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Comparison of the *Oilseed rape mosaic virus* and *Tobacco mosaic virus* movement proteins (MP) reveals common and dissimilar MP functions for tobamovirus spread

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ABSTRACT

Tobacco mosaic virus (TMV) is a longstanding model for studying virus movement and macromolecular transport through plasmodesmata (PD). Its movement protein (MP) interacts with cortical microtubule (MT)-associated ER sites (C-MERs) to facilitate the formation and transport of ER-associated viral replication complexes (VRCs) along the ER–actin network towards PD. To investigate whether this movement mechanism might be conserved between tobamoviruses, we compared the functions of *Oilseed rape mosaic virus* (ORMV) MP with those of MP^{TMV}. We show that MP^{ORMV} supports TMV movement more efficiently than MP^{TMV}. Moreover, MP^{ORMV} localizes to C-MERs like MP^{TMV} but accumulates to lower levels and does not localize to larger inclusions/VRCs or along MTs, patterns regularly seen for MP^{TMV}. Our findings extend the role of C-MERs in viral cell-to-cell transport to a virus commonly used for functional genomics in Arabidopsis. Moreover, accumulation of tobamoviral MP in inclusions or along MTs is not required for virus movement.

Introduction

Plant viruses depend on specialized non-cell autonomous movement proteins to transport their genome within and between cells to achieve systemic infection. Similar to viruses and their movement proteins, certain plant endogenous proteins such as specific transcription factors important for plant development and also certain plant RNA molecules move between cells (Chen et al., 2013; Dunoyer et al., 2010a, 2010b; Kong et al., 2012; Kurata et al., 2005; Lucas et al., 1995; Molnar et al., 2010; Rim et al., 2011; Urbanus et al., 2010; Vatén et al., 2011; Wu and Gallagher, 2013). Thus, viral movement proteins likely hitchhike on existing host pathways for the selective transport of macromolecules and hence are excellent tools to study intra- and intercellular macromolecular transport processes in plants. Support for this hypothesis was presented by the finding that two host proteins, CmPP36 and CmPP16 are immunologically related with a viral movement protein and traffic between cells through PD (Xoconostle-Cázares et al., 2000, 1999).

Tobamoviruses are single-stranded RNA viruses encoding proteins important for viral replication, movement and encapsidation. *Tobacco mosaic tobamovirus* (TMV) was the first virus discovered more than a century ago (Beijerinck, 1898; Ivanovski, 1892) and has been extensively used as a model system to study viral intra- and intercellular movement. This virus replicates in association with endoplasmic reticulum (ER) membranes and exploits this membrane network for spread through plasmodesmata (PD), symplastic communication channels through cell walls between neighboring cells (Laliberté and Sanfaçon, 2010; Liu and Nelson, 2013; Niehl and Heinlein, 2011; Niehl et al., 2013b). The 30 kDa movement protein of TMV (MP^{TMV}) is present in viral replication complexes (VRCs) (Asurmendi et al., 2004; Heinlein et al., 1998), associates with RNA (Beachy and Heinlein, 2000; Brill et al., 2000; Citovsky et al., 1990; Más and Beachy, 1999; Sambade et al., 2008)

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and acts as a microtubule (MT)-associated protein (Ashby et al., 2006; Boyko et al., 2000, 2007; Ferralli et al., 2006; Heinlein et al., 1995). Studies addressing the involvement of the cytoskeleton during TMV intra- and intercellular movement revealed that the tightly ER-associated actomyosin network facilitates targeting of MP^{TMV} to PD (Wright et al., 2007), intracellular movement of replicase complexes (Liu et al., 2005), and the spread of infection (Harries et al., 2009; Hofmann et al., 2009; Kawakami et al., 2004; Liu et al., 2005). It has to be noted, however, that TMV movement occurs unaffected within 24 h after the onset of inhibition of the actin cytoskeleton (Hofmann et al., 2009). Collectively, these data indicate that movement occurs primarily along the ER though with assistance by the actin network. The interaction of the protein with MTs plays a role during early infection when ER-membrane-associated VRCs localize to local intersections of MTs with the ER ((Boyko et al., 2007), reviewed in (Niehl et al., 2013b; Peña and Heinlein, 2013)). These sites, recently termed "cortical MT-associated ER sites" (C-MERs), are proposed to function as specific platforms for the recruitment of host factors and membranes in order to facilitate the maturation of the VRCs into movement-competent VRCs and, later, into virus factories (Peña and Heinlein, 2013). Upon formation, the MP-tagged VRCs are visualized as very small cortical particles that either remain attached to C-MERs or get detached to move in a directional stop-and-go fashion between C-MERs, in a manner depending on a dynamic actin and MT cytoskeleton (Boyko et al., 2007; Kawakami et al., 2004; Sambade et al., 2008). At PD, the MP facilitates the spread of VRCs into the adjacent cell by directly or indirectly causing an increase in the size exclusion limit (SEL) of the channel, presumably by a mechanism that involves the recruitment or activation of beta-glucanase to degrade SEL-restricting callose deposits in the PD neck regions (Beffa et al., 1996; Iglesias and Meins, 2000; Ueki et al., 2010; Zavaliev et al., 2013). Consistent with being VRCs in nature, the formation of the mobile MP particles is correlated with MP function in virus movement (Boyko et al., 2007; Sambade et al., 2008) and MP-particles contain replicase in early stages of infection (Asurmendi et al., 2004; Szécsi et al., 1999). During late infection stages, VRCs remaining in the infected cell may grow into virus factories that produce progeny virus (Asurmendi et al., 2004; Heinlein et al., 1998; Tilsner et al., 2009). Over time, the MP leaves the factories and accumulates along MTs to which the virus factories are aligned before it is finally degraded (Gillespie et al., 2002; Heinlein et al., 1995, 1998; Más and Beachy, 1999; Niehl et al., 2012, 2013a). However, accumulation of MP along MTs at late infection stages is dispensable for virus movement (Gillespie et al., 2002; Heinlein et al., 1998; Kawakami et al., 2004; Niehl et al., 2012; Szécsi et al., 1999). Whether the accumulation of MP along MTs plays a regulatory role or only reflects the functional MT-interacting activity of the protein playing a role during earlier stages is not known.

