Intercellular Targeting Of A Viral Movement Protein To Plasmodesmata
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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 truly 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 0.1 mM colchicine solution (a concentration that was known to disrupt MTs).

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.

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.

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