through domain of CP-RT is a major determinant of the pathobiological properties of different strains.

Multiple sequence alignment of the CP-RT sequences revealed that some of the Peruvian isolates have internal in-frame deletions (Figure 5, Paper I). The internal deletions in the CP-RT region were previously reported in few isolates that were manually propagated for a long time, and also in some field isolates. These internal deletions had no effect on the systemic movement of the PMTV (Torrance et al., 2009). However, the reason why the virus loses this region upon serial mechanical transmission was not clear. Here, we showed that the isolates with the internal in-frame deletions accumulate slightly higher (Figure 6B, Paper I), suggesting a faster replication of the genome.

The isolates with the internal deletions in the CP-RT sequences were unable to be transmitted by its natural vector when tested experimentally (Reavy et al., 1998). Previously it has been shown that many genera of viruses with plasmodiophorid vectors contain transmembrane domains in the CP-RT region. These transmembrane domains are suggested to be involved in the attachment of the CP-RT to the plasma membrane of the vector, and thereby supporting movement from the cytoplasm of the host and the vector (Adams et al., 2001). Consistent with the previous studies, our *in-silico* analysis identified the presence of two transmembrane domains in the CP-RT region of PMTV, supporting the idea that the CP-RT protein is a membrane protein which is inserted into the lipid bilayer in a U-shaped orientation (Figure 5C, Paper I). We noticed that the isolates with internal deletion contain only one of the transmembrane domains, which is also consistent with the previous studies showing the loss of transmembrane domain in the nontransmissible deletion mutants, further supporting the idea that these transmembrane domains are important for the virus transmission by the vector. Future studies may experimentally address the importance of these transmembrane domains in the vector transmission.

3.2 RNA silencing suppression activity by PMTV 8K protein (Paper I and II)

3.2.1 Variability and selection pressure acting on the 8K gene (Paper I)

Multiple sequence alignment of the 8K amino acid sequences showed an extraordinary variability, with 23 variable amino acid positions in a 68 amino acid protein (Figure 4B, Paper I). The phylogenetic analysis of the 8K amino acid sequence revealed three clades and a novel distinct genotype (Figure 4A, Paper I). Peruvian isolates grouped into all four clades indicating higher variability of 8K in Peru than other parts of the world. While the clade I of 8K phylogenetic tree was represented by the majority of isolates from Europe, Asia, and two isolates from Colombia, and one isolate from Peru, clade II was represented by isolates from Peru, Colombia, and some isolates from Europe. The clade III and the novel genotype was exclusively represented by Peruvian isolates. The sequences in clade I shared 89 – 98% identity with clade II, 88 – 95% identity with clade III and 77 – 85% identity with novel genotype.

To address the question if there is any selection pressure acting on the PMTV genome, we calculated the ratio of non-synonymous to synonymous substitutions (dN/dS) using SLAC analysis. We found that the *8K* genomic region (dN/dS ration 1.415; dN/dS > 1, positive selection), but not any other cistrons are under positive selection. Interestingly, previous studies indicated no strong positive selection on the *8K* gene of the PMTV isolates from Europe, North America and Colombia. Moreover, we found that the the dN/dS value is even higher (dN/dS value 1.863) among *8K* sequences of isolates from Peru.

3.2.2 RNA silencing suppression activity of the 8K protein of various PMTV isolates (Paper II)

Although 8K protein is dispensable for the movement of the PMTV, it is required for efficient virus accumulation (Lukhovitskaya et al. 2005). The 8K protein was previously reported to be a weak suppressor of RNA silencing (Lukhovitskaya et al., 2013). Previous studies on the VSR of *Rice yellow motile virus* indicated that sites under positive selection modulate the RNA silencing suppression activity (Sereme et al., 2014). Indeed, a strong counter-counter-defence by hosts might impose strong selection pressure on the viruses that might favour the acceleration in the divergence of the VSRs. As our study indicated that the 8K protein has high variability and is under positive selection, we compared the VSR activity of seven most diverse alleles representing four

major clades of the 8K phylogenetic tree. These analyses showed that the 8K protein of one of the isolates from Peru, $8K^{P1}$ has stronger suppression of RNA silencing activity compared to the 8K protein of the rest of the isolates. The 8K protein of Swedish isolate, $8K^{SwH}$ showed weak VSR activity as reported previously (Lukhovitskaya et al., 2013). Some Peruvian isolates, $8K^{P118}$ and $8K^{P157}$ also showed weak VSR activity, followed by $8K^{P11}$, $8K^{P13}$, $8K^{P125}$ which showed weakest VSR activity among the isolates characterized (Figure 2, Paper II).

Interestingly, the $8K^{P125}$, one of the weakest VSR, differ only by two amino acid residues - $^{P1}Gly18Cys^{P125}$ and $^{P1}Ser50Asn^{P125}$ - from the $8K^{P1}$, a relatively strong VSR (Figure 1, Paper II). To identify the key amino acid residue contributing to the efficient RNA silencing suppression activity of $8K^{P1}$, we carried out site-directed mutagenesis in the $8K^{P125}$ coding sequence to generate two mutant alleles, *C18G* ($8K^{C18G}$) and *N50S* ($8K^{N50S}$), and evaluated the RNA silencing suppression activity. We found that the $8K^{N50S}$ allele has stronger RNA silencing suppression activity than $8K^{C18G}$ and $8K^{P125}$ (Figure 3, Paper II), suggesting that Ser-50 is critical for efficient VSR activity of the 8K protein.

Through multiple sequence analysis of 86 8K amino acid sequences, we identified that the 8K protein has a conserved C_{14} x C_{16} x_n C_{34} x C_{36} (where x denotes any amino acid) type SWIM zinc-finger motif (Figure 4, Paper I). To examine the importance of putative SWIM zinc-finger motif, we carried out site-directed mutagenesis at C_{34} and C_{36} to generate a mutant allele *C34A C36A*. Comparison the RNA silencing suppression activity of the wild-type 8K protein ($8K^{SwH}$) with the zinc-finger mutant ($8K^{C34A\ C36A}$), revealed that disruption of zinc-finger motif abolished the RNA silencing suppression activity, signifying that the integrity of the zinc-finger is essential for the VSR activity of the 8K protein (Figure 4, Paper II).

In order to get an insight into the mechanisms of 8K-mediated RNA silencing suppression, we carried out deep sequencing of small RNAs (sRNA) to compare the sRNA profiles between the 8K proteins of two PMTV isolates with contrasting VSR abilities, $8K^{P1}$, a moderate VSR, and $8K^{P125}$, a weak VSR. These proteins were transiently expressed in the *N. benthamiana* leaves together with GFP. Transient expression of an empty plasmid (EP) was used as a negative control, while, HcPro, a known strong VSR, as a positive control. Alignment of total reads obtained from the sRNA sequencing to the *GFP* transgene sequence indicated an overall reduction in the amount of GFP specific sRNA reads in the presence of VSRs (Figure 5, Paper II). The 21-nt class was most abundant (30-48%) siRNA class, followed by 22-nt (22-34%) and 24-nt (11-32%) sRNAs. There was an almost equal number of sense and antisense strands of siRNAs

distributed throughout the GFP transgene sequence. However, the amount of 22-nt sRNA class was slightly reduced in the presence of $8K^{P1}$ compared to the $8K^{P125}$ (Figure 5, Paper II). In order to validate the observed differences in the NGS data, we performed stem-loop RT-qPCR for detection of the antisense strand of sRNAs. To this end, we randomly selected six abundant 21-nt and 22-nt size class siRNAs scatted along GFP ORF sequence. The stem-loop RT-qPCR revealed that the expression of 22-nt sRNAs were significantly lower in the presence of VSRs (Figure 6, Paper II).

In plants, the 5'terminal nucleotide in the sRNAs directs the loading of them into specific AGO proteins, which is an important step in the functioning of the RISC. In Arabidopsis, it has been identified that the AGO1 preferentially binds to sRNAs with 5' terminal U residue (Mi et al., 2008). Our analysis revealed that in the presence of an empty plasmid control, U was the most abundant nucleotide at the 5' end, suggesting that these sRNAs are preferentially loaded into AGO1 containing RISC complex. Interestingly, this pattern was similar in the presence of weak VSR, $8K^{P125}$. However, in the presence of HcPro, and $8K^{P1}$ there were a reduction siRNAs with the U residue at their 5' end. Previously it has been shown that modifications in the 5'terminal nucleotide in the miRNA resulted in the failure of proper loading into the RISC, preventing the biological activity of the miRNA (Mi et al., 2008). The data of our study suggests that the $8K^{P1}$ protein and HcPro interfere with the RNA silencing pathway by interfering with AGO1 functioning. Interestingly, it has been shown that 22-nt miRNAs, but not 21-nt miRNAs bound to AGO1 recruit RDR6 to generate double-stranded RNA substrates for subsequent DCL processing, leading to the increased secondary siRNA production, and thus amplification of the signal (Schwab and Voinnet, 2010). Hence, it is tempting to hypothesize that VSRs such as HcPro and $8K^{P1}$ might destabilize the sRNAs with U at the 5'terminal end, inhibit their recruitment to AGO1, and thus prevent RDR6 recruitment. The observation of a reduction in 22-nt siRNAs, but not 21-nt siRNAs through stem-loop qRT-PCR further supports this hypothesis. Taken together, these results show several novel features of the VSR activity of the 8K protein and provides new insights on how variability and selection pressure modulate the activities of VSR.

3.3 Movement of potato mop-top virus

3.3.1 Role of the acto-myosin network in the cell-to-cell movement of PMTV (Paper III)

To address the role of acto-myosin network in the movement of PMTV, we inoculated the plants with a modified PMTV variant that expresses GFP-fused TGB1 and infiltrated with LatB, an actin depolymerizing agent. This disruption in the actin network led to the impaired cell-to-cell movement of the PMTV (Figure 1, Paper III). It is worth noting that the disruption of microtubular network using oryzalin or colchicine had no effect on the intercellular movement of the virus (Wright et al., 2010), suggesting that PMTV depends on the actin microfilaments for its cell to cell movement. Previously, it has been shown that the MPs of certain viruses, such as the 30K protein of TMV, TGB1 protein of PVX; and 2b of GFLV uses actin network for their cell-to-cell movement (Amari et al., 2014, 2011; Harries et al., 2009).

To assess the role of molecular motors behind the actin-mediated intercellular movement of PMTV, we used dominant negative inhibition constructs of six myosins belonging to two classes, VIII and XI. Transient expression of these dominant negative constructs in *N. benthamiana* leaves were carried out followed by inoculation with the PMTV.TGB1-GFP.

Our analysis revealed that there was a significant decrease in the size of infection foci area when certain class VIII myosins were inhibited while inhibiting class XI myosins did not have a significant effect (Figure 2, Paper III). Inhibition of Class VIII myosins drastically reduced the localization of TGB1-GFP protein to plasmodesmata. The presence of TGB1-GFP at the plasma membrane suggests that the intracellular movement of TGB1 was not affected. To examine if the efficiency of delivery of the TGB1 at the plasmodesmata is affected when the Class VIII myosins were inhibited, we performed Fluorescence Recovery After Photobleaching (FRAP) assay. As the name suggests, upon bleaching of the TGB1-GFP fluorescence at plasmodesmata, the rate with which the fluorescence is recovered reflects the efficiency of the TGB1-GFP movement. As expected, the recovery of TGB1-GFP fluorescence at the plasmodesmata was severely reduced upon overexpression of tails class VIII myosins (Figure 3, Paper III), indicating that class VIII myosins are required for efficient delivery of the TGB1 to the plasmodesmata.

Previously it was reported that both class XI and class VIII myosins are required for the intercellular movement of the TMV, however, inhibiting class

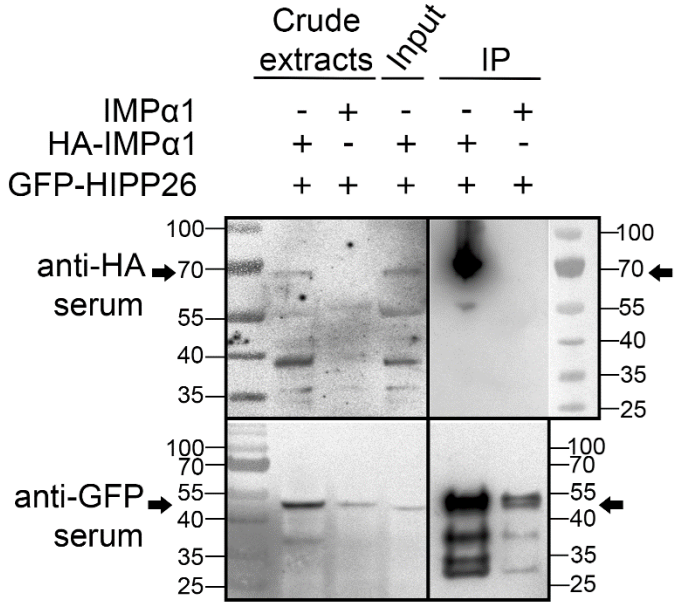
VIII myosins specifically resulted in the abolishment of plasmodesmata localization, suggesting that the class VIII myosins are specifically required for MP targeting and movement through the plasmodesmata (Amari et al., 2014). A similar result has been observed in a previous study with BYV MP, where class VIII myosins, but not class XI myosins resulted in inhibition of plasmodesmata localization (Avisar et al., 2008). Overall, these results suggest a specific role of class VIII myosins in the virus movement, probably, by altering the permeability of the plasmodesmata as suggested by Pitzalis and Heinlein (2018). This idea is further supported by the fact that inhibiting class VIII myosins had no effect on the tubule guided movement of GFLV, where the virus MPs transform the plasmodesmata into specialized tunnels, whereas inhibiting class XI myosins resulted in the impaired intercellular movement of the GFLV (Amari et al., 2011).

