Intercellular Targeting Of A Viral Movement Protein To Plasmodesmata

Alison Roberts, Kath Wright, Nikki Wood, Trudi Gillespie, Petra Boevink, Sophie Haupt & Karl Oparka

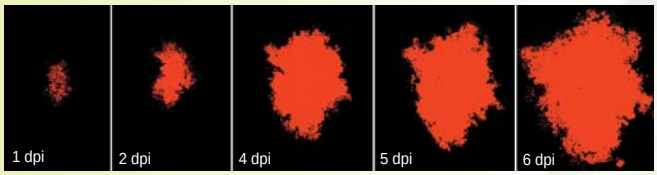
Unit of Cell-Cell Communication, Scottish Crop Research Institute, Invergowrie, Scotland, DD2 5DA.

Introduction

We have been studying the role of the cytoskeleton and endoplasmic reticulum (ER) in trafficking the tobacco mosaic virus (TMV) movement protein (MP) to and through plasmodesmata. A wide variety of biologically important macromolecules, such as transcription factors, move through plasmodesmata, but little is understood about the intercellular pathways that are used to move cytosolic proteins to plasmodesmata in the first place. Since the advent of fluorescent reporter proteins, viral MPs have been tagged and imaged as they target to and pass through the plasmodesmata, and there have been many published reports to suggest that microtubules (MTs) are the route used for the transport of the TMV MP. This study used chemical inhibitors to perturb the cytoskeleton and ER to determine their function in MP targeting.

Colchicine treatment of growing TMV lesions.

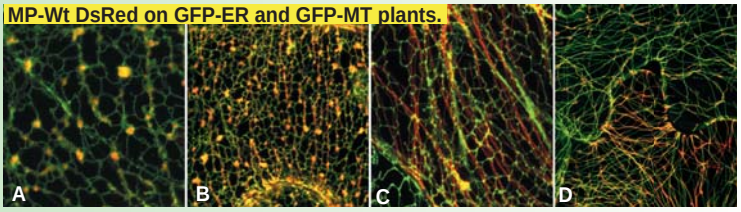
If MTs are trully required for the movement of TMV, then it would seem logical that disruption of the MT network would prevent viral spread. However, as the images below show, TMV lesions were able to spread over a 6-day period when the leaf was saturated with 0.1mM colchicine solution (a concentration that was known to disrupt MTs).



An enhanced movement protein does not target MTs

A functionally enhanced, DNA-shuffled TMV MP (MP-R3) was found to allow more rapid viral spread, but, unlike the wild-type MP (MP-Wt) did not target MTs.

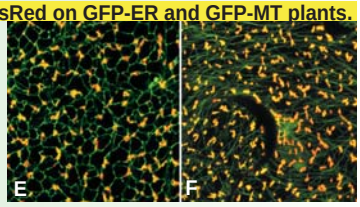
MP-Wt DsRed on GFP-ER and GFP-MT plants.



Early in infection, the MP-Wt was associated with the vertices of the cortical ER (A). It then spread along the ER network, causing the ER to show linear distortion (B). Later in the infection, the MP was transferred to underlying MTs and the ER restored its polygonal structure (C). The co-localisation of MP-Wt DsRed and GFP-MTs is shown in D.

MP-R3 DsRed on GFP-ER and GFP-MT plants.

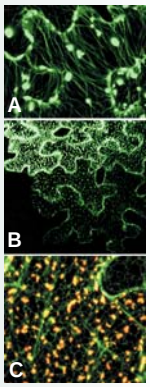
The MP-R3 looks very similar to a Wt infection in the early stages, being associated with the vertices of the ER (E). However, this MP was not transferred to the underlying network of MTs (F). In time, larger, static aggregates built up and were maintained at the ER vertices.

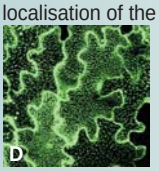


Proteasome inhibitor treatment of MP-Wt reproduces the MP-R3 phenotype.

Treatment of MP-Wt lesions (showing MT targeting) (A) with *clasto*-lactacystin b-lactone, an inhibitor of the 26S proteasome, produced the punctate localisation (B) of the MP that had previously been seen for MP-R3. These aggregates were again found to localise to the vertices of the ER (C).

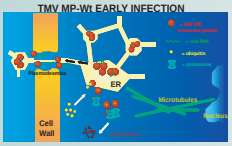
MP-R3 lesions maintained the same punctate localisation of the MP after treatment with the proteasome inhibitor (D). These results suggest that degredation of MP-R3 is impaired, and that MT targeting in Wt infections could be part of the degredation pathway.





