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Vincenzo Lionetti[a], Alessandro Raiola[b], Felice Cervone[a] & Daniela Bellincampi[a]
[a] Dipartimento di Biologia e Biotecnologie 'C. Darwin'; 'Sapienza' Università di Roma; Roma, Italy
[b] Dipartimento Territorio e Sistemi Agroforestali; Università di Padova; Legnaro (PD), Italy
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How do pectin methylesterases and their inhibitors affect the spreading of tobamovirus?

Vincenzo Lionetti[1], Alessandro Raiola[2], Felice Cervone[1], and Daniela Bellincampi[1,*]

[1]Dipartimento di Biologia e Biotecnologie 'C. Darwin'; 'Sapienza' Università di Roma; Roma, Italy; [2]Dipartimento Territorio e Sistemi Agroforestali; Università di Padova; Legnaro (PD), Italy

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Abbreviations: PME, pectin methylesterase; PMEI, 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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*Correspondence to: Daniela Bellincampi; Email: daniela.bellincampi@uniroma1.it

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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) interacts 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.

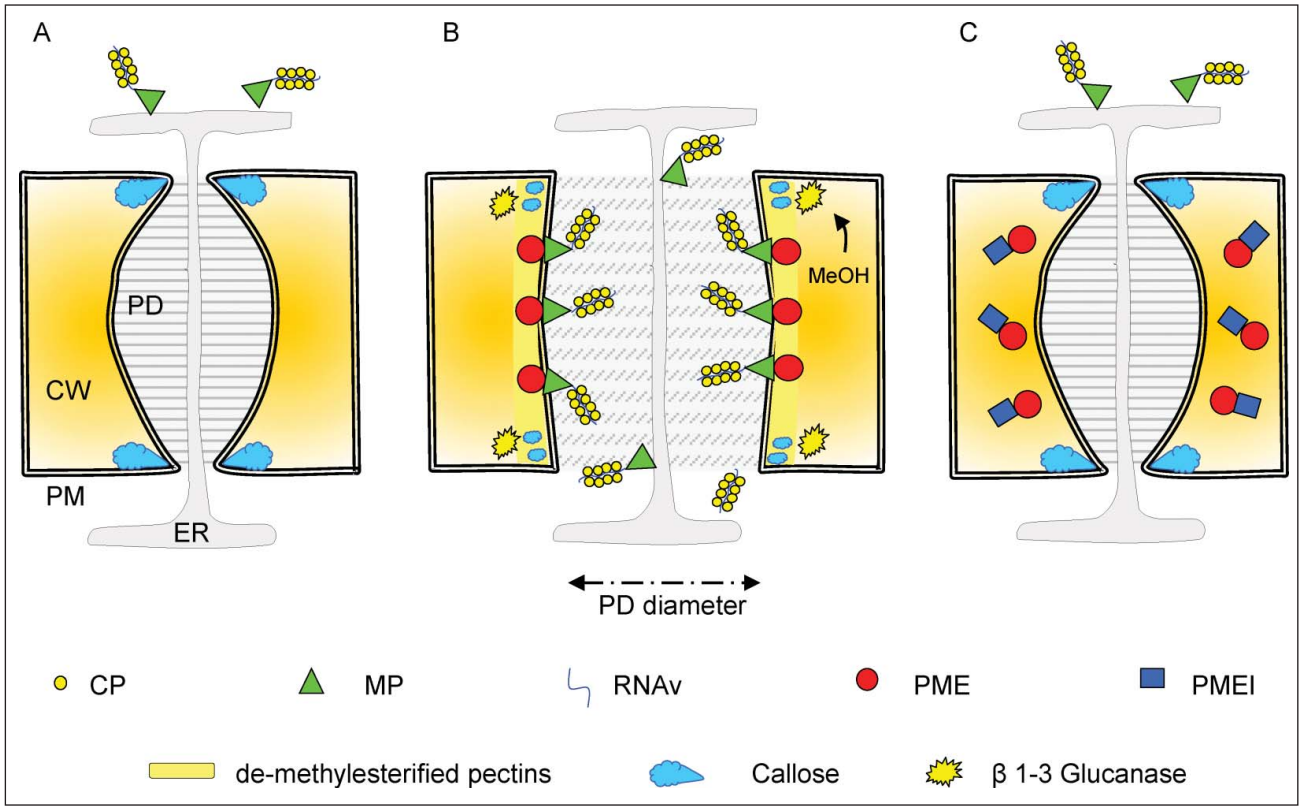

Figure 1. Dynamics of pectin methylesterase (PME) and pectin methylesterase inhibitors (PMEIs) in the viral cell-to-cell movement through the plasmodesmata (PD). (**A**) After viral penetration plants reduce size exclusion limit of PD by locally depositing callose at the neck regions of PD. (**B**) Virus 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 methylsterification 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.

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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.[5]

PMEs are a large class of cell wall-remodelling enzymes induced during growth and upon pathogen infection.[8,20] Specific PME isoforms are up-regulated upon infection by different viruses.[21-23] The accumulation of PME transcripts is induced by TMV in infected tobacco leaves.[23] 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.[5]

PMEs catalyze the de-methylesterification of pectin and release both protons and methanol. PME activity is considered the main metabolic source of methanol *in planta*.[24] It has been recently demonstrated that PME-dependent methanol emission triggers PD dilation and facilitates cell-to-cell communication and viral spreading.[23] This effect has been related to expression of methanol-induced genes including β-1,3-glucanases cooperating to PD dilation by degrading callose, which is locally deposited at the cell wall embedded neck region of PD to restrict cell-to-cell movement of viruses.[23,25] The overexpression of PMEI in transgenic plants limits cell-to-cell viral spreading by affecting the viral-induced PME activity and possibly by reducing the methanol-activated degradation of callose. PMEI expression has been shown to be induced by virus and after methanol treatment suggesting that the production of the inhibitor may be considered a defense strategy of the plant to hamper the activity of PME during viral infection.[23,26,27]

Immunoelectron microscopy studies indicate that PME is present in pectin-rich cell wall micro-domains around PD where acidic pectin and PME colocalize.[1,4,27] Protons produced by PME activity, accumulate in the apoplast during pectin de-methylesterification and lead to acidification of the wall.[28] A lower pH can

promote the cell wall loosening by stimulating the activity of several cell wall-degrading enzymes (CWDEs), such as polygalacturonases, pectate lyases and expansins.[29-31] In addition, a lower degree of methylesterification caused by PME may render the pectin more susceptible to the degradation by plant derived pectic enzymes.[17,20,32] It can be postulated that the virus exploits the MP-PME interaction to recruit additional PMEs to perform a localized decrease of pH 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 PMEIs 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.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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