3.3.2 Role of HIPP26 in the long-distance movement of PMTV (Paper IV)

PMTV TGB1 plays an important role in the long-distance movement of PMTV. The Importin-α mediated nucleolar localization of TGB1 is necessary for the virus long-distance movement (Lukhovitskaya et al., 2015). However, the role of this nucleolar accumulation for the long-distance movement is not clearly understood. The yeast-two-hybrid screening of TGB1 with *N. benthamiana* cDNA library identified an interaction between the TGB1 and *N. benthamiana* HIPP26, a metallochaperone (Figure 2, Paper IV). The HIPP26 protein is unique to vascular plants, that act in the heavy metal homeostasis, regulating the transcriptional response to the biotic and abiotic stress (Barth et al., 2009; de Abreu-Neto et al., 2013).

The TGB1 interacts with the c-terminal prenyl motif, CVVM, of NbHIPP26. The bimolecular fluorescence complementation assay (BiFC) confirmed the interaction between the TGB1 and HIPP26 and revealed that this complex accumulates in the nucleolus, and associates with microtubules (Figure 3, Paper IV). Interestingly, the HIPP26, when expressed alone, does not localize to the microtubules (Figure 3, Paper IV). HIPP26 protein, like many other membrane-associated proteins that are involved in abiotic or biotic stresses, are modified posttranslationally by the addition of lipid moieties through a reversible linkage. Mutations in the lipidations domains resulted in weaker binding of HIPP26 to plasma membrane, suggesting that the lipidation is required to maintain the HIPP26 association with the membrane. Loss of association with membrane lead to increased accumulation of HIPP26 in the

nucleus and nucleolus. Co-immunoprecipitation (CoIP) showed that the HIPP26 protein interacts with the nuclear import protein, importin-α (unpublished results) (Figure 9), suggesting that Importin-α mediates the nucleolar localization of the HIPP26. Taken together, our results support a model where the TGB1 interacts with HIPP26 at the C-terminal prenyl motif (Figure 2, Paper IV), reversing its association with the plasma membrane. Following that, the



TGB1 translocates HIPP26 to the nucleus via cytoskeletal components.

Figure 9. CoIP of extracts from *N. benthamiana* leaves co-infiltrated with GFP-TGB1 and HA-IMPα1, or IMPα1 and GFP-TGB1, using anti-GFP microbeads, followed by immunoblot analysis with anti-HA and anti- GFP antibodies. The coexpression of nonfused IMPα1 and GFP-TGB1 was used as a control in the CoIP experiment.

To test the importance of the HIPP26 in the long-distance movement of the virus, Tobacco Rattle Virus (TRV) based virus-induced gene silencing (VIGS) vectors were used to knock down the expression of NbHIPP26. The knock down of *NbHIPP26* was confirmed using RT-qPCR. The *NbHIPP26* silenced plants were then inoculated with PMTV. Two weeks post inoculation RNA was extracted from the upper non-inoculated leaves, and subsequently used to quantify the viral RNA. We detected a reduced accumulation of all three RNA segments of PMTV in the upper leaves upon knock down of *NbHIPP26*, suggesting that NbHIPP26 is necessary for the virus long-distance movement. Quantification of PMTV accumulation in the leaves by ELISA revealed a

significant reduction in the virus accumulation in the systemically-infected leaves as compared to the control plants (Figure 9, Paper IV) further supporting the idea that the HIPP26 is necessary for the virus long-distance movement. However, quantification of PMTV accumulation in inoculated leaves by ELISA revealed no difference in the viral accumulation upon silencing of *NbHIPP26*, suggesting that TGB1-NbHIPP26 interaction is required for the systemic movement, but not cell-to-cell movement of PMTV.

Interestingly, PMTV infection resulted in increased drought tolerance in the *N. benthamiana* plants, suggesting a possible role of TGB1-HIPP26 association in activating the drought response (Figure 8, Paper IV). It was shown that in *Arabidopsis*, HIPP26 interacts with a transcriptional activator, ZFHD1 in the nucleus, thereby regulating its response to the stress (Barth et al., 2009). It is hypothesized that nuclear accumulation of the TGB1-HIPP26 complex triggers the activation of ATHB29 transcription factor and thereby initiates transcription of drought response genes, even under normal, non-drought conditions.

4 Concluding remarks

The findings of this thesis contribute to a better understanding of PMTV variability and its interactions with the host. The main findings include:

- PMTV has high genetic variability in the Andean region of Peru.
- Based on the phylogenetic analyses, and the pathobiological differences, our work shows that the RNA-CP segment of all the isolates sequenced so far can be grouped into two genotypes: S-type (Severe) and M-type (Mild).
- All of the previously characterized isolates from Europe, Asia, and North America belong to S-type, along with some newly characterized isolates from Peru. M-type, so far was found in Peru.
- We suggested a novel classification of PMTV isolates based genetic constellations.
- Our findings establish that PMTV has undergone continued evolutionary divergence in Peru.
- The ORF encoding 8K protein is under positive selection.
- Through characterization of RNA silencing suppression activity of diverse 8K variants, we identified $8K^{P1}$ as a much stronger VSR compared to other natural variants of 8K. Mutants of the weak *P125* allele allowed us to identify that Ser-50 is critical for the activity.
- Comparison of small RNA profiles upon transient expression of *P1* and *P125* alleles in *N. benthamiana* plants revealed lower accumulation of certain classes of siRNAs the presence of $8K^{P1}$.
- Our findings set new grounds for future research to address the mechanism of the $8K^{P1}$ suppressor activity. This study also provides new insights on how genetic variability and positive selection modulate the activities of VSRs.
- We demonstrated that PMTV utilizes the acto-myosin network for the cell-to-cell movement.

- Our analysis indicates that two myosins, namely, VIII-1 and VIII-B from the class-VIII family, play a major role in the intercellular movement of PMTV.
- Although class XI myosins had no effect in the intercellular movement of PMTV, knockdown of *NbMyosin XI-K* expression indicates that this myosin might have a functional role in the long-distance movement of the virus. However, this data must be interpreted with caution as knockdown of individual myosin gene expression often influenced expression of other myosin genes, probably due to the high level of redundancy among the myosin genes.
- Further research is needed to clarify the role of acto-myosin network in the movement of PMTV.
- TGB1 protein, a major protein facilitating the long-distance movement of PMTV, interacts with HIPP26, a vascular-expressed plant stress sensor, which acts as signal from plasma membrane-to-nucleus during abiotic stress.
- Our results indicate that the interaction between TGB1 and HIPP26 reverses the association of HIPP26 with the plasma membrane, followed by translocation of HIPP26 to the nucleus via microtubules.
- Knockdown of *NbHIPP26* expression resulted in the inhibition of PMTV long-distance movement.
- We demonstrated that PMTV infection leads to increased drought tolerance in *N. benthamiana*.
- Based on our results, we propose a model where the nuclear accumulation of the TGB1-HIPP26 complex induces the expression of dehydration-responsive genes in the vasculature, even under normal irrigation conditions, establishing a drought-tolerant state. These changes also allow the virus particles or RNPs enter the phloem for their long-distance movement.

References

Abreu-Neto, João Braga de, Andreia C. Turchetto-Zolet, Luiz Felipe Valter de Oliveira, Maria Helena Bodanese Zanettini, and Marcia Margis-Pinheiro. 2013. “Heavy Metal-Associated Isoprenylated Plant Protein (HIPP): Characterization of a Family of Proteins Exclusive to Plants.” *FEBS Journal* 280 (7): 1604–16. https://doi.org/10.1111/febs.12159.

Adams, J M, F J and Antoniw, and L J G Mullins. 2001. “Plant Virus Transmission by Plasmodiophorid Fungi Is Associated with Distinctive Transmembrane Regions of Virus-Encoded Proteins.” *Archives of Virology* 146 (6): 1139–53. https://doi.org/10.1007/s007050170111.

Adams, Michael J., Scott Adkins, Claude Bragard, David Gilmer, Dawei Li, Stuart A. MacFarlane, Sek-Man Wong, et al. 2017. “ICTV Virus Taxonomy Profile: Virgaviridae.” *Journal of General Virology* 98 (8): 1999–2000. https://doi.org/10.1099/jgv.0.000884.

Adams, Michael J, C Heinze, A Jackson, Jan F Kreuze, S Macfarlane, and Lesley Torrance. 2012. “Family Virgaviridae.” In *Virus Taxonomy: Classification and Nomenclature of Viruses - Ninth Report of the International Committee on Taxonomy of Viruses*, edited by AMQ King, MJ Adams, EB Carstens, and EJ Lefkowitz, 1139–62. Burlington: Elsevier Academic Press.

Amari, Khalid, Emmanuel Boutant, Christina Hofmann, Corinne Schmitt-Keichinger, Lourdes Fernandez-Calvino, Pascal Didier, Alexander Lerich, et al. 2010. “A Family of Plasmodesmal Proteins with Receptor-Like Properties for Plant Viral Movement Proteins.” Edited by Peter Nagy. *PLoS Pathogens* 6 (9): e1001119. https://doi.org/10.1371/journal.ppat.1001119.

Amari, Khalid, Martin Di Donato, Valerian V Dolja, and Manfred Heinlein. 2014. “Myosins VIII and XI Play Distinct Roles in Reproduction and Transport of Tobacco Mosaic Virus.” https://doi.org/10.1371/journal.ppat.1004448.

Amari, Khalid, Alexander Lerich, Corinne Schmitt-Keichinger, Valerian V Dolja, and Christophe Ritzenthaler. 2011. “Tubule-Guided Cell-to-Cell Movement of a Plant Virus Requires Class XI Myosin Motors.” *PLoS Pathog* 7 (10). https://doi.org/10.1371/journal.ppat.1002327.

Andrianifahanana, Mahefa, Karrie Lovins, Roland Dute, Edward Sikora, and John F. Murphy. 1997. “Pathway for Phloem-Dependent Movement of Pepper Mottle Potyvirus in the Stem of *Capsicum Annuum*.” *Phytopathology* 87 (9): 892–98. https://doi.org/10.1094/PHYTO.1997.87.9.892.

Arif, Muhammad, Murad Ali, Anayatur Rehman, and Muhammad Fahim. 2014. “Detection of Potato Mop-Top Virus in Soils and Potato Tubers Using Bait-Plant Bioassay, ELISA and RT-PCR.” *Journal of Virological Methods* 195. https://doi.org/10.1016/j.jviromet.2013.10.022.

Atabekov, J.G., and Yu. L. Dorokhov. 1984. “Plant Virus-Specific Transport Function And Resistance

Of Plants To Viruses." *Advances in Virus Research* 29 (January): 313–64. https://doi.org/10.1016/S0065-3527(08)60412-1.

Avisar, Dror, Alexey I Prokhnevsky, and Valerian V Dolja. 2008. "Class VIII Myosins Are Required for Plasmodesmatal Localization of a Closterovirus Hsp70 Homolog." *JOURNAL OF VIROLOGY* 82 (6): 2836–43. https://doi.org/10.1128/JVI.02246-07.

Bajczyk, Mateusz, Susheel Sagar Bhat, Lukasz Szewc, Zofia Szweykowska-Kulinska, Artur Jarmolowski, and Jakub Dolata. 2019. "Novel Nuclear Functions of Arabidopsis ARGONAUTE1: Beyond RNA Interference." *Plant Physiology* 179 (3): 1030–39. https://doi.org/10.1104/pp.18.01351.

Bamunusinghe, D., S. Chaturvedi, J.-K. Seo, and A. L. N. Rao. 2013. "Mutations in the Capsid Protein of Brome Mosaic Virus Affecting Encapsidation Eliminate Vesicle Induction In Planta: Implications for Virus Cell-to-Cell Spread." *Journal of Virology* 87 (16): 8982–92. https://doi.org/10.1128/jvi.01253-13.

Barth, Olaf, Sebastian Vogt, Ria Uhlemann, Wiebke Zschiesche, and Klaus Humbeck. 2009. "Stress Induced and Nuclear Localized HIPP26 from Arabidopsis Thaliana Interacts via Its Heavy Metal Associated Domain with the Drought Stress Related Zinc Finger Transcription Factor ATHB29." *Plant Molecular Biology* 69 (1–2): 213–26. https://doi.org/10.1007/s11103-008-9419-0.

Baumberger, Nicolas, Ching-Hsui Tsai, Miranda Lie, Ericka Havecker, and David C. Baulcombe. 2007. "The Polerovirus Silencing Suppressor P0 Targets ARGONAUTE Proteins for Degradation." *Current Biology* 17 (18): 1609–14. https://doi.org/10.1016/j.cub.2007.08.039.

Belin, Christophe, Corinne Schmitt, Fabien Gaire, Bernard Walter, Ge ! Rard Demangeat, and Lothaire Pinck. 1999. "Printed in Great Britain The Nine C-Terminal Residues of the Grapevine Fanleaf Nepovirus Movement Protein Are Critical for Systemic Virus Spread." *Journal of General Virology*. Vol. 80. www.microbiologyresearch.org.

Bennett, C W. 1940. "Acquisition and Transmission of Viruses by Dodder (Cuscuta Subinclusa)." *Phytopathology* 30:2.

Bertens, P., W. Heijne, N. Van der Wel, J. Wellink, and A. Van Kammen. 2003. "Studies on the C-Terminus of the Cowpea Mosaic Virus Movement Protein." *Archives of Virology* 148 (2): 265–79. https://doi.org/10.1007/s00705-002-0918-z.

Beuch, Ulrike. 2013. *Distribution and Diversity of Potato Mop-Top Virus in Sweden*. https://pub.epsilon.slu.se/10852/1/beuch_u_131015.pdf.

Beuch, Ulrike, Sofia Berlin, Jonas Åkerblom, Mogens Nicolaisen, Steen Lykke Nielsen, James M Crosslin, Philip B Hamm, Johanna Santala, Jari P T Valkonen, and Anders Kvarnheden. 2015. "Diversity and Evolution of Potato Mop-Top Virus." *Archives of Virology* 160 (5): 1345–51. https://doi.org/10.1007/s00705-015-2381-7.

Björnstad, A. 1969. "Spredning Av Potet-Mop-Topp-Virus (PMTV) Med Settepotater." *Jord Og Avling* 2: 2–4.

Blume, Yaroslav B., Yuliya A. Krasylenko, and Alla I. Yemets. 2017. "The Role of the Plant Cytoskeleton in Phytohormone Signaling under Abiotic and Biotic Stresses." In *Mechanism of Plant Hormone Signaling under Stress*, 127–85. Hoboken, NJ, USA: John Wiley & Sons, Inc. https://doi.org/10.1002/9781118889022.ch23.

