Plasmodesmatal targeting of TMV movement protein utilises the ER/actin network

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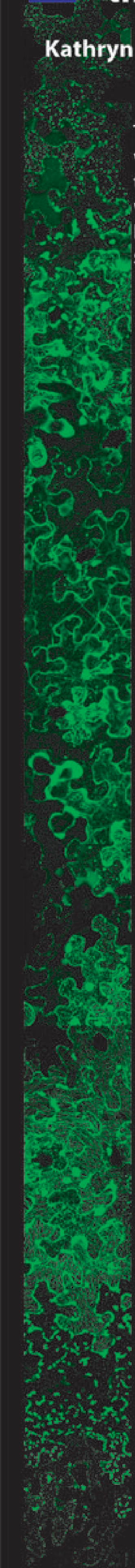
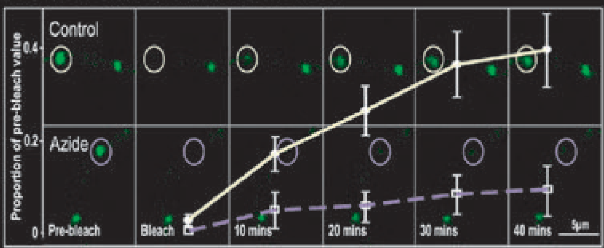


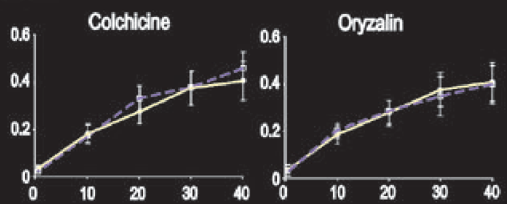
Tobacco mosaic virus (TMV) is one of the most extensively studied viral pathogens of plants. The mechanism by which the viral movment protein (MP) targets and accumulates within plasmodesmata (PD) and enables the trafficking of viral RNA remains the subject of some debate. During a TMV infection, MP becomes associated with microtubules (MT) leading to the hypothesis that MT are required for the movement of TMV-MP[1]. However, disruption of MT does not impede viral cell-to-cell movement , and a TMV vector expressing a DNA-shuffled MP gene which shows enhanced movement, does not associate with MT[2].

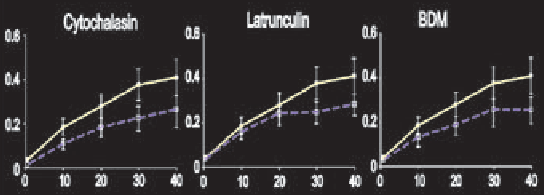
FRAP of PD

Using the technique of fluorescence recovery after photbleaching (FRAP) we investigated the targeting of MP to PD. At the leading edge of an infection, MP associated with PD was bleached, either under control conditions or in the presence of a range of pharmacological agents. Since photobleaching does not remove MP from the PD but only bleaches the GFP, the recovery of fluorescence within the PD represents the addition of new MP into the PD.

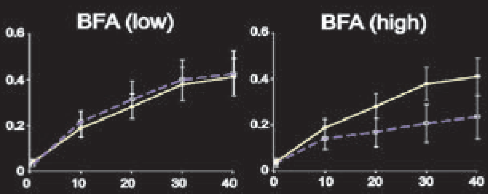


When followed for 40 min. the fluorescence recovery was severely inhibited in the presence of azide, indicating an energy dependence. In the presence of either colchicine or oryzalin (which disrupt MT function) there was no significant effect.

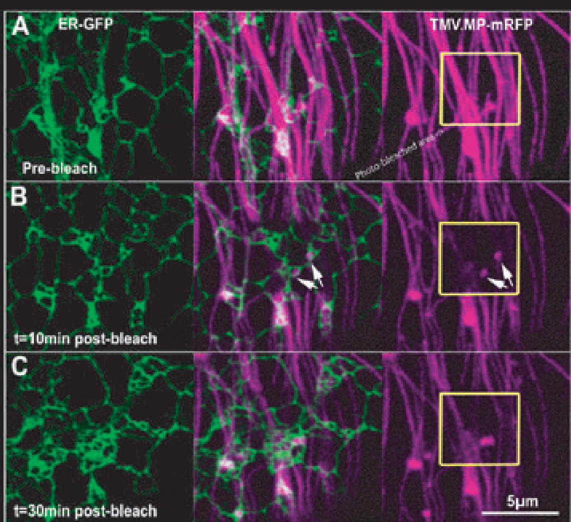




In the presence of the actin inhibitors cytochalasin or oryzalin and the myosin motor inhibitor, BDM there was a significant reduction in fluorescence recovery.There was also a significant reduction when cells were treated with high concentrations of BFA, but not with low concentrations.



FRAP of MT



Using transgenic plants expressing GFP in the ER that have been infected with TMV.MP-mRFP (purple) it can be shown that MP does not migrate along MT. Instead it appears that additional MP accumulates first on the ER vertices. Arrows show spots on ER in B, before MTs reappear in C.

Photoactivation of MP

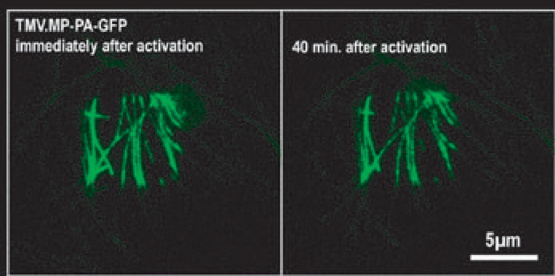
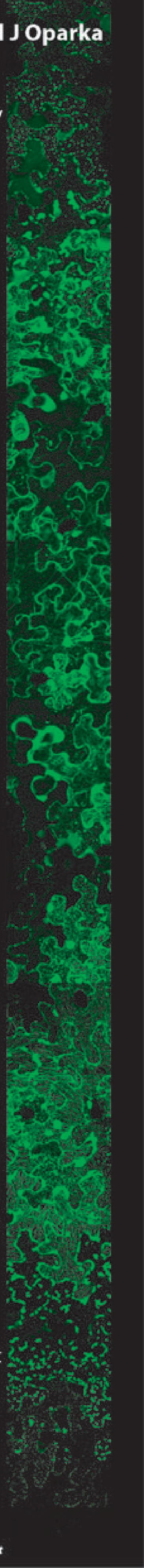


Photo-activation of TMV.MP-PA-GFP on MT confirms that MP does not move along MT. There is no movement of fluorescence along MTs outside the activated area.

Conclusions

The targeting of TMV-MP to PD is energy dependent and involves the ER/actin network.
The targeting of TMV-MP to PD does not require MT.
MP accumulates on the vertices of the ER before transfer to MT.
MP does not migrate along MT.



Side panel
Across a TMV.MP-GFP lesion

1. Heinlein M (2002) Cell Mol Life Sci **59**:58-82
2. Gillespie T et al. (2002) Plant Cell 14:1207-1222

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