How do pectin methylesterases and their inhibitors affect the spreading of tobamovirus?

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Abstract: After replication in the cytoplasm, viruses spread from the infected cell into the neighboring cells through plasmodesmata, membranous channels embedded by the cell wall. As obligate parasites, viruses have acquired the ability to utilize host factors that unwillingly cooperate for the viral infection process. For example, the viral movement proteins (MP) interact with the host pectin methylesterase (PME) and both proteins cooperate to sustain the viral spread. However, how and where PMEs interact with MPs and how the PME/MP complexes favor the viral translocation is not well understood. Recently, we demonstrated that the overexpression of PME inhibitors (PMEIs) in tobacco and Arabidopsis plants limits the movement of Tobacco mosaic virus and Turnip vein clearing virus and reduces plant susceptibility to these viruses. Here we discuss how overexpression of PMEI may reduce tobamovirus spreading.

After penetration through damaged cells, plant viruses utilize host proteins that assist their infection process. The viral cell-to-cell movement goes through the plasmodesmata (PD), dynamic and complex membranous channels surrounded by specialized cell wall regions.¹,² The movement is supported by virus-encoded movement proteins (MPs), which are able to increase the size exclusion limit of PD.³ MPs perform multiple interactions with host intracellular proteins, among which the cell wall-associated pectin methylesterases (PMEs).⁴,⁵ Specific interactions of MP of Tobacco mosaic virus (TMV) and Turnip vein clearing virus (TVCV) with PMEs from tomato, citrus and tobacco and, more recently, between MP of TVCV with PMEs from Arabidopsis have been characterized.⁴,⁵ Although both MP and PME have been found associated to PD structures the definition of the subcellular localization of the PME-MP complex is under debate.⁴,⁶,⁷ Plant PMEs contain a transmembrane (TM) domain preceding the mature enzymes that is considered a membrane-anchor domain required for targeting the enzyme to cell wall (CW).⁸ MP was found in cell wall where it is phosphorylated by wall associated kinases to regulate PD transport.⁹ MP of TMV has 2 putative transmembrane regions that enable the protein to expose its cytosolic and ER luminal domains.¹⁰ It can be hypothesized that these structural features enable MP to interact with membrane-associated PME at ER luminal face and/or in the apoplastic compartment. Consistently, the interaction between the MP of Chinese wheat mosaic virus and PME from Nicotiana benthamiana has been showed to occur at the plasma membrane-CW level of N. benthamiana epidermal cells.⁶

Several experimental evidences suggest that PMEs, by interacting with MP, play a functional role in tobamovirus local spreading.⁴,⁵,¹¹ PME is also involved in TMV systemic movement mainly participating in the viral outcome from the vascular system.¹² The activity of PME is modulated in the cell wall by pectin methylesterase inhibitors (PMEIs).¹³–¹⁸ PMEIs are targeted to the extracellular matrix and inhibit plant PMEs by forming a specific stoichiometric 1:1 complex.¹⁹ We have recently demonstrated that PMEIs affect plant susceptibility toward viruses by counteracting the action of plant PMEs.

Keywords: cell wall, methanol, pectin methylesterase, pectin methylesterase inhibitors, pectin methylesterification, plasmodesmata, virus spreading

Abbreviations: PME, pectin methylesterase; PME, pectin methylesterase inhibitor; MP, movement protein; PD, plasmodesmata; TMV, Tobacco mosaic virus; CW, cell wall; MeOH, methanol; PM, Plasma membrane; ER, Endoplasmic Reticulum; CP, coat protein.

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We overexpressed genes encoding 2 well-characterized PMEIs in tobacco and Arabidopsis plants and showed that overexpression of AcPMEI in tobacco and AtPMEI-2, in Arabidopsis, causes a significant reduction of PME activity, an increase of cell wall methylesterification and, as a consequence, the reduction of the local and systemic translocation of TMV and TVCV.

PMEs are a large class of cell wall-remodelling enzymes induced during growth and upon pathogen infection. Specific PME isoforms are up-regulated upon infection by different viruses. The accumulation of PME transcripts is induced by TMV in infected tobacco leaves. We have found that PME activity is strongly induced in tobacco and Arabidopsis leaves during TMV and TVCV infection and we demonstrated, that the overexpression of PMEIs in tobacco and Arabidopsis transgenic plants, not only affects the existing PME activity but also inhibits the PME activity induced during viral infection. The overexpression of PMEIs in transgenic plants counteracts these processes by limiting PME/MP-mediated PD pore dilatation and cell-to-cell viral spreading. PM, plasma membrane; ER, endoplasmic reticulum; CW, cell wall; PD, plasmodesmata; CP, coat protein; MP, movement protein; RNAv, viral RNA; MeOH, methanol.