Bortolamiol, Diane, Maghsoud Pazhouhandeh, Katia Marrocco, Pascal Genschik, and Véronique Ziegler-Graff. 2007. "The Polerovirus F Box Protein P0 Targets ARGONAUTE1 to Suppress RNA Silencing." *Current Biology* 17 (18): 1615–21. https://doi.org/10.1016/j.cub.2007.07.061.

Bos, L. 1999. *Plant Viruses, Unique and Intriguing Pathogens - a Textbook of Plant Virology*. Backhuys

Publishers.

Bouzoubaa, S., V. Ziegler, D. Beck, H. Guilley, K. Richards, and G. Jonard. 1986. “Nucleotide Sequence of Beet Necrotic Yellow Vein Virus RNA-2.” *Journal of General Virology* 67 (8): 1689–1700. https://doi.org/10.1099/0022-1317-67-8-1689.

Boyko, Vitaly, Jacqueline Ferralli, Jamie Ashby, Paul Schellenbaum, and Manfred Heinlein. 2000. “Function of Microtubules in Intercellular Transport of Plant Virus RNA.” *Nature Cell Biology*. Vol. 2. https://doi.org/10.1038/35041072.

Boyko, Vitaly, Quanan Hu, Mark Seemanpillai, Jamie Ashby, and Manfred Heinlein. 2007. “Validation of Microtubule-Associated Tobacco Mosaic Virus RNA Movement and Involvement of Microtubule-Aligned Particle Trafficking.” *The Plant Journal* 51 (4): 589–603. https://doi.org/10.1111/j.1365-313X.2007.03163.x.

Brodersen, P, L Sakvarelidze-Achard, M Bruun-Rasmussen, P Dunoyer, Y Y Yamamoto, L Sieburth, and O Voinnet. 2008. “Widespread Translational Inhibition by Plant MiRNAs and SiRNAs.” *Science* 320 (5880): 1185–90. https://doi.org/10.1126/science.1159151.

Budziszewska, M., P. Wieczorek, K. Nowaczyk, N. Borodynko, H. Pospieszny, and A. Obrêpalska-Stêplowska. 2010. “First Report of *Potato Mop-Top Virus* on Potato in Poland.” *Plant Disease* 94 (7): 920–920. https://doi.org/10.1094/PDIS-94-7-0920A.

Burckhardt, Christoph J., and Urs F. Greber. 2009. “Virus Movements on the Plasma Membrane Support Infection and Transmission between Cells.” Edited by Marianne Manchester. *PLoS Pathogens* 5 (11): e1000621. https://doi.org/10.1371/journal.ppat.1000621.

Burgyán, József, and Zoltán Havelda. 2011. “Viral Suppressors of RNA Silencing.” *Trends in Plant Science* 16 (5): 265–72. https://doi.org/10.1016/j.tplants.2011.02.010.

Byszewska, Magdalena, Miroslaw Smieta Nski, El_ Zbieta Purta, and Janusz M Bujnicki. 2014. “RNA Methyltransferases Involved in 5’ Cap Biosynthesis.” *RNA Biology* 11 (12): 1597–1607. https://doi.org/10.1080/15476286.2015.1004955.

Calvert, E. L., and B. D. Harrison. 1966. “Potato Mop-Top, a Soil-Borne Virus.” *Plant Pathology* 15 (3): 134–39. https://doi.org/10.1111/j.1365-3059.1966.tb00333.x.

Calvert, E.L. 1968. “The Reaction of Potato Varieties to Potato Mop-Top Virus.” *Record of Agricultural Research, Ministry of Agriculture, Northern Ireland* 17 (1): 31–40.

Carmen Herranz, Mari, Vicente Pallas, and Frederic Aparicio. 2009. “Emerging Concepts in Effector Biology.” *Molecular Plant-Microbe Interactions* 25 (8): 1093–1103. https://doi.org/10.1094/mpmi.

Carnegie, S. F., A. M. Cameron, and M. McCreath. 2010. “Foliar Symptoms Caused by Potato Mop-Top Virus on Potato Plants During Vegetative Propagation in Scotland and Their Association With Tuber Yield, Spraing and Tuber Infection.” *Potato Research* 53 (2): 83–93. https://doi.org/10.1007/s11540-010-9153-2.

Carr, John P., Mathew G. Lewsey, and Peter Palukaitis. 2010. “Signaling in Induced Resistance.” *Advances in Virus Research* 76 (January): 57–121. https://doi.org/10.1016/S0065-3527(10)76003-6.

Carrington, James C, Kristin D Kasschau, Sunita K Mahajan, and Mary C Schaad. 1996. “Cell-to-Cell and Long-Distance Transport of Viruses in Plants.” *The Plant Cell*. Vol. 8. American Society of Plant Physiologists. http://www.plantcell.org/content/plantcell/8/10/1669.full.pdf.

Chen, Cheng, Yan Zhang, Lei Zhu, and Ming Yuan. 2010. “The Actin Cytoskeleton Is Involved in the Regulation of the Plasmodesmal Size Exclusion Limit.” *Plant Signaling & Behavior* 5 (12): 1663–65. https://doi.org/10.4161/psb.5.12.14018.

Chen, H Y, J Yang, C Lin, and Y A Yuan. 2008. “Structural Basis for RNA-Silencing Suppression by Tomato Aspermy Virus Protein 2b.” *EMBO Rep* 9 (8): 754–60. https://doi.org/10.1038/embor.2008.118.

Cheng, Ning-Hui, Chun-Lin Su, Shelly A. Carter, and Richard S. Nelson. 2000. “Vascular Invasion Routes and Systemic Accumulation Patterns of Tobacco Mosaic Virus in *Nicotiana Benthamiana*.” *The Plant Journal* 23 (3): 349–62. https://doi.org/10.1046/j.1365-313x.2000.00788.x.

Chinnusamy, Viswanathan, Jianjun Zhu, Tao Zhou, and Jian-Kang Zhu. 2007. “Small Rnas: Big Role In Abiotic Stress Tolerance Of Plants.” In *Advances in Molecular Breeding Toward Drought and Salt Tolerant Crops*, 223–60. Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-1-4020-5578-2_10.

Chiu, Meng-Hsuen, I-Hsuan Chen, David C. Baulcombe, and Ching-Hsiu Tsai. 2010. “The Silencing Suppressor P25 of Potato Virus X Interacts with Argonaute1 and Mediates Its Degradation through the Proteasome Pathway.” *Molecular Plant Pathology* 11 (5): no-no. https://doi.org/10.1111/j.1364-3703.2010.00634.x.

Citovsky, V, M L Wong, A L Shaw, B V Prasad, and P Zambryski. 1992. “Visualization and Characterization of Tobacco Mosaic Virus Movement Protein Binding to Single-Stranded Nucleic Acids.” *The Plant Cell* 4 (4): 397–411. https://doi.org/10.1105/tpc.4.4.397.

Cowan, G H, L Torrance, and B Reavy. 1997. “Detection of Potato Mop-Top Virus Capsid Readthrough Protein in Virus Particles.” *Journal of General Virology* 78 (7): 1779–83. https://doi.org/10.1099/0022-1317-78-7-1779.

Cowan, Graham H, Alison G Roberts, Sean N Chapman, Angelika Ziegler, Eugene I Savenkov, and Lesley Torrance. 2012. “The Potato Mop-Top Virus TGB2 Protein and Viral RNA Associate with Chloroplasts and Viral Infection Induces Inclusions in the Plastids.” *Frontiers in Plant Science* 3 (December): 290. https://doi.org/10.3389/fpls.2012.00290.

Csorba, Tibor, Levente Kontra, and József Burgyán. 2015. “Viral Silencing Suppressors: Tools Forged to Fine-Tune Host-Pathogen Coexistence.” *Virology* 479–480 (May): 85–103. https://doi.org/10.1016/j.virol.2015.02.028.

Dalmay, T, A Hamilton, S Rudd, S Angell, and D C Baulcombe. 2000. “An RNA-Dependent RNA Polymerase Gene in Arabidopsis Is Required for Posttranscriptional Gene Silencing Mediated by a Transgene but Not by a Virus.” *Cell* 101 (5): 543–53. https://www.ncbi.nlm.nih.gov/pubmed/10850496.

Day, Brad, Jessica L Henty, Katie J Porter, and Christopher J Staiger. 2011. “The Pathogen-Actin Connection: A Platform for Defense Signaling in Plants.” *Annu. Rev. Phytopathol* 49: 483–506. https://doi.org/10.1146/annurev-phyto-072910-095426.

Deleris, A, J Gallego-Bartolome, J Bao, K D Kasschau, J C Carrington, and O Voinnet. 2006. “Hierarchical Action and Inhibition of Plant Dicer-like Proteins in Antiviral Defense.” *Science* 313 (5783): 68–71. https://doi.org/10.1126/science.1128214.

Diaz-Pendon, Juan A., Feng Li, Wan-Xiang Li, and Shou-Wei Ding. 2007. “Suppression of Antiviral Silencing by Cucumber Mosaic Virus 2b Protein in *Arabidopsis* Is Associated with Drastically Reduced Accumulation of Three Classes of Viral Small Interfering RNAs.” *The Plant Cell* 19 (6): 2053–63. https://doi.org/10.1105/tpc.106.047449.

Ding, B. 1998. “Intercellular Protein Trafficking through Plasmodesmata.” *Plant Molecular Biology* 38 (1–2): 279–310. http://www.ncbi.nlm.nih.gov/pubmed/9738972.

Ding, Biao, Myoung-Ok Kwon, and Leif Warnberg. 1996. “Evidence That Actin Filaments Are Involved

in Controlling the Permeability of Plasmodesmata in Tobacco Mesophyll." *The Plant Journal*. Vol. 10. http://www.botanik.kit.edu/botzell/downloads/intern_Ding_et_al_1996.pdf.

Ding, Shou-Wei, and Olivier Voinnet. 2007. "Antiviral Immunity Directed by Small RNAs." *Cell* 130 (3): 413–26. https://doi.org/10.1016/j.cell.2007.07.039.

Dolja, V V, R Haldeman, N L Robertson, W G Dougherty, and J C Carrington. 1994. "Distinct Functions of Capsid Protein in Assembly and Movement of Tobacco Etch Potyvirus in Plants." *The EMBO Journal* 13 (6): 1482–91. http://www.ncbi.nlm.nih.gov/pubmed/7511101.

Dolja, Valerian V., Ruth Haldeman-Cahill, Amanda E. Montgomery, Kathryn A. Vandenbosch, and James C. Carrington. 1995. "Capsid Protein Determinants Involved in Cell-to-Cell and Long Distance Movement of Tobacco Etch Potyvirus." *Virology* 206 (2): 1007–16. https://doi.org/10.1006/viro.1995.1023.

Donaire, Livia, Daniel Barajas, Belén Martínez-García, Llucia Martínez-Priego, Israel Pagán, and César Llave. 2008. "Structural and Genetic Requirements for the Biogenesis of Tobacco Rattle Virus-Derived Small Interfering RNAs." *Journal of Virology* 82 (11): 5167–77. https://doi.org/10.1128/JVI.00272-08.

Ehlers, K., and R. Kollmann. 2001. "Primary and Secondary Plasmodesmata: Structure, Origin, and Functioning." *Protoplasma* 216 (1–2): 1–30. https://doi.org/10.1007/BF02680127.

Elvira, M. I., M. M. Galdeano, P. Gilardi, I. Garcia-Luque, and M. T. Serra. 2008. "Proteomic Analysis of Pathogenesis-Related Proteins (PRs) Induced by Compatible and Incompatible Interactions of Pepper Mild Mottle Virus (PMMoV) in Capsicum Chinense L3 Plants." *Journal of Experimental Botany* 59 (6): 1253–65. https://doi.org/10.1093/jxb/ern032.

Epel, Bernard L. 2009. "Plant Viruses Spread by Diffusion on ER-Associated Movement-Protein-Rafts through Plasmodesmata Gated by Viral Induced Host β-1,3-Glucanases." *Seminars in Cell & Developmental Biology* 20 (9): 1074–81. https://doi.org/10.1016/J.SEMCDB.2009.05.010.

Fagard, M, S Boutet, J B Morel, C Bellini, and H Vaucheret. 2000. "AGO1, QDE-2, and RDE-1 Are Related Proteins Required for Post-Transcriptional Gene Silencing in Plants, Quelling in Fungi, and RNA Interference in Animals." *Proc Natl Acad Sci U S A* 97 (21): 11650–54. https://doi.org/10.1073/pnas.200217597.

Fang, Yuan-Yuan, Jian-Hua Zhao, Shang-Wu Liu, Sheng Wang, Cheng-Guo Duan, and Hui-Shan Guo. 2016. "CMV2b-AGO Interaction Is Required for the Suppression of RDR-Dependent Antiviral Silencing in Arabidopsis." *Frontiers in Microbiology* 7: 1329. https://doi.org/10.3389/fmicb.2016.01329.

Faulkner, Christine, Ozgur E Akman, Karen Bell, Chris Jeffree, and Karl Oparka. 2008. "Peeking into Pit Fields: A Multiple Twinning Model of Secondary Plasmodesmata Formation in Tobacco W." Accessed August 3, 2019. https://doi.org/10.1105/tpc.107.056903.

Fire, Andrew, SiQun Xu, Mary K Montgomery, Steven A Kostas, Samuel E Driver, and Craig C Mello. 1998. "Potent and Specific Genetic Interference by Double-Stranded RNA in Caenorhabditis Elegans." *Nature* 391 (February): 806. http://dx.doi.org/10.1038/35888.

Foxe, M.J. 1980. "An Investigation of the Distribution of Potato Mop-Top Virus in Country Donegal." *Journal of Life Sciences, Royal Dublin Society* 1 (2): 149–55.

Fridborg, Ingela, Jef Grainger, Anthony Page, Mark Coleman, Kim Findlay, and Susan Angell. 2003. "TIP, A Novel Host Factor Linking Callose Degradation with the Cell-to-Cell Movement of *Potato Virus X*." *Molecular Plant-Microbe Interactions* 16 (2): 132–40. https://doi.org/10.1094/MPMI.2003.16.2.132.