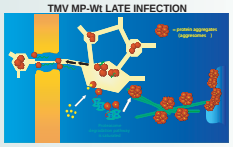
MODELS:

TMV MP-Wt EARLY INFECTION



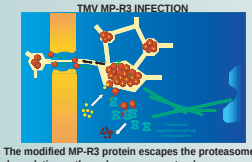
At low levels of protein, the degradation pathway is not saturated and ubiquitination removes the MP from the ER to allow cytosolic proteasome-mediated degredation.

TMV MP-Wt LATE INFECTION



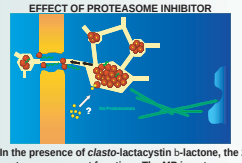
As protein accumulates, the proteasome pathway becomes saturated. At this point, the excess MP is moved on to MTs and also targeted to the perinuclear region of the cell.

TMV MP-R3 INFECTION



The modified MP-R3 protein escapes the proteasome degradation pathway by some as-yet unknown means. The pathway does not become saturated, protein is not transferred to MT and builds up at the ER vertices.

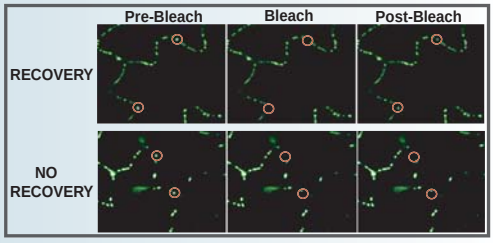
EFFECT OF PROTEASOME INHIBITOR

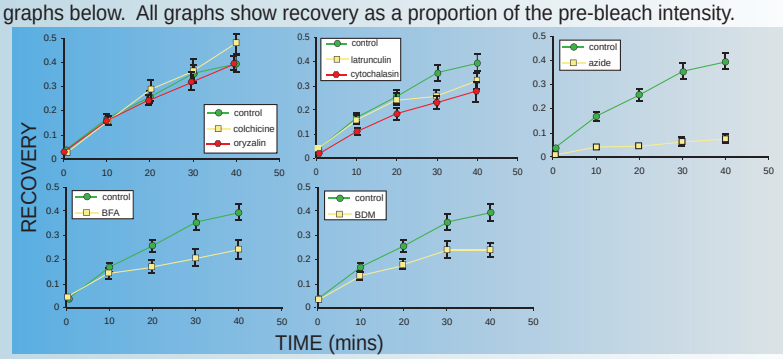


In the presence of *clasto*-lactacystin b-lactone, the 26s proteasome cannot function. The MP is not removed from the ER and so again accumulates on the vertices.

Effect of cellular inhibitors on the targeting of MP-Wt to plasmodesmata - FRAP studies.

Leaves infected with TMV MP-Wt were infiltrated and incubated with a range of cellular inhibitors for two hours prior to bleaching. Individual plasmodesmata were then bleached and followed over a 40 minute time period to see if there was any recovery of fluorescence - i.e. if there was any movement of unbleached protein into the bleached region. The results are shown in the graphs below. All graphs show recovery as a proportion of the pre-bleach intensity.





The MT inhibitors (colchicine & oryzalin) had no effect on the recovery of fluorescence - signal returned as quickly as in the water controls. The actin inhibitor latrunculin had no significant effect although cytochalasin (another actin inhibitor) did reduce the rate of recovery. This highlights differences in the mode of inhibitor action and seems to indicate some requirement for actin in subcellular MP transport. Azide, a metabolic inhibitor, prevented recovery showing that MP targeting requires active transport. Both BFA, an endomembrane inhibitor, and BDM, an inhibitor of myosin motor function, caused significant reductions in the recovery of fluorescence.

Summary

Our results suggest that TMV MP targeting to MTs occurs as part of the 26s proteasome degredation pathway, and not as a functional phase of the TMV movement process. From our results, we can find no evidence that MTs are involved in targeting viral MP to the plasmodesmal pore, or in viral spread. At present, our results suggest an involvement of the endomembrane system in protein transport, and we suggest that the ER, possibly moving in association with myosin and on actin cables, is the most likely mechanism for intracellular transport of proteins to, and potentially through, plasmodesmata.

References

Reichel, C. & Beachy, R.N. (2000). Degredation of tobacco mosaic virus movement protein by the 26s proteasome. *Journal of Virology*, **74**, 3330-3337.

Gillespie, T., Boevink, P., Haupt, S., Roberts, A. G., Toth, R., Valentine, T., Chapman, S. and Oparka, K. J. (2002). Functional analysis of a DNA-shuffled movement protein reveals that microtubules are dispensable for cell-to-cell movement of tobacco mosaic virus. *Plant Cell*, **14**, 1207-1222.

Roberts, A.G. and Oparka, K.J. (2003). Plasmodesmata and the control of symplastic transport. *Plant Cell & Environment,* **26**, 103-124.