Figure 1. Dynamics of pectin methyltransferase (PME) and pectin methyltransferase inhibitors (PMEIs) in the viral cell-to-cell movement through the plasmodesmata (PD). (A) After viral penetration plants reduce size exclusion limit of PD by locally depositing callose at the neck regions of PD. (B) Virus infection alters the PD gating capacity by inducing PME that in cooperation with MP enlarges the pore diameter of PD by affecting the cell wall microdomains embedding PD. PME activity, localized by MP at the level of the cell wall embedding PD, can decrease pH and pectin degree of methylesterification which, in turn, favor the cell wall degradation by CWDEs. In addition viruses degrade the callose ring by inducing a methanol-mediated expression of β 1–3 glucanases. (C) The overexpression of PMEIs in transgenic plants counteracts these processes by limiting PME/MP-mediated PD pore dilatation and cell-to-cell viral spreading. PM, plasma membrane; ER, endoplasmic reticulum; CW, cell wall; PD, plasmodesmata; CP, coat protein; MP, movement protein; RNAv, viral RNA; MeOH, methanol.
promote the cell wall loosening by stimulating the activity of several cell wall-degrading enzymes (CWDEs), such as polygalacturonases, pectate lyases and expansins. In addition, a lower degree of methylesterification caused by PME may render the pectin more susceptible to the degradation by plant derived pectic enzymes. It can be postulated that the virus exploits the MP-PME interaction to recruit additional PMEs to perform a local decrease of pectin and pectin degree of esterification and to loosen the cell wall around PD to assist PD opening during infection. The overexpression of PMEI in transgenic plants may counteract this process and consequently limit viral spreading.