Garcia-Ruiz, Hernan, Atsushi Takeda, Elisabeth J. Chapman, Christopher M. Sullivan, Noah Fahlgren,

Katherine J. Brempelis, and James C. Carrington. 2010. "*Arabidopsis* RNA-Dependent RNA Polymerases and Dicer-Like Proteins in Antiviral Defense and Small Interfering RNA Biogenesis during *Turnip Mosaic Virus* Infection." *The Plant Cell* 22 (2): 481–96. https://doi.org/10.1105/tpc.109.073056.

Gau, Rebecca D., Ueli Merz, Richard E. Falloon, and Patrick C. Brunner. 2013. "Global Genetics and Invasion History of the Potato Powdery Scab Pathogen, Spongospora Subterranea f.Sp. Subterranea." Edited by Nadia Singh. *PLoS ONE* 8 (6): e67944. https://doi.org/10.1371/journal.pone.0067944.

Gibbs, Adrian. 1976. "Viruses and Plasmodesmata." In *Intercellular Communication in Plants: Studies on Plasmodesmata*, 149–64. Berlin, Heidelberg: Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-66294-2_8.

Gil, J. F., I. Adams, N. Boonham, S. L. Nielsen, and M. Nicolaisen. 2016. "Molecular and Biological Characterization of *Potato Mop-Top Virus* (PMTV, *Pomovirus*) Isolates from the Potato-Growing Regions of Colombia." *Plant Pathology* 65 (7): 1210–20. https://doi.org/10.1111/ppa.12491.

Gil, J. F., Pablo A. Gutiérrez, José M. Cotes, Elena P. González, and Mauricio Marín. 2011. "Genotypic Characterization of Colombian Isolates of Potato Mop-Top Virus (Pmtv, Pomovirus)." *Actualidades Biológicas* 33 (94): 69–84. http://www.scielo.org.co/scielo.php?pid=S0304-35842011000100005&script=sci_arttext&tlng=pt.

Glick, Efrat, Avi Zrachya, Yael Levy, Anahit Mett, David Gidoni, Eduard Belausov, Vitaly Citovsky, and Yedidya Gafni. 2008. "Interaction with Host SGS3 Is Required for Suppression of RNA Silencing by Tomato Yellow Leaf Curl Virus V2 Protein." *Proceedings of the National Academy of Sciences of the United States of America* 105 (1): 157–61. https://doi.org/10.1073/pnas.0709036105.

Goddard, R. H., S. M. Wick, C. D. Silflow, and D. P. Snustad. 1994. "Microtubule Components of the Plant Cell Cytoskeleton." *Plant Physiology*. Vol. 104. https://doi.org/10.1104/pp.104.1.1.

Gorovits, Rena, Fouad Akad, Hila Beery, Favi Vidavsky, Assaf Mahadav, and Henryk Czosnek. 2007. "Expression of Stress-Response Proteins Upon Whitefly-Mediated Inoculation of *Tomato Yellow Leaf Curl Virus* in Susceptible and Resistant Tomato Plants." *Molecular Plant-Microbe Interactions* 20 (11): 1376–83. https://doi.org/10.1094/MPMI-20-11-1376.

Gosalvez-bernal, Blanca, Ainhoa Genoves, Jose Antonio navarro, Vicente Pallas, and m. Amelia Sanchez-pina. 2008. "Distribution and Pathway for Phloem-Dependent Movement of Melon Necrotic Spot Virus in Melon Plants." *Molecular Plant Pathology* 9 (4): 447–61. https://doi.org/10.1111/j.1364-3703.2008.00474.x.

Government of NZ. 2018. "Potato Mop-Top Virus | Biosecurity NZ | NZ Government." 2018. https://www.biosecurity.govt.nz/protection-and-response/responding/alerts/potato-mop-top-virus/.

Guo, Hongyan, Xiaoguang Song, Chuanmiao Xie, Yan Huo, Fujie Zhang, Xiaoying Chen, Yunfeng Geng, and Rongxiang Fang. 2013. "*Rice Yellow Stunt Rhabdovirus* Protein 6 Suppresses Systemic RNA Silencing by Blocking RDR6-Mediated Secondary SiRNA Synthesis." *Molecular Plant-Microbe Interactions* 26 (8): 927–36. https://doi.org/10.1094/MPMI-02-13-0040-R.

Guo, Zhongxin, Yang Li, and Shou-Wei Ding. 2019. "Small RNA-Based Antimicrobial Immunity." *Nature Reviews Immunology* 19 (1): 31–44. https://doi.org/10.1038/s41577-018-0071-x.

Haas, G, J Azevedo, G Moissiard, A Geldreich, C Himber, M Bureau, T Fukuhara, M Keller, and O Voinnet. 2008. "Nuclear Import of CaMV P6 Is Required for Infection and Suppression of the

RNA Silencing Factor DRB4." *EMBO J* 27 (15): 2102–12. https://doi.org/10.1038/emboj.2008.129.

Hamilton, A J, and D C Baulcombe. 1999. "A Species of Small Antisense RNA in Posttranscriptional Gene Silencing in Plants." *Science* 286 (5441): 950–52. https://www.ncbi.nlm.nih.gov/pubmed/10542148.

Harries, Phillip A., J.-W. Park, N. Sasaki, K. D. Ballard, A. J. Maule, and R. S. Nelson. 2009. "Differing Requirements for Actin and Myosin by Plant Viruses for Sustained Intercellular Movement." *Proceedings of the National Academy of Sciences* 106 (41): 17594–99. https://doi.org/10.1073/pnas.0909239106.

Harrison, B. D., and R. A. C. Jones. 1971. "Factors Affecting the Development of Spraing in Potato Tubers Infected with Potato Mop-Top Virus." *Annals of Applied Biology* 68 (3): 281–89. https://doi.org/10.1111/j.1744-7348.1971.tb04647.x.

Haupt, W Sophie, Graham H Cowan, Angelika Ziegler, Alison G Roberts, Karl J Oparka, and Lesley Torrance. 2005. "Two Plant–Viral Movement Proteins Traffic in the Endocytic Recycling Pathway." *The Plant Cell* 17: 164–81. https://doi.org/10.1105/tpc.104.027821.

Hepler, P. K. 1982. "Endoplasmic Reticulum in the Formation of the Cell Plate and Plasmodesmata." *Protoplasma* 111 (2): 121–33. https://doi.org/10.1007/BF01282070.

Hinostroza, Ana Maria, and E. R. French. 1972. "Potato Mop-Top Virus in Cork-Diseased Peruvian Potatoes." *American Potato Journal* 49 (6): 234–39. https://doi.org/10.1007/BF02864833.

Hiraguri, A, R Itoh, N Kondo, Y Nomura, D Aizawa, Y Murai, H Koiwa, M Seki, K Shinozaki, and T Fukuhara. 2005. "Specific Interactions between Dicer-like Proteins and HYL1/DRB-Family DsRNA-Binding Proteins in Arabidopsis Thaliana." *Plant Mol Biol* 57 (2): 173–88. https://doi.org/10.1007/s11103-004-6853-5.

Hofmann, Christina, Annette Niehl, Adrian Sambade, André Steinmetz, and Manfred Heinlein. 2009. "Inhibition of Tobacco Mosaic Virus Movement by Expression of an Actin-Binding Protein." *Plant Physiology* 149 (4): 1810–23. https://doi.org/10.1104/pp.108.133827.

Holland, John, Katherine Spindler, Frank Horodyski, Elizabeth Grabau, Stuart Nichol, and Scott Vandepol. 1982. "Rapid Evolution of RNA Genomes." *Source: Science, New Series*. Vol. 215. https://www.jstor.org/stable/pdf/1687612.pdf?refreqid=excelsior%3Aeaa449a58b73423f085f2733c1785c15.

Hollander, P W den, S N Kieper, J W Borst, and J W M van Lent. 2016. "The Role of Plasmodesma-Located Proteins in Tubule-Guided Virus Transport Is Limited to the Plasmodesmata." *Archives of Virology* 161 (9): 2431–40. https://doi.org/10.1007/s00705-016-2936-2.

Hoof, A.A. van, and A. Rozendaal. 1969. "Het Voorkomen van ´Potato Mop-Top Virus´ in Nederland." *Netherlands Journal of Plant Pathology* 75 (1): 275.

Howard, Amanda R., Marty L. Heppler, Ho-Jong Ju, Konduru Krishnamurthy, Mark E. Payton, and Jeanmarie Verchot-Lubicz. 2004. "Potato Virus X TGBp1 Induces Plasmodesmata Gating and Moves between Cells in Several Host Species Whereas CP Moves Only in N. Benthamiana Leaves." *Virology* 328 (2): 185–97. https://doi.org/10.1016/j.virol.2004.06.039.

Hu, Xinxi, Virginia Dickison, Yan Lei, Changzheng He, Mathuresh Singh, Yanli Yang, Xingyao Xiong, and Xianzhou Nie. 2016. "Molecular Characterization of *Potato Mop-Top Virus* Isolates from China and Canada and Development of RT-PCR Differentiation of Two Sequence Variant Groups." *Canadian Journal of Plant Pathology* 38 (2): 231–42. https://doi.org/10.1080/07060661.2016.1189968.

Huang, Zhong, Yu Han, and Stephen H. Howell. 2001. "Effects of Movement Protein Mutations on the

Formation of Tubules in Plant Protoplasts Expressing a Fusion Between the Green Fluorescent Protein and *Cauliflower Mosaic Virus* Movement Protein." *Molecular Plant-Microbe Interactions* 14 (8): 1026–31. https://doi.org/10.1094/MPMI.2001.14.8.1026.

Hull, Roger. 2013a. "Movement of Viruses Within Plants." In *Plant Virology*. https://doi.org/10.1016/b978-0-12-384871-0.00010-8.

Hull, Roger. 2013b. "Virus–Plant Interactions." In *Plant Virology*. https://doi.org/10.1016/b978-0-12-384871-0.00009-1.

Iglesias, Victor Alejandro, and Frederick Meins. 2000. "Movement of Plant Viruses Is Delayed in a Beta-1,3-Glucanase-Deficient Mutant Showing a Reduced Plasmodesmatal Size Exclusion Limit and Enhanced Callose Deposition." *The Plant Journal* 21 (2): 157–66. https://doi.org/10.1046/j.1365-313x.2000.00658.x.

Iki, T, M A Tschopp, and O Voinnet. 2017. "Biochemical and Genetic Functional Dissection of the P38 Viral Suppressor of RNA Silencing." *RNA* 23 (5): 639–54. https://doi.org/10.1261/rna.060434.116.

Imoto, M., H. Toclfihara, M. Iwaki, and H. Nakamura. 1986. "The Occurrence of Potato Mop Top Virus in Japan and Its Some Properties." *Japanese Journal of Phytopathology* 52 (5): 752–57.

Islam, Waqar, Hassan Naveed, Madiha Zaynab, Zhiqun Huang, and Han Y. H. Chen. 2019. "Plant Defense against Virus Diseases; Growth Hormones in Highlights." *Plant Signaling & Behavior* 14 (6): 1596719. https://doi.org/10.1080/15592324.2019.1596719.

Jackson, Andrew O., Hyoun-Sub Lim, Jennifer Bragg, Uma Ganesan, and Mi Yeon Lee. 2009. "Hordeivirus Replication, Movement, and Pathogenesis." *Annual Review of Phytopathology* 47 (1): 385–422. https://doi.org/10.1146/annurev-phyto-080508-081733.

Jankowsky, Eckhard. 2011. "RNA Helicases at Work: Binding and Rearranging." *Trends Biochem Sci* 36 (1): 19–29. https://doi.org/10.1016/j.tibs.2010.07.008.

Jones, R. A.C., and B. D. Harrison. 1969. "The Behaviour of Potato Mop-top Virus in Soil, and Evidence for Its Transmission by Spongospora Subterranea (Wallr.) Lagerh." *Annals of Applied Biology* 63 (1): 1–17. https://doi.org/10.1111/j.1744-7348.1969.tb05461.x.

Jones, R.A.C. 1975. "Presence of Potato Mop-Top Virus in Bolivia." *Plant Disease Reporter* 59 (2): 193.

Kalantidis, Kriton, Heiko Tobias Schumacher, Tasos Alexiadis, and Jutta Maria Helm. 2008. "RNA Silencing Movement in Plants." *Biology of the Cell* 100 (1): 13–26. https://doi.org/10.1042/BC20070079.

Kashiwazaki, Satoshi, Karen P. Scott, Brian Reavy, and Bryan D. Harrison. 1995. "Sequence Analysis and Gene Content of Potato Mop-Top Virus RNA 3: Further Evidence of Heterogeneityin the Genome Organization of Furoviruses." *Virology*. Vol. 206. https://doi.org/10.1016/S0042-6822(95)80092-1.

Kawakami, Shigeki, Yuichiro Watanabe, Roger N Beachy, and Donald Danforth. 2004. "Tobacco Mosaic Virus Infection Spreads Cell to Cell as Intact Replication Complexes." www.pnas.orgcgidoi10.1073pnas.0401221101.

Khraiwesh, B., JK. Zhu, and J. Zhu. 2012 "Role of MiRNAs and SiRNAs in Biotic and Abiotic Stress Responses of Plants." *Biochimica et Biophysica Acta. Protein Structure and Molecular Enzymology* 1819 (2): 137–48. https://doi.org/10.1016/j.bbagrm.2011.05.001.

Khvorova, Anastasia, Angela Reynolds, and Sumedha D Jayasena. 2003. "Functional SiRNAs and MiRNAs Exhibit Strand Bias." *Cell* 115 (2): 209–16. https://doi.org/10.1016/s0092-8674(03)00801-8.

Kinkema, Mark, and John Schiefelbein. 1994. "A Myosin from a Higher Plant Has Structural Similarities

to Class V Myosins." *Journal of Molecular Biology* 239 (4): 591–97. https://doi.org/10.1006/jmbi.1994.1400.

Kitajima, E.W., and J.A. Lauritis. 1969. "Plant Virions in Plasmodesmata." *Virology* 37 (4): 681–85. https://doi.org/10.1016/0042-6822(69)90288-8.