Although the model for tobamovirus movement is built on an extensive amount of observations from different laboratories, it is currently largely limited to TMV. Thus, it remains uncertain whether the specific interactions of the MP with host factors and the specific importance of these interactions for viral spread also apply to other tobamoviruses and to macromolecular transport in general. In an attempt to compare the requirements for cytoskeletal elements for different tobamoviruses, it has been shown that the distantly TMV-related, but closely ORMV-related Arabidopsis-infecting tobamovirus *Turnip vein clearing virus* (TVCV) does not require the actomyosin system for spread (Harries et al., 2009), indicating that the TMV-based model might not be fully transferable to other tobamoviruses and to other plant species. In support of this notion, it was very recently shown that MP^{TVCV} does not accumulate along MT, and requires nuclear localization to mediate efficient cell-to-cell movement of the virus (Levy et al., 2013).

To further dissect differences and similarities in the movement of tobamoviruses, we here investigated the subcellular localization and activity of the MP of *Oilseed rape mosaic virus* (ORMV, also called *Youcai mosaic virus* or *Chinese rape mosaic virus*, or TMV-Cg). Whereas TMV belongs to subgroup 1 of the tobamoviruses, which causes disease in solanacious species, ORMV, like TVCV, belongs to the viruses of subgroup 3 that cause symptoms in crucifers and are commonly used virus models for studies in Arabidopsis (Lartey et al., 1996). We show here that the MP of ORMV forms particles that move along the ER–actin network and in association with C-MERs like the MP of TMV, thus indicating a general role of C-MERs in viral transport. The results also indicate that the accumulation of MP in viral factories and along MTs is dispensable for virus movement and may rather restrict the spread of the virus.

Results

MP^{ORMV} complements TMV movement

Previous research indicated that $MP^{TMV\text{-}Cg}$ (TMV-Cg is very similar to ORMV (Gibbs et al., 2008)) can complement MP^{TMV} movement in trans in tobacco, and chimeric TMV constructs expressing $MP^{TMV\text{-}Cg}$ and MP^{ORMV} were shown to spread efficiently in tobacco (Diaz-Griffero et al., 2006) and to infect *N. benthamiana* and Arabidopsis (Mansilla et al., 2009). In order to compare the function of MP^{ORMV} with that of MP^{TMV}, we first established that also our MP^{ORMV} was able to functionally complement a MP-deficient TMV derivative for movement. *N. benthamiana* leaves were infected with a movement deficient, fluorescent TMV (TMV-ΔMPΔCP-GFP) and agroinfiltrated at 1 day post inoculation (dpi) to express either MP^{TMV}:RFP or MP^{ORMV}:RFP, or free RFP as control. Whereas expression of either MP^{TMV}:RFP or MP^{ORMV}:RFP led to the formation of expanding GFP-fluorescent infection sites at 5 dpi,

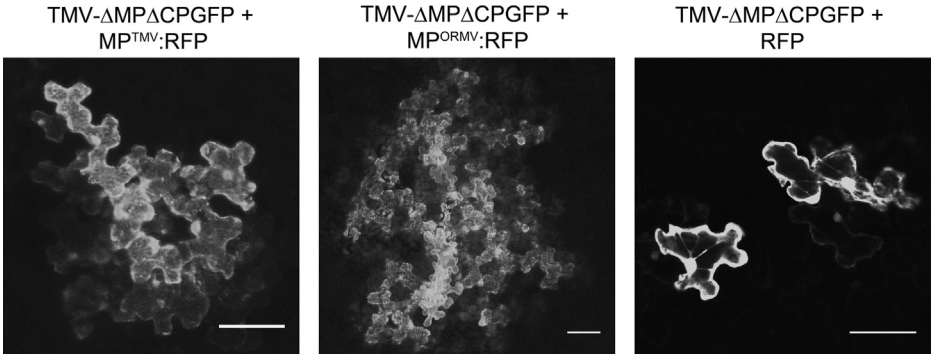

Fig. 1. MP^{ORMV} trans-complements MP-deficient TMV for movement. *N. benthamiana* leaves infected with TMVΔMPΔCP-GFP and agroinfiltrated to express either MP^{TMV}:RFP (left), MP^{ORMV}:RFP (center), or RFP (right), at 5 dpi. Images show GFP fluorescence. Scale bars, 100 μm.