In conclusion a scenario is proposed that might explain the role of PME and PMEI in tobamovirus spreading. After viral penetration, plants respond to viral infection by depositing callose at the PD level to restrict the viral cell-to-cell diffusion (Fig. 1A). Viruses produce MPs and induce host PMEs and the interaction between the 2 proteins is exploited to localize additional PME activity and loosen the cell wall around PDs to promote the PD enlargement (Fig. 1B). The overexpression of PMEs in transgenic plants may counteract the process by limiting PME/MP-mediated PD pore dilation and cell-to-cell viral spreading (Fig. 1C). Although this model proposes a novel vision on the impact of PME and PMEI on tobamovirus spreading, further experimental evidence is required to support this hypothesis and to clarify the mechanisms at the base of this process.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References
1. Orfila C, Knox JP. Spatial regulation of pectic polysaccharides in relation to pit fields in cell walls of tomato fruit pericarp. Plant Physiol 2000; 122:775-81; PMID:10712541; http://dx.doi.org/10.1104/pp.122.3.775
2. Liu C, Nelson RS. The cell biology of Tobacco mosaic virus replication and movement. Front Plant Sci 2013; 4: 12; PMID:23403525; http://dx.doi.org/10.3389/fpls.2013.00012
3. Benitez-Alfonso Y, Faulkner C, Ritzenhaler C, Maude AJ. Plasmaemoderm: gateways to local and systemic virus infection. Mol Plant-Microbe Interact 2010; 23:1403-12; PMID:20687788; http://dx.doi.org/10.1094/MPMI-05-10-0116
4. Chen MH, Sheng J, Hind G, Handa AK, Citovsky V. Interaction between the tobacco mosaic virus movement protein and host cell pectin methylesterases is required for viral cell-to-cell movement. EMBO J 2000; 19:913-20; PMID:10698933; http://dx.doi.org/10.1093/emboj/19.5.913
5. Lionetti V, Raioa A, Cervone F, Bellincampi D. Transgenic expression of pectin methylesterase inhibitors limits its tobamovirus spread in tobacco and Arabidopsis. Mol Plant Pathol 2014; 15:265-74; PMID:24127644; http://dx.doi.org/10.1111/mpp.12090
6. Andika IB, Zheng SL, Tan ZL, Sun LY, Kondo H, Zhou XP, Chen JP. Endoplasmic reticulum export and vesicle formation of the movement protein of Chinese beet mosaic virus are regulated by two transmembrane domains and depend on the secretory pathway. Virulology 2013; 435:493-503; PMID:23137810; http://dx.doi.org/10.1016/j.virul.2012.10.024
7. Morvan O, Quentin M, Jaunea A, Mareea A, Morvan C. Immunogold localization of pectin methylesterases in the cortical tissues of flux hypocory. Proteoplasma 1998; 202:175-84; http://dx.doi.org/10.1007/BF01282545
8. Pellosj J, Ruuterucci C, Mellerowicz EJ. New insights into pectin methylesterase structure and function. Trends Plant Sci 2007; 12:677-8; PMID:17499007; http://dx.doi.org/10.1016/j.tplants.2007.04.001
9. Waigmann E, Chen MH, Bachmaier R, Ghoshroy S, Citovsky V. Regulation of plasmaemodermal transport by phoshorylation of tobacco mosaic virus cell-to-cell movement protein. EMBO J 2000; 19:4875-84; PMID:10990451; http://dx.doi.org/10.1093/emboj/19.18.4875
10. Brill LM, Nunn RS, Kahn TW, Yeager M, Beachy RN. Recombinant tobacco mosaic virus movement protein is an RNA-binding, alpha-helical membrane protein. Proc Nat Acad Sci USA 2000; 97:7112-7; PMID:10840061; http://dx.doi.org/10.1073/pnas.101381789
11. Dorokhov YL, Komarova TV, Petrunia IV, Frolova OY, Pozdyshev DV, Gleba YY. Airborne signals from a wounded leaf facilitate viral spreading and induce anti-bacterial resistance in neighboring plants. Plos Pathogens 2012; 8: e1002665; PMID:2249658; http://dx.doi.org/10.1371/journal.ppat.1002665
12. Fall R, Benson AA. Leaf methanol–the simplest natural product from plants. Trends Plant Sci 1996; 1:296-301; http://dx.doi.org/10.1016/S1360-1385(96)87187-0
13. Zavalev R, Levy A, Gera A, Epel BL. Subcellular dynamics and role of Arabidopsis b-1,3-glucanases in cell-to-cell movement of tobamoviruses. Mol Plant Microbe Interact 2013; 26: 1016-30; PMID:23656331; http://dx.doi.org/10.1094/MPMI-03-13-0062-R
14. Korenovsk P, Pumpe-Novak M, Baebler S, Roter A, Gom L, Gruden K, Fosner GD, Boomhun N, Ravnikar M. Aggressive and mild Potato virus Y isolates trigger different specific responses in susceptible potato plants. Plant Pathol 2010; 59:1121-32; http://dx.doi.org/10.1111/j.1365-3059.2010.02540.x
15. Yadav RK, Chatterjady H. Differential soybean gene expression during early phase of infection with Mung bean yellow mosaic India virus. Mol Biol Rep 2014; 41:5123-34; PMID:24752408; http://dx.doi.org/10.1007/s11033-014-3378-0
28. Gaffe J, Tieman DM, Handa AK. Pectin methylesterase isoforms in tomato (Lycopersicon esculentum) tissues. Effects of expression of a pectin methylesterase antisense gene. Plant Physiol 1994; 105:199-203; PMID: 12232199

29. Wen FS, Zhu YM, Hawes MC. Effect of pectin methylesterase gene expression on pea root development. Plant Cell 1999; 11:1129-40; PMID:10368183; http://dx.doi.org/10.1105/tpc.11.6.1129

30. Ren C, Kermode AR. An increase in pectin methyl esterase activity accompanies dormancy breakage and germination of yellow cedar seeds. Plant Physiol 2000; 124:231-42; PMID:10982438; http://dx.doi.org/10.1104/pp.124.1.231

31. Bellincampi D, Cervone F, Lionetti V. Plant cell wall dynamics and wall-related susceptibility in plant-pathogen interactions. Front Plant Sci 2014; 5:228; PMID:24904628; http://dx.doi.org/10.3389/fpls.2014.00228

32. Pogorelko G, Lionetti V, Bellincampi D, Zabolotina O. Cell wall integrity: targeted post-synthetic modifications to reveal its role in plant growth and defense against pathogens. Plant Signal Behav 2013; 8: e25435; PMID:23857352; http://dx.doi.org/10.4161/psb.25435