Koenig, R, U Commandeur, S Loss, C Beier, A Kaufmann, and D E Lesemann. 1997. "Beet Soil-Borne Virus RNA 2: Similarities and Dissimilarities to the Coat Protein Gene-Carrying RNAs of Other Furoviruses." *The Journal of General Virology* 78 (Pt 2) (February): 469–77. https://doi.org/10.1099/0022-1317-78-2-469.

Kurppa, A. 1989a. "The Distribution and Incidence of Potato Mop-Top Virus in Finland as Determined in 1987 and on the Variation of Disease Symptoms in Infected Potatoes." *Annales Agriculturae Fenniae 28. Seria Phytopathologia* 28 (4): 285–95.

Kurppa, A. 1989b. "Reaction of Potato Cultivars to Primary and Secondary Infection by Potato Mop-Top Furovirus and Strategies for Virus Detection." *EPPO Bulletin* 19 (3): 593–98. https://doi.org/10.1111/j.1365-2338.1989.tb00437.x.

Lakatos, Lóránt, György Szittya, Dániel Silhavy, and József Burgyán. 2004. "Molecular Mechanism of RNA Silencing Suppression Mediated by P19 Protein of Tombusviruses." *The EMBO Journal* 23 (4): 876–84. https://doi.org/10.1038/sj.emboj.7600096.

Lambert, D. H., L. Levy, V. A. Mavrodieva, S. B. Johnson, M. J. Babcock, and M. E. Vayda. 2003. "First Report of *Potato Mop-Top Virus* on Potato from the United States." *Plant Disease* 87 (7): 872–872. https://doi.org/10.1094/PDIS.2003.87.7.872A.

Latvala-Kilby, Satu, Johanna M. Aura, Neda Pupola, Asko Hannukkala, and Jari P. T. Valkonen. 2009. "Detection of Potato Mop-Top Virus in Potato Tubers and Sprouts: Combinations of RNA2 and RNA3 Variants and Incidence of Symptomless Infections." *Phytopathology* 99 (5): 519–31. https://doi.org/10.1094/PHYTO-99-5-0519.

Lee, Jung-Youn, and Hua Lu. 2011. "Plasmodesmata: The Battleground against Intruders." *Trends in Plant Science* 16 (4): 201–10. https://doi.org/10.1016/j.tplants.2011.01.004.

Leisner, Scott M., and Robert Turgeon. 1993. "Movement of Virus and Photoassimilate in the Phloem: A Comparative Analysis." *BioEssays* 15 (11): 741–48. https://doi.org/10.1002/bies.950151107.

Lemoine, Remi, Sylvain La Camera, Rossitza Atanassova, Fabienne Dédaldéchamp, Thierry Allario, Nathalie Pourtau, Jean-Louis Bonnemain, et al. 2013. "Source-to-Sink Transport of Sugar and Regulation by Environmental Factors." *Frontiers in Plant Science* 4: 272. https://doi.org/10.3389/fpls.2013.00272.

Li, F, C Huang, Z Li, and X Zhou. 2014. "Suppression of RNA Silencing by a Plant DNA Virus Satellite Requires a Host Calmodulin-like Protein to Repress RDR6 Expression." *PLoS Pathog* 10 (2): e1003921. https://doi.org/10.1371/journal.ppat.1003921.

Li, Feng, and Shou-Wei Ding. 2006. "Virus Counterdefense: Diverse Strategies for Evading the RNA-Silencing Immunity." *Annual Review of Microbiology* 60 (1): 503–31. https://doi.org/10.1146/annurev.micro.60.080805.142205.

Lim, Hyoun-Sub, Jennifer N Bragg, Uma Ganesan, Steven Ruzin, Denise Schichnes, Mi Yeon Lee, Anna Maria Vaira, Ki Hyun Ryu, John Hammond, and Andrew O Jackson. 2009. "Subcellular Localization of the Barley Stripe Mosaic Virus Triple Gene Block Proteins." *Journal of Virology* 83 (18): 9432–48. https://doi.org/10.1128/JVI.00739-09.

Liu, Jian-Zhong, Feng Li, and Yule Liu. 2017. "Plant Immunity against Viruses." *Frontiers in Microbiology* 8 (March): 520. https://doi.org/10.3389/fmicb.2017.00520.

Lough, Tony J., Khalid Shash, Beatriz Xoconostle-Cázares, Katrina R. Hofstra, David L. Beck, Ezequiel

Balmori, Richard L. S. Forster, and William J. Lucas. 1998. “Molecular Dissection of the Mechanism by Which Potexvirus Triple Gene Block Proteins Mediate Cell-to-Cell Transport of Infectious RNA.” *Molecular Plant-Microbe Interactions* 11 (8): 801–14. https://doi.org/10.1094/MPMI.1998.11.8.801.

Lucas, William J. 2006. “Plant Viral Movement Proteins: Agents for Cell-to-Cell Trafficking of Viral Genomes.” *Virology*. https://doi.org/10.1016/j.virol.2005.09.026.

Lucas, William J, Byung-Kook Ham, and Jae-Yean Kim. 2009. “Plasmodesmata - Bridging the Gap between Neighboring Plant Cells.” *Trends in Cell Biology* 19 (10): 495–503. https://doi.org/10.1016/j.tcb.2009.07.003.

Lukhovitskaya, N I, G H Cowan, R R Vetukuri, J Tilsner, L Torrance, and E I Savenkov. 2015. “Importin-Alpha-Mediated Nucleolar Localization of Potato Mop-Top Virus TRIPLE GENE BLOCK1 (TGB1) Protein Facilitates Virus Systemic Movement, Whereas TGB1 Self-Interaction Is Required for Cell-to-Cell Movement in Nicotiana Benthamiana.” *Plant Physiol* 167 (3): 738–52. https://doi.org/10.1104/pp.114.254938.

Lukhovitskaya, N I, S Thaduri, S K Garushyants, L Torrance, and E I Savenkov. 2013. “Deciphering the Mechanism of Defective Interfering RNA (DI RNA) Biogenesis Reveals That a Viral Protein and the DI RNA Act Antagonistically in Virus Infection.” *J Virol* 87 (11): 6091–6103. https://doi.org/10.1128/JVI.03322-12.

Lukhovitskaya, N I, N E Yelina, A A Zamyatnin Jr., M V Schepetilnikov, A G Solovyev, M Sandgren, S Y Morozov, J P Valkonen, and E I Savenkov. 2005. “Expression, Localization and Effects on Virulence of the Cysteine-Rich 8 KDa Protein of Potato Mop-Top Virus.” *J Gen Virol* 86 (Pt 10): 2879–89. https://doi.org/10.1099/vir.0.81099-0.

Makarov, Valentin V., Ekaterina N. Rybakova, Alexander V. Efimov, Eugene N. Dobrov, Marina V. Serebryakova, Andrey G. Solovyev, Igor V. Yaminsky, Michael E. Taliansky, Sergey Yu Morozov, and Natalia O. Kalinina. 2009. “Domain Organization of the N-Terminal Portion of Hordeivirus Movement Protein TGBp1.” *Journal of General Virology* 90 (12): 3022–32. https://doi.org/10.1099/vir.0.013862-0.

Mandadi, Kranthi K, and Karen-Beth G Scholthof. 2013. “Plant Immune Responses Against Viruses: How Does a Virus Cause Disease? OA.” *The Plant Cell* 25: 1489–1505. https://doi.org/10.1105/tpc.113.111658.

Manova, Vasilissa, and Damian Gruszka. 2015. “DNA Damage and Repair in Plants – from Models to Crops.” *Frontiers in Plant Science* 6 (October): 885. https://doi.org/10.3389/fpls.2015.00885.

Marcos, Jose F., Marçal Vilar, Enrique Pérez-Payá, and Vicente Pallás. 1999. “In VivoDetection, RNA-Binding Properties and Characterization of the RNA-Binding Domain of the P7 Putative Movement Protein from Carnation Mottle Carmovirus (CarMV).” *Virology* 255 (2): 354–65. https://doi.org/10.1006/viro.1998.9596.

Margis, R, A F Fusaro, N A Smith, S J Curtin, J M Watson, E J Finnegan, and P M Waterhouse. 2006. “The Evolution and Diversification of Dicers in Plants.” *FEBS Lett* 580 (10): 2442–50. https://doi.org/10.1016/j.febslet.2006.03.072.

Matthews, R. E. F. (Richard Ellis Ford), and Roger Hull. 2002. *Matthews' Plant Virology.* Academic Press.

Mérai, Zsuzsanna, Zoltán Kerényi, Sándor Kertész, Melinda Magna, Lóránt Lakatos, and Dániel Silhavy. 2006. “Double-Stranded RNA Binding May Be a General Plant RNA Viral Strategy To Suppress RNA Silencing Downloaded From.” *Journal Of Virology* 80 (12): 5747–56. https://doi.org/10.1128/JVI.01963-05.

Mérai, Zsuzsanna, Zoltán Kerényi, Attila Molnár, Endre Barta, Anna Válóczi, György Bisztray, Zoltán Havelda, József Burgyán, and Dániel Silhavy. 2005. “Aureusvirus P14 Is an Efficient RNA Silencing Suppressor That Binds Double-Stranded RNAs without Size Specificity.” *Journal of Virology* 79 (11): 7217–26. https://doi.org/10.1128/JVI.79.11.7217-7226.2005.

Mermigka, Glykeria, Frédéric Verret, and Kriton Kalantidis. 2016. “RNA Silencing Movement in Plants.” *Journal of Integrative Plant Biology* 58 (4): 328–42. https://doi.org/10.1111/jipb.12423.

Mi, S J, T Cai, Y G Hu, Y Chen, E Hodges, F R Ni, L Wu, et al. 2008. “Sorting of Small RNAs into Arabidopsis Argonaute Complexes Is Directed by the 5 ’ Terminal Nucleotide.” *Cell* 133 (1): 116–27. https://doi.org/10.1016/j.cell.2008.02.034.

Mitra, Ruchira, Konduru Krishnamurthy, Elison Blancaflor, Mark Payton, Richard S Nelson, and Jeanmarie Verchot-Lubicz. 2003. “The Potato Virus X TGBp2 Protein Association with the Endoplasmic Reticulum Plays a Role in but Is Not Sufficient for Viral Cell-to-Cell Movement.” *Virology* 312 (1): 35–48. https://doi.org/10.1016/s0042-6822(03)00180-6.

Mølgaard, Jens Peter, and Steen Lykke Nielsen. 1996. “Influence of Post Harvest Temperature Treatments, Storage Period and Harvest Date on Development of Spraing Caused by Tobacco Rattle Virus and Potato Mop-Top Virus.” *Potato Research* 39 (4): 571–79. https://doi.org/10.1007/BF02358476.

Molnár, Attila, Tibor Csorba, Lóránt Lakatos, Eva Várallyay, Christophe Lacomme, and József Burgyán. 2005. “Plant Virus-Derived Small Interfering RNAs Originate Predominantly from Highly Structured Single-Stranded Viral RNAs.” *Journal of Virology* 79 (12): 7812–18. https://doi.org/10.1128/JVI.79.12.7812-7818.2005.

Montero-Astúa, Mauricio, Viviana Vasquéz, William W. Turechek, Ueli Merz, and Carmen Rivera. 2008. “Incidence, Distribution, and Association of *Spongospora Subterranea* and *Potato Mop-Top Virus* in Costa Rica.” *Plant Disease* 92 (8): 1171–76. https://doi.org/10.1094/PDIS-92-8-1171.

Morozov, S. Yu., and Andrey G. Solovyev. 2003. “Triple Gene Block: Modular Design of a Multifunctional Machine for Plant Virus Movement.” *Journal of General Virology* 84 (6): 1351–66. https://doi.org/10.1099/vir.0.18922-0.

Napoli, Carolyn, Christine Lemieux, and Richard Jorgensen. 2007. “Introduction of a Chimeric Chalcone Synthase Gene into Petunia Results in Reversible Co-Suppression of Homologous Genes in Trans.” *The Plant Cell*. Vol. 2. American Society of Plant Physiologists. https://doi.org/10.2307/3869076.

Neben Uhr, Andreas, and Ram Dixit. 2018. “Kinesins and Myosins: Molecular Motors That Coordinate Cellular Functions in Plants,” no. 1. https://doi.org/10.1146/annurev-arplant-042817.

Niehl, Annette, and Manfred Heinlein. 2011. “Cellular Pathways for Viral Transport through Plasmodesmata.” *Protoplasma* 248 (1): 75–99. https://doi.org/10.1007/s00709-010-0246-1.

Nishiguchi, M., F. Motoyoshi, and N. Oshima. 1978. “Behaviour of a Temperature Sensitive Strain of Tobacco Mosaic Virus in Tomato Leaves and Protoplasts.” *Journal of General Virology* 39 (1): 53–61. https://doi.org/10.1099/0022-1317-39-1-53.

Novak, J.B., V. Rasocha, and J. Lanzova. 1983. “Demonstration of Potato Mop-Top Virus in Czechoslovak Socialist Republic.” *Sbornik UVTIZ Ochrana Rostlin* 19: 161–67.

Ohno, T., N. Takamatsu, T. Meshi, Y. Okada, M. Nishiguchi, and Y. Kiho. 1983. “Single Amino Acid Substitution in 30K Protein of TMV Defective in Virus Transport Function.” *Virology* 131 (1): 255–58. https://doi.org/10.1016/0042-6822(83)90551-2.

Okano, Y, H Senshu, M Hashimoto, Y Neriya, O Netsu, N Minato, T Yoshida, et al. 2014. “In Planta

Recognition of a Double-Stranded RNA Synthesis Protein Complex by a Potexviral RNA Silencing Suppressor." *Plant Cell* 26 (5): 2168–83. https://doi.org/10.1105/tpc.113.120535.

Oparka, K.J., D.A.M. Prior, S. Santa Cruz, H.S Padgett, and R.N. Beachy. 1997. "Gating of Epidermal Plasmodesmata Is Restricted to the Leading Edge of Expanding Infection Sites of Tobacco Mosaic Virus (TMV)." *The Plant Journal* 12 (4): 781–89. https://doi.org/10.1046/j.1365-313X.1997.12040781.x.

Oparka, Karl J, Alison G Roberts, Petra Boevink, Simon Santa Cruz, Ian Roberts, Katja S Pradel, Astrid Imlau, Guy Kotlizky, Norbert Sauer, and Bernard Epel. 1999. "Simple, but Not Branched, Plasmodesmata Allow the Nonspecific Trafficking of Proteins in Developing Tobacco Leaves." *Cell* 97 (6): 743–54. https://doi.org/10.1016/S0092-8674(00)80786-2.

Ortega, C.E., and D.O. Leopardi. 1989. "Potato Mop Top, Nueva Enfermedad Viral de La Papa En Venezuela." *Fitopatologia Venezolana* 2 (2): 43.

Overall, Robyn L., and Leila M. Blackman. 1996. "A Model of the Macromolecular Structure of Plasmodesmata." *Trends in Plant Science* 1 (9): 307–11. https://doi.org/10.1016/S1360-1385(96)88177-0.

Ozeki, Johji, Masayoshi Hashimoto, Ken Komatsu, Kensaku Maejima, Misako Himeno, Hiroko Senshu, Takeshi Kawanishi, Satoshi Kagiwada, Yasuyuki Yamaji, and Shigetou Namba. 2009. "The N-Terminal Region of the *Plantago Asiatica Mosaic Virus* Coat Protein Is Required for Cell-to-Cell Movement but Is Dispensable for Virion Assembly." *Molecular Plant-Microbe Interactions* 22 (6): 677–85. https://doi.org/10.1094/MPMI-22-6-0677.

Palauqui, J C, T Elmayan, J M Pollien, and H Vaucheret. 1997. "Systemic Acquired Silencing: Transgene-Specific Post-Transcriptional Silencing Is Transmitted by Grafting from Silenced Stocks to Non-Silenced Scions." *The EMBO Journal* 16 (15): 4738–45. https://doi.org/10.1093/emboj/16.15.4738.

Perez, R A, G M Langford, W R Eckberg, and W A Anderson. 1986. "Contractile Proteins (Actin, Myosin) and Tubulin Are Revealed within DNA-Containing Nucleocytoplasm in Mature Spermatozoa of Libinia Emarginata L." *Journal of Submicroscopic Cytology* 18 (3): 471–80. http://www.ncbi.nlm.nih.gov/pubmed/2427742.

Perraki, Artemis, Maria Binaghi, Martin A. Mecchia, Julien Gronnier, Sylvie German-Retana, Sébastien Mongrand, Emmanuelle Bayer, Alicia M. Zelada, and Véronique Germain. 2014. "StRemorin1.3 Hampers Potato Virus X TGBp1 Ability to Increase Plasmodesmata Permeability, but Does Not Interfere with Its Silencing Suppressor Activity." *FEBS Letters* 588 (9): 1699–1705. https://doi.org/10.1016/j.febslet.2014.03.014.

Pitzalis, Nicolas, and Manfred Heinlein. 2018. "The Roles of Membranes and Associated Cytoskeleton in Plant Virus Replication and Cell-to-Cell Movement." *Journal of Experimental Botany* 69 (1): 117–32. https://doi.org/10.1093/jxb/erx334.

Preller, Matthias, and Dietmar J. Manstein. 2013. "Myosin Structure, Allostery, and Mechano-Chemistry." *Structure* 21 (11): 1911–22. https://doi.org/10.1016/J.STR.2013.09.015.

Prokhnevsky, Alexey I, Valera V Peremyslov, and Valerian V Dolja. 2005. "Actin Cytoskeleton Is Involved in Targeting of a Viral Hsp70 Homolog to the Cell Periphery." *Journal of Virology* 79 (22): 14421–28. https://doi.org/10.1128/JVI.79.22.14421-14428.2005.

Qu, F, X Ye, and T J Morris. 2008. "Arabidopsis DRB4, AGO1, AGO7, and RDR6 Participate in a DCL4-Initiated Antiviral RNA Silencing Pathway Negatively Regulated by DCL1." *Proc Natl Acad Sci U S A* 105 (38): 14732–37. https://doi.org/10.1073/pnas.0805760105.

Rabobank. 2019. "World Potato Map 2019: Fries Are on the Menu Globally." 2019.

https://research.rabobank.com/far/en/sectors/regional-food-agri/world_potato_map_2019.html.

Raffaele, Sylvain, Emmanuelle Bayer, David Lafarge, Stéphanie Cluzet, Sylvie German Retana, Tamy Boubekeur, Nathalie Leborgne-Castel, et al. 2009. "Remorin, a Solanaceae Protein Resident in Membrane Rafts and Plasmodesmata, Impairs *Potato Virus X* Movement." *The Plant Cell* 21 (5): 1541–55. https://doi.org/10.1105/tpc.108.064279.

Raja, P., B. C. Sanville, R. C. Buchmann, and D. M. Bisaro. 2008. "Viral Genome Methylation as an Epigenetic Defense against Geminiviruses." *Journal of Virology* 82 (18): 8997–9007. https://doi.org/10.1128/JVI.00719-08.

Ramesh, S V, G Raikhy, C R Brown, J L Whitworth, and H R Pappu. 2014. "Complete Genomic Characterization of a Potato Mop-Top Virus Isolate from the United States." *Archives of Virology* 159 (12): 3427–33. https://doi.org/10.1007/s00705-014-2214-0.

Reavy, B, M Arif, G H Cowan, and L Torrance. 1998. "Association of Sequences in the Coat Protein/Readthrough Domain of Potato Mop-Top Virus with Transmission by Spongospora Subterranea." *Journal of General Virology* 79: 2343–47.

Reddy, Anireddy SN, and Irene S Day. 2001. "Analysis of the Myosins Encoded in the Recently Completed Arabidopsis Thaliana Genome Sequence." http://genomebiology.com/2001/2/7/research/0024.1.

Reichelt, Stefanie, Alex E. Knight, Tony P. Hodge, Frantisek Baluska, Jozef Samaj, Dieter Volkmann, and John Kendrick-Jones. 1999. "Characterization of the Unconventional Myosin VIII in Plant Cells and Its Localization at the Post-Cytokinetic Cell Wall." *The Plant Journal* 19 (5): 555–67. https://doi.org/10.1046/j.1365-313X.1999.00553.x.

Robards, A W, and W J Lucas. 1990. "Plasmodesmata." *Annu. Rev. Plant Physiol. Plant Mol. Bioi*. Vol. 41. www.annualreviews.org.

Roberts, A. G., S. S. Cruz, I. M. Roberts, DAM. Prior, R. Turgeon, and K. J. Oparka. 1997. "Phloem Unloading in Sink Leaves of Nicotiana Benthamiana: Comparison of a Fluorescent Solute with a Fluorescent Virus." *The Plant Cell* 9 (8): 1381–96. https://doi.org/10.1105/tpc.9.8.1381.

Rojas, Maria R., Minor R. Maliano, Juliana O. de Souza, Marcela Vasquez-Mayorga, Mŏnica A. de Macedo, Byung Kook Ham, and Robert L. Gilbertson. 2016. "Cell-to-Cell Movement of Plant Viruses: A Diversity of Mechanisms and Strategies." In *Current Research Topics in Plant Virology*. https://doi.org/10.1007/978-3-319-32919-2_5.

Roossinck, Marilyn J. 2015. "Plants, Viruses and the Environment: Ecology and Mutualism." *Virology* 479–480 (May): 271–77. https://doi.org/10.1016/j.virol.2015.03.041.

Rubio, Luis, José Guerri, Pedro Moreno, Ricardo Flores, Scott J Harper, and Ioannis E Tzanetakis. 2013. "Genetic Variability and Evolutionary Dynamics of Viruses of the Family Closteroviridae." https://doi.org/10.3389/fmicb.2013.00151.

Ryan, Jennifer M, and Andreas Nebenführ. 2018. "Update on Myosin Motors: Molecular Mechanisms and Physiological Functions." *Plant Physiology* 176 (1): 119–27. https://doi.org/10.1104/pp.17.01429.

Ryden, K., B. Eriksson, and V. Insunza. 1986. "Spraing in Potato Tubers Caused by Potato Mop-Top Virus (PMTV)." *Växtskyddsnotiser* 50 (4–5): 97–102.

Sager, Ross, and Jung-Youn Lee. 2014. "Plasmodesmata in Integrated Cell Signalling: Insights from Development and Environmental Signals and Stresses." *Journal of Experimental Botany* 65 (22): 6337–58. https://doi.org/10.1093/jxb/eru365.

Sahana, N, H Kaur, R K Jain, P Palukaitis, T Canto, and S Praveen. 2014. "The Asparagine Residue in the FRNK Box of Potyviral Helper-Component Protease Is Critical for Its Small RNA Binding

and Subcellular Localization." *J Gen Virol* 95 (Pt 5): 1167–77. https://doi.org/10.1099/vir.0.060269-0.

Salazar, L F, and R A C Jones. 1975. "Some Studies on the Distribution and Incidence of Potato Mop-Top Virus in Peru." *American Potato Journal* 52 (5): 143–50. https://doi.org/10.1007/BF02838112.

Samuel, Geoffrey. 1934. "The Movement of Tobacco Mosaic Virus Within the Plant." *Annals of Applied Biology* 21 (1): 90–111. https://doi.org/10.1111/j.1744-7348.1934.tb06891.x.

Sandgren, M., E. I. Savenkov, and J. P. T. Valkonen. 2001. "The Readthrough Region of Potato Mop-Top Virus (PMTV) Coat Protein Encoding RNA, the Second Largest RNA of PMTV Genome, Undergoes Structural Changes in Naturally Infected and Experimentally Inoculated Plants." *Archives of Virology* 146 (3): 467–77. https://doi.org/10.1007/s007050170156.

Sandgren, M, I E Savenkov, and T J P Valkonen. 2001. "The Readthrough Region of Potato Mop-Top Virus (PMTV) Coat Protein Encoding RNA, the Second Largest RNA of PMTV Genome, Undergoes Structural Changes in Naturally Infected and Experimentally Inoculated Plants." *Archives of Virology* 146 (3): 467–77. https://doi.org/10.1007/s007050170156.

Sandgren, Maria. 1995. "Potato Mop-Top Virus (PMTV): Distribution in Sweden, Development of Symptoms during Storage and Cultivar Trials in Field and Glasshouse." *Potato Research* 38 (4): 379–89. https://doi.org/10.1007/BF02357743.

Santala, J., O. Samuilova, A. Hannukkala, S. Latvala, H. Kortemaa, U. Beuch, A. Kvarnheden, et al. 2010. "Detection, Distribution and Control of Potato Mop-Top Virus, a Soil-Borne Virus, in Northern Europe." *Annals of Applied Biology* 157 (2): 163–78. https://doi.org/10.1111/j.1744-7348.2010.00423.x.

Sasaki, Nobumitsu, Eita Takashima, and Hiroshi Nyunoya. 2018. "Altered Subcellular Localization of a Tobacco Membrane Raft-Associated Remorin Protein by Tobamovirus Infection and Transient Expression of Viral Replication and Movement Proteins." *Frontiers in Plant Science* 9 (May): 619. https://doi.org/10.3389/fpls.2018.00619.

Savenkov, E I, A Germundsson, A A Zamyatnin Jr., M Sandgren, and J P T Valkonen. 2003. "Potato Mop-Top Virus: The Coat Protein-Encoding RNA and the Gene for Cysteine-Rich Protein Are Dispensable for Systemic Virus Movement in Nicotiana Benthamiana." *Journal of General Virology* 84 (4): 1001–5. https://doi.org/10.1099/vir.0.18813-0.

Savenkov, Eugene I, Maria Sandgren, and Jari P T Valkonen. 1999. "Complete Sequence of RNA 1 and the Presence of TRNA-like Structures in All RNAs of Potato Mop-Top Virus, Genus Pomovirus." *Journal of General Virology* 80: 2779–84.

Schneider, W L, and M J Roossinck. 2001. "Genetic Diversity in RNA Virus Quasispecies Is Controlled by Host-Virus Interactions." *Journal of Virology* 75 (14): 6566–71. https://doi.org/10.1128/JVI.75.14.6566-6571.2001.

Schoelz, James E., Phillip A. Harries, and Richard S. Nelson. 2011. "Intracellular Transport of Plant Viruses: Finding the Door out of the Cell." *Molecular Plant* 4 (5): 813–31. https://doi.org/10.1093/mp/ssr070.

Schwab, Rebecca, and Olivier Voinnet. 2010. "RNA Silencing Amplification in Plants: Size Matters." *Proceedings of the National Academy of Sciences of the United States of America* 107 (34): 14945–46. https://doi.org/10.1073/pnas.1009416107.

Schwarz, Dianne S, György Hutvágner, Tingting Du, Zuoshang Xu, Neil Aronin, and Phillip D Zamore. 2003. "Asymmetry in the Assembly of the RNAi Enzyme Complex." *Cell* 115 (2): 199–208. https://doi.org/10.1016/s0092-8674(03)00759-1.

Schwärzel, R. 2002. “Sensibilité Des Raciness et Tubercules Des Variétés de Pommes de Terre à La Gale Poudreuse et Qualques Résultats de Lutte Chimique.” *Revue Suisse D´ Agriculture* 34 (4): 261–66.

Scott, K. P., S. Kashiwazaki, B. Reavy, and B. D. Harrison. 1994. “The Nucleotide Sequence of Potato Mop-Top Virus RNA 2: A Novel Type of Genome Organization for a Furovirus.” *Journal of General Virology* 75 (12): 3561–68. https://doi.org/10.1099/0022-1317-75-12-3561.

Seemanpillai, Mark, Rabab Elamawi, Christophe Ritzenthaler, and Manfred Heinlein. 2006. “Challenging the Role of Microtubules in Tobacco Mosaic Virus Movement by Drug Treatments Is Disputable.” *Journal of Virology* 80 (13): 6712–15. https://doi.org/10.1128/JVI.00453-06.

Sereme, D, S Lacombe, M Konate, M Bangratz, A Pinel-Galzi, D Fargette, A S Traore, G Konate, and C Brugidou. 2014. “Sites under Positive Selection Modulate the RNA Silencing Suppressor Activity of Rice Yellow Mottle Virus Movement Protein P1.” *Journal of General Virology* 95: 213–18. https://doi.org/10.1099/vir.0.057026-0.

Shiboleth, Yoel Moshe, Elina Haronsky, Diana Leibman, Tzahi Arazi, Michael Wassenegger, Steven A Whitham, Victor Gaba, and Amit Gal-On. 2007. “The Conserved FRNK Box in HC-Pro, a Plant Viral Suppressor of Gene Silencing, Is Required for Small RNA Binding and Mediates Symptom Development.” *Journal of Virology* 81 (23): 13135–48. https://doi.org/10.1128/JVI.01031-07.

Shirako, Yukio, and T.Michael A. Wilson. 1993. “Complete Nucleotide Sequence and Organization of the Bipartite RNA Genome of Soil-Borne Wheat Mosaic Virus.” *Virology* 195 (1): 16–32. https://doi.org/10.1006/viro.1993.1342.

Solovyev, A G, D A Zelenina, E I Savenkov, V Z Grdzelishvili, S Yu Morozov, D.-E Lesemann, E Maiss, R Casper, and J G Atabekov. 1996. “Movement of a Barley Stripe Mosaic Virus Chimera with a Tobacco Mosaic Virus Movement Protein.” *Virology*. Vol. 217. https://core.ac.uk/download/pdf/82654365.pdf.

Solovyev, Andrey G., Natalia O. Kalinina, and Sergey Y. Morozov. 2012. “Recent Advances in Research of Plant Virus Movement Mediated by Triple Gene Block.” *Frontiers in Plant Science* 3 (December): 276. https://doi.org/10.3389/fpls.2012.00276.

Spooner, David M, Karen McLean, Gavin Ramsay, Robbie Waugh, and Glenn J Bryan. 2005. “A Single Domestication for Potato Based on Multilocus Amplified Fragment Length Polymorphism Genotyping.” *Proceedings of the National Academy of Sciences of the United States of America* 102 (41): 14694–99. https://doi.org/10.1073/pnas.0507400102.

Staiger, Chris J. 2000. “Signaling to the Actin Cytoskeleton in Plants.” www.annualreviews.org.

Su, Shengzhong, Zhaohui Liu, Cheng Chen, Yan Zhang, Xu Wang, Lei Zhu, Long Miao, Xue-Chen Wang, and Ming Yuan. 2010. “Cucumber Mosaic Virus Movement Protein Severs Actin Filaments to Increase the Plasmodesmal Size Exclusion Limit in Tobacco W OA.” *The Plant Cell* 22: 1373–1387. https://doi.org/10.1105/tpc.108.064212.

Takeda, Atsushi, Shintaro Iwasaki, Toshiaki Watanabe, Maki Utsumi, and Yuichiro Watanabe. 2008. “The Mechanism Selecting the Guide Strand from Small RNA Duplexes Is Different Among Argonaute Proteins.” *Plant Cell Physiology* 49 (4): 493–500. https://doi.org/10.1093/pcp/pcn043.

Takeda, Atsushi, Misato Tsukuda, Hiroyuki Mizumoto, Kimiyuki Okamoto, Masanori Kaido, Kazuyuki Mise, and Tetsuro Okuno. 2005. “A Plant RNA Virus Suppresses RNA Silencing through Viral RNA Replication.” *The EMBO Journal* 24 (17): 3147–57. https://doi.org/10.1038/sj.emboj.7600776.

Takemoto, Daigo, and Adrienne R Hardham. 2004. “The Cytoskeleton as a Regulator and Target of Biotic Interactions in Plants.” *Plant Physiology* 136 (4): 3864–76.

https://doi.org/10.1104/pp.104.052159.

Tamai, Atsushi, Kenji Kubota, Hideaki Nagano, Motoyasu Yoshii, Masayuki Ishikawa, Kazuyuki Mise, and Tetsuo Meshi. 2003. “Cucumovirus- and Bromovirus-Encoded Movement Functions Potentiate Cell-to-Cell Movement of Tobamo- and Potexviruses.” *Virology* 315 (1): 56–67. https://doi.org/10.1016/S0042-6822(03)00480-X.

Tenorio, J., Y. Franco, C. Chuquillanqui, R. A. Owens, and L. F. Salazar. 2006. “Reaction of Potato Varieties to Potato Mop-Top Virus Infection in the Andes.” *American Journal of Potato Research* 83 (5): 423–31. https://doi.org/10.1007/BF02872018.

Tilsner, J., G. H. Cowan, A. G. Roberts, S. N. Chapman, A. Ziegler, E. Savenkov, and L. Torrance. 2010. “Plasmodesmal Targeting and Intercellular Movement of Potato Mop-Top Pomovirus Is Mediated by a Membrane Anchored Tyrosine-Based Motif on the Lumenal Side of the Endoplasmic Reticulum and the C-Terminal Transmembrane Domain in the TGB3 Movement Protein.” *Virology* 402 (1): 41–51. https://doi.org/10.1016/j.virol.2010.03.008.

Tominaga, Motoki, and Akihiko Nakano. 2012. “Plant-Specific Myosin XI, a Molecular Perspective.” *Frontiers in Plant Science* 3 (September): 1–11. https://doi.org/10.3389/fpls.2012.00211.

Torrance, Lesley, Nina I. Lukhovitskaya, Mikhail V. Schepetilnikov, Graham H. Cowan, Angelika Ziegler, and Eugene I. Savenkov. 2009. “Unusual Long-Distance Movement Strategies of *Potato Mop-Top Virus* RNAs in *Nicotiana Benthamiana*.” *Molecular Plant-Microbe Interactions* 22 (4): 381–90. https://doi.org/10.1094/MPMI-22-4-0381.

Ueki, Shoko, Roman Spektor, Danielle M. Natale, and Vitaly Citovsky. 2010. “ANK, a Host Cytoplasmic Receptor for the Tobacco Mosaic Virus Cell-to-Cell Movement Protein, Facilitates Intercellular Transport through Plasmodesmata.” Edited by Biao Ding. *PLoS Pathogens* 6 (11): e1001201. https://doi.org/10.1371/journal.ppat.1001201.

Valli, A A, A Gallo, B Rodamilans, J J Lopez-Moya, and J A Garcia. 2018. “The HCPro from the Potyviridae Family: An Enviable Multitasking Helper Component That Every Virus Would like to Have.” *Mol Plant Pathol* 19 (3): 744–63. https://doi.org/10.1111/mpp.12553.

Varallyay, E, and Z Havelda. 2013. “Unrelated Viral Suppressors of RNA Silencing Mediate the Control of ARGONAUTE1 Level.” *Mol Plant Pathol* 14 (6): 567–75. https://doi.org/10.1111/mpp.12029.

Vargason, J M, G Szittya, J Burgyan, and T M Hall. 2003. “Size Selective Recognition of SiRNA by an RNA Silencing Suppressor.” *Cell* 115 (7): 799–811. https://www.ncbi.nlm.nih.gov/pubmed/14697199.

Vatén, Anne, Jan Dettmer, Shuang Wu, York-Dieter Stierhof, Shunsuke Miyashima, Shri Ram Yadav, Christina J. Roberts, et al. 2011. “Callose Biosynthesis Regulates Symplastic Trafficking during Root Development.” *Developmental Cell* 21 (6): 1144–55. https://doi.org/10.1016/j.devcel.2011.10.006.

Venkataraman, Sangita, Burra V L S Prasad, and Ramasamy Selvarajan. 2018. “RNA Dependent RNA Polymerases: Insights from Structure, Function and Evolution.” https://doi.org/10.3390/v10020076.

Verchot-Lubicz, Jeanmarie, Lesley Torrance, Andrey G Solovyev, Sergey Yu Morozov, Andrew O Jackson, and David Gilmer. 2010. “Varied Movement Strategies Employed by Triple Gene Block-Encoding Viruses.” */ 1231 MPMI* 23 (10): 1231–47. https://doi.org/10.1094/MPMI.

Vogler, Hannes, Rashid Akbergenov, Padubidri V Shivaprasad, Vy Dang, Monika Fasler, Myoung-Ok Kwon, Saule Zhanybekova, Thomas Hohn, and Manfred Heinlein. 2007. “Modification of Small RNAs Associated with Suppression of RNA Silencing by Tobamovirus Replicase Protein.” *Journal of Virology* 81 (19): 10379–88. https://doi.org/10.1128/JVI.00727-07.

Voinnet, O, P Vain, S Angell, and D C Baulcombe. 1998. “Systemic Spread of Sequence-Specific Transgene RNA Degradation in Plants Is Initiated by Localized Introduction of Ectopic Promoterless DNA.” *Cell* 95 (2): 177–87. https://www.ncbi.nlm.nih.gov/pubmed/9790525.

Wang, Aiming. 2015. “Dissecting the Molecular Network of Virus-Plant Interactions: The Complex Roles of Host Factors.” *Annu. Rev. Phytopathol* 53: 45–66. https://doi.org/10.1146/annurev-phyto-080614-120001.

Wang, Che, Li-Jun Zhang, and Rui-Dong Huang. 2011. “Cytoskeleton and Plant Salt Stress Tolerance.” *Plant Signaling & Behavior* 6 (1): 29–31. https://doi.org/10.4161/psb.6.1.14202.

Wang, Xu, Ross Sager, Weier Cui, Chong Zhang, Hua Lu, and Jung-Youn Lee. 2013. “Salicylic Acid Regulates Plasmodesmata Closure during Innate Immune Responses in Arabidopsis.” *The Plant Cell* 25 (6): 2315–29. https://doi.org/10.1105/tpc.113.110676.

Wang, Zhengyuan, and Thomas C. Pesacreta. 2004. “A Subclass of Myosin XI Is Associated with Mitochondria, Plastids, and the Molecular Chaperone Subunit TCP-1? In Maize.” *Cell Motility and the Cytoskeleton* 57 (4): 218–32. https://doi.org/10.1002/cm.10168.

White, R. G., and D. A. Barton. 2011. “The Cytoskeleton in Plasmodesmata: A Role in Intercellular Transport?” *Journal of Experimental Botany* 62 (15): 5249–66. https://doi.org/10.1093/jxb/err227.

Wolf, S., W. J. Lucas, C. M. Deom, and R. N. Beachy. 1989. “Movement Protein of Tobacco Mosaic Virus Modifies Plasmodesmatal Size Exclusion Limit.” *Science* 246 (4928): 377–79. https://doi.org/10.1126/science.246.4928.377.

Wright, Kathryn M., Graham H. Cowan, Nina I. Lukhovitskaya, Jens Tilsner, Alison G. Roberts, Eugene I. Savenkov, and Lesley Torrance. 2010. “The N-Terminal Domain of PMTV TGB1 Movement Protein Is Required for Nucleolar Localization, Microtubule Association, and Long-Distance Movement.” *Molecular Plant-Microbe Interactions* 23 (11): 1486–97. https://doi.org/10.1094/MPMI-05-10-0105.

Xiong, Z., K.H. Kim, D. Giesman-Cookmeyer, and S.A. Lommel. 1993. “The Roles of the Red Clover Necrotic Mosaic Virus Capsid and Cell-to-Cell Movement Proteins in Systemic Infection.” *Virology* 192 (1): 27–32. https://doi.org/10.1006/VIRO.1993.1004.

Xu, H., T.-L. DeHaan, and S. H. De Boer. 2004. “Detection and Confirmation of Potato Mop-Top Virus in Potatoes Produced in the United States and Canada.” *Plant Disease* 88 (4): 363–67. https://doi.org/10.1094/PDIS.2004.88.4.363.

Zamyatnin, Andrey A, Andrey G Solovyev, Eugene I Savenkov, Anna Germundsson, Maria Sandgren, Jari P T Valkonen, and Sergey Y Morozov. 2004. “Transient Coexpression of Individual Genes Encoded by the Triple Gene Block of Potato Mop-Top Virus Reveals Requirements for TGBp1 Trafficking” 17 (8): 921–30.

Popular science summary

Plant diseases due to pathogens pose a serious threat to crop production worldwide. Shortage of food was responsible for the death of millions of people and animals. Among the plant pathogens, viruses are the least understood and known to be the most difficult to control. Potato production is affected by a number of virus like Potato mop-top virus (PMTV). PMTV causes a disease called potato 'spraing', which results in necrotic arcs in the tubers making them not marketable. In Sweden alone, it causes about 80-100 million/SEK losses per year. The virus has its distribution in many parts of the world including Nordic countries, North and South America, and parts of Asia. Increasing detection in many new countries in the recent years suggests that PMTV poses a significant epidemiological risk. However, no viable options that are currently available for the control of PMTV and the chemical control methods are largely ineffective on virus infections.

In this study, we collected samples from the Andean regions of Peru, which is considered as the centre of domestication of potato. By analysing these isolates we identified that compared to the rest of the world, PMTV has high genetic diversity in the Andean regions of Peru. Our result supports a notion that PMTV was first introduced into Europe from South America, which served as a source for subsequent spread to the other regions in the world.

Viruses have highly diverse mechanisms in taking over the host's machinery for their functionality. Understanding how viral proteins interact with the plant cellular components is critical to develop sustainable methods for disease control. In this study, we found that one of the genes that codes for a protein that counters the plant defence system against the virus is evolving rapidly. Through gene-editing method, we identified that the changes in this gene can enhance its counter-defence activity, suggesting that the evolutionary pressure modulates the viral counter-defence activity.

Movement of the virus is paramount for establishing successful infection in the plant. In this study, we also identified key components involved in the local and long-distance movement of the virus. We showed that the virus hijacks key cellular components like myosin motors that transport cellular organelles in and out of the cells. We also showed that PMTV hijacks a plant abiotic stress signalling protein for its long-distance movement. Our study indicated that PMTV can induce and enhance drought resilience in plants. The main reason for this could be that helping the plant survive adverse conditions could, in turn, help the survival of the virus itself. Further studies are required to enhance our understanding of this virus-induced drought tolerance in the plants so that we can explore the possibilities of improving drought tolerance in the agricultural crops.

Taken together, this thesis contributed to a better understanding of the diversity of PMTV and how it hijacks the host proteins, and defends itself during the process of infection.

అధ్యయన సారాంశం

మొక్కల వ్యాధులు ప్రపంచవ్యాప్తంగా పంట ఉత్పత్తికి తీవ్రమైన ముప్పు కలిగిస్తాయి. చారిత్రాత్మకంగా, మొక్కల వ్యాధులు కోట్ల మంది ప్రజలు మరియు జంతువుల మరణాలకు కారణమయ్యాయి. ఇతర వ్యాధికారక కారకాలతోకంటే, వైరస్లు తక్కువగా అర్థం చేసుకోబడినవి మరియు నియంత్రించటానికి చాలా కష్టమయినవి. ఇతర పంటల మాదిరిగానే, బంగాళాదుంప ఉత్పత్తి కూడా 'పాటాటో స్పిండిల్ ' వంటి అనేక వైరల్ వ్యాధుల ద్వారా ప్రభావితమవుతుంది. పాటాటో మాప్-టాప్ వైరస్ (పిఎమ్‌టివి) వల్ల కలిగే ఈ వ్యాధి దుంపలలో నలలటి చారలు కలిగిస్తుంది. స్వీడన్‌లో మాత్రమే, ఇది సంవత్సరానికి 80-100 మిలియన్ SEK నష్టాలను కలిగిస్తుంది. ఈ వైరస్ ప్రపంచంలోని అనేక ప్రాంతాలలో నార్డిక్ దేశాలు, ఉత్తర మరియు దక్షిణ అమెరికా మరియు ఆసియాలోని కొన్ని ప్రాంతాలలో కనుగొనబడినది మరియు ఇటీవలి సంవత్సరాలలో అనేక కొత్త దేశాలలో కూడా కనుగొనబడినది. అయినప్పటికీ, ప్రస్తుతం పిఎమ్‌టివి నియంత్రణకు ఎలాంటి మార్గాలు అందుబాటులో లేవు, ఎందుకంటే రసాయన నియంత్రణ పద్ధతులు వైరస్ వ్యాధులపై ఎక్కువగా పనిచేయవు.

ఈ అధ్యయనంలో, మేము బంగాళాదుంప యొక్క పెంపకం కేంద్రంగా పరిగణించబడుతున్న పెరూలోని ఆండియన్ ప్రాంతాల నుండి నమూనాలను సేకరించి పరీక్షించాము. ఈ నమూనాలను విశ్లేషించడం ద్వారా, మిగతా ప్రపంచంతో పోలిస్తే, పెరూలోని ఆండియన్ ప్రాంతాలలో పిఎమ్‌టివికి అధిక జన్యు వైవిధ్యం ఉందని మేము గుర్తించాము. దక్షిణ అమెరికా నుండి పిఎమ్‌టివిని మొదట యూరప్‌లోకి ప్రవేశించి, ఆ తరువాత ఇక్కడినుండి ప్రపంచంలోని ఇతర ప్రాంతాలకు వ్యాప్తిచెందిందన్న భావనకు ఈ అధ్యయనం మద్దతు ఇస్తుంది.

వైరస్లు వాటి కార్యాచరణ కోసం మొక్క యొక్క ప్రోటీన్ల పనితీరును స్వాధీనం చేసుకోవడంలో చాలా విభిన్నమైన విధానాలను కలిగి ఉంటాయి. వైరల్ ప్రోటీన్లు మొక్క కణాల భాగాలతో ఎలా సంకర్షణ చెందుతాయో అర్థం చేసుకోవడం వ్యాధిని నియంత్రించడానికి స్థిరమైన పద్ధతులను అభివృద్ధి చేయడంలో కీలకం. ఈ అధ్యయనంలో, మొక్కల రక్షణ వ్యవస్థను ఎదుర్కునే ప్రోటీన్ ఒకటి పరిణామ

క్రమములో వేగంగా మార్పు చెందుతోందని మేము కనుగొన్నాము. దాని ద్వారా జన్యువులో కలిగే మార్పులు వైరస్ యొక్క స్వీయ-రక్షణ కార్యకలాపాలను మెరుగుపరుస్తాయని మేము గుర్తించాము.

మొక్కలో విజయవంతమైన వ్యాధిని కలుగచేయటానికి వైరస్ యొక్క కదలిక చాలా ముఖ్యమైనది. ఈ అధ్యయనంలో, వైరస్ ఒక కణం నుండి మర్కక కణానికి, మరియు ఆ కణజాలమును వీడి వేరె భాగమునకు అవసరమైన ముఖ్య ప్రోటీన్లను కూడా మేము గుర్తించాము. కణాల లోపల మరియు వెలుపల రవాణా చేసే మైయోసిన్ మోటార్లు వంటి కీలకమైనవాటిని వైరస్ హైజాక్ చేస్తుందని మేము చూపించాము. పిఎమ్‌టివి దాని సుదూర కదలిక కోసం మొక్కలో కరువు సమయములో మాత్రమే ఎక్కువగా పనిచేసే ఒక ప్రోటీన్‌ను హైజాక్ చేస్తుందని మేము కనుగొన్నాము. మా అధ్యయనం వల్ల పిఎమ్‌టివి మొక్కలలో కరువు సహనాన్ని ప్రేరేపించగలదని నిరూపించబడినది. దీనికి ప్రధాన కారణం ఏమిటంటే, ప్రతికూల పరిస్థితుల నుండి బయటపడటానికి మొక్కకు సహాయపడటం వల్ల, వైరస్ యొక్క మనుగడ కూడా మెరుగుదల అవ్వగలదు. మొక్కలలో ఈ వైరస్-ప్రేరిత కరువు సహనం గురించి మన అవగాహన పెంచడానికి మరిన్ని అధ్యయనాలు అవసరం, తద్వారా వ్యవసాయ పంటలలో కరువు సహనాన్ని మెరుగుపరిచే అవకాశాలను అన్వేషించవచ్చు.

Acknowledgements

गुरुर्ब्रह्मा गुरुर्विष्णुः गुरुर्देवो महेश्वरः ।
गुरु साक्षात परंब्रह्म तस्मै श्रीगुरवे नमः ॥

As this famous verse in Sanskrit says, Guru is the direct form of any god. Throughout my life, I have been lucky to have many such teachers who supported me in my journey until now. Thank you to all of them starting from my first teacher Smt. Vasundhara to Eugene Savenkov, my PhD supervisor. Each one of them were amazing people with enormous commitment.

I would like to thank Eugene Savenkov for giving me this wonderful opportunity to do PhD under his supervision. The idea of doing a PhD goes way back to my schooling, and it has been a huge dream ever since. These four and half years have been truly incredible learning period of my life and I can't thank you enough for the support you gave me throughout this period. Thank you very for teaching me designing experiments, and critically commenting manuscripts and thesis. You really are a huge source of inspiration for me, and I'm very glad that I did PhD with you.

Thanks to my co-supervisors Annelie Carlsbecker, Panagiotis Moschou and Jose Gil for all the support during these years, and for going through the text of my thesis and suggesting improvements.

Special thanks to Annelie Carlsbecker for your continued support and encouragement from my day 1 in Sweden (actually day 0, even before my arrival). You were there for me whenever I needed some advice before and during my PhD. Also, thank you for organizing the journal club. Panos, you are an excellent teacher. You encouragement had immense impact in not feeling stressful in the beginning of my PhD. That was a great foundation for the rest of the PhD. Thank you for your suggestions whenever I hit a road block with the experiments. Jose, thank you very much for being a wonderful lab mate. You joining our group was one of the best things that happened in my PhD. Thanks

for your constant encouragement, teaching me a lot through your questions. You are like Socrates. I'm sure none of the students we had in our lab would deny that.

I can't thank Ramesh Vetukuri enough for teaching me many of the things that I have been using in my PhD. Working with you is always a great delight. You are the first person that comes to my mind when I need any help.

I acknowledge Anders Hafrén, German Martinez Arias, and Daniel Hofius for being part of my PhD evaluation committee and for their advice during the evaluation.

I want to thank Anders Kvarnheden for his support with the Swedish translation whenever I asked, and for organizing the group meetings. I want to thank all the present and past members of whole virology group. Adérito Monjane, for your great support with phylogenetic analysis for Paper I, Merike Sõmera, for giving me nice RNA extraction protocol, Sana Bashir, Laila, Mehdi Kamali, and Asmaa Youssef for nice discussions in the lab and outside. Asmaa, thanks for the gifts from Egypt.

Efstratia, thanks for listening to me whenever I needed to talk to someone, and thanks for all your help in the lab since my first year of PhD. You are the best! PhD gave me an opportunity to meet some wonderful people who became great friends like Prashanth Ramachandran, Stefan Schwarzbach, Elham Yazdkhasti, Amela Kujovic, Chandra Shekar Kenchappa, Vikash Yadav, Siddi, Dr. Liu Chen, Sheena Rasmussen, Jitka, Shirin, Frauke Augstein, Aayushi Shukla, and Nirbhay Kushwaha. I feel very lucky having met you guys. I will forever remember all the fun times we had. Thanks a lot for being there for me, and listening to me. Just saying thanks is not enough to you guys.

Chen, Anders Hafrén and Suayib Üstün, thanks a lot for your advice with my experiments when things didn't work sometimes. Minerva, thanks a lot for patiently answering my questions and for your support during the last few days. It has been a great help. Thank you.

Prashanth, Daniel Uddenberg and Frauke, it has been wonderful to share the office space with you guys over the years. Thanks for all the interesting discussions from science to religion, philosophy etc and fun moments in the office.

I would like to thank all the past members of Savenkov group Salam Kamalmaz, Aminalah Tahmasebi, Daan Langendoen, and Nihan Gunes for your support during my PhD.

I'm very grateful for SLU and the Dept. of plant biology, for not just giving me on platform to do PhD but also for giving an opportunity teach, something that I started loving very much over the years. Obviously, students are one of the best teachers, and it has been great learning experience to teach at SLU.

Thanks a lot to Salim Hossain Reza, Maria Luz Annacondia, Lejon Kralemann, Minerva Susana Trejo Arellano, Marcelinus Rocky Hatorangan, Rami Petteri Apuli and all others with whom I shared teaching assignments. I would like to thank Anna Westerberg for her encouraging words since I was in my master's program.

Thank you very much to Eva Sundberg, and all other colleagues at the Dept of Plant Biology for making the Department such a friendly and pleasant place to work. Special thanks to Björn Nicander, for being a great support with computing, Cecelia and Marie for great help in the lab, Lotta Olsson and Qing Heijkenskjöld, Monica Beergrehn for helping with administrative aspects, Anna Ståhlsten and Anneli Rimmer for being very helpful in ordering supplies, Urban Pettersson and Per Linden for graciously helping with Phytotron and green house.

I would also like to thank my microbiology professor at Kalasalingam University, Dr Sundar Krishnan for inspiring me go ahead in science and mathematics professor Dr S Bala Murali for his incredible help during my bachelors.

My life in Sweden would not have been so easy without two amazing people. Phani Rama Krishna Behra, and my sister Kalyani Vemuri.

Thank you Phani *anna* for helping me from my day 1 in Sweden. You were there for me whenever I asked for any help. Thanks to both you and Sirisha *akka* for all the great times we had.

Kalyani, you are truly god's gift for me. Looking back, I cannot imagine my life in Sweden without you, and cannot thank you enough for the incredible support you gave me. So many things you, me and Phani *anna*, and both of us did together, whether it is going to work, having dinners, watching movies, or traveling, we had a great time. Thanks a lot for all your support ra. Thanks a lot also for nice western blot techniques.

I also want to thank Punya and Smruti for being such a wonderful friends, and the great support you gave over the years. Thanks to Nagarjun Vijay, I really enjoy talking to you. Thanks to Sunel Rachapudi for a great help during my initial days in Sweden. Thanks to all my friends in Uppsala for making my life exciting and fun. It was great to have met all you guys!

Thanks a lot to my family members for being very supportive. Many thanks to my cousin and mentor, Vijay Krishna for teaching me to think big, and Teju *akka* for her great support when I was pretty clueless about what to do. Thanks to Krishna Chaitanya, my uncle, for supporting me during my time in Hyderabad. His support was immense. I will forever be grateful and cherish those times I spent in Hyderabad with you and Kishore *mama*. Thanks to my uncle, Ravi *mama* for his supporting words whenever I felt I get struck. He gave

me some advice to think beyond. My family had given me greatest support during my education. Credit also goes to Subramanyam *Pedannanna*, and Sridevi *Pedamma* for supporting me in my education, while Seshaphani *chinnana* for his support while studying, and Bharathi *pinamma* for our discussions on science since schooling days. I'm deeply grateful to my paternal uncles and aunts as well for teaching me a lot of things.

To all my coaches at 'Landmark worldwide' Jaya, Sashi, Venkat Baddipudi, Mahesh Nambiar, and friends Komal and Kishore Garikapaty, Amarnath, Rajesh and Jyothi, Anita, Karthick, Swechha and Garima, thanks a lot. Your contribution has been immense in my life. A huge part of who I'm is because of you guys.

Prathyu, Thanks a lot for being so understanding and supportive since you were a little girl. Can't wait to come back and play with my little niece Sankeerthana.

Sadhakshi, words fail me to thank you, of course. I cannot believe that it has been one year and eight months since we have been married, and I have been incredibly grateful for that. Thank you for tolerating me and my disorganized ways of being. Thanks in advance also because you will have to continue doing that. Over the past few months you kept a lot of effort to allow me peacefully work, thank you very much. I love you ra.

Nothing in the whole world is enough to thank my parents. While my mom was always there for me listening to ALL my dramas, my father stood there for me as an example to go beyond any obstacle. I'm very sure that he was concerned about what I would do in my life, but he never told me what to do, and what to become. He gave me freedom to choose what I wanted and just supported that in spite of being a huge burden for him sometimes.

My grandfather and grandmother have been the biggest source of inspiration in my life. While his way of questioning and thinking inspired me the most, her compassion beats everything. I think one of the happiest persons about my PhD was my grandfather. We had so many discussions about science, spirituality and society whenever we had phone calls and even on the day I met him for the last time. As I am about to finish my PhD, one person I miss the most is him.

To him who inspired me and to my parents who supported me!

Like Saint Tyagaraja says, ఎందరో మహానుభావులు అందరికీ వందనములు (so many great people, salutations to all of them). My apologies if I missed anyone.

Finally, to myself. I did it.