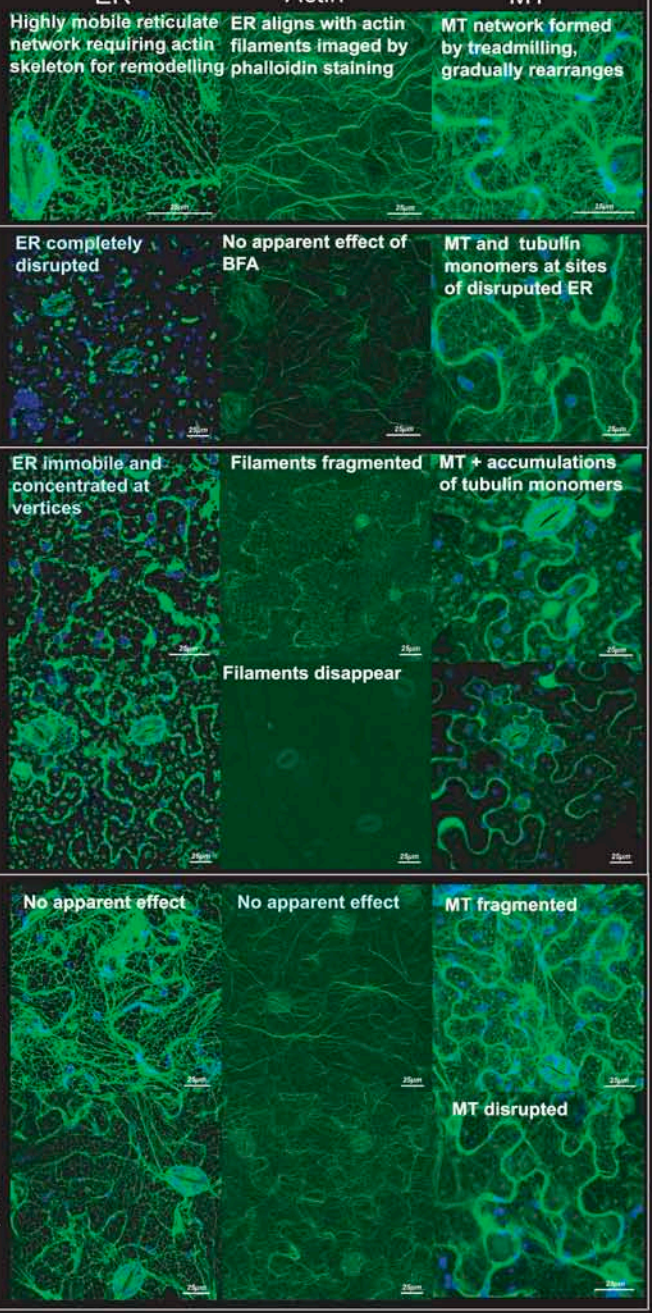
The effect of commonly used inhibitors on tobacco epidermal cell structure.

Kathryn M Wright, Sean Chapman & Karl J Oparka*

Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA Scotland.
*Present address: Institute of Molecular Plant Sciences, University of Edinburgh, Edinburgh EH9 3JR Scotland.

Introduction

During the course of our investigations into the interaction of Tobacco mosaic virus movement protein (TMV-MP) with plasmodesmata (PD) and microtubules (MT) we have utilised a range of commonly used inhibitors. Here we report the effect of these treatments on tobacco epidermal cell structure.



FRAP of PDs

At the edge of TMV.MP-GFP infection site, GFP labelling is first detected in PDs.
Using FRAP techiniques we have shown that the targeting of MP to PDs involves the actin/ER network, but not the MTs.

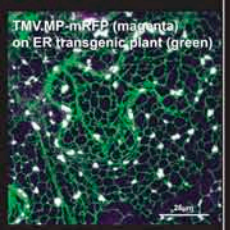
Inhibitor	Proportion FRAP	P-value	No. of replicates examined.
Control	0.41 ± 0.08	-	19
BFA	0.23 ± 0.10	0.01	14
Cytochalasin	0.27 ± 0.08	0.05	14
Latrunculin	0.28 ± 0.05	0.05	13
Colchicine	0.46 ± 0.07	ns	18
Oryzalin	0.40 ± 0.08	ns	10

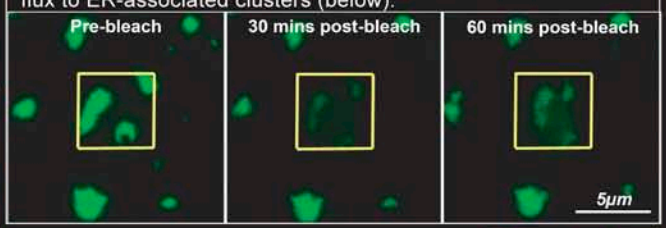
Materials and methods

We have used transgenic plants expressing GFP in the endoplasmic reticulum (ER), or fused to α-tubulin on MT, and Alexa-phalloidin staining of actin to see the various cell components. The leaf tissue was treated with 100μgml^{-1} BFA, 200μM cytochalasin B, 25μM latrunculin, 20μgml^{-1} oryzalin or 500μM colchicine for 2h prior to imaging. Control tissue was infiltrated with water.
We investigated the effects of GFP-, mRFP-, and PS-CFP-tagged TMV-MP on various cell components.

FRAP of ER clusters.

Near the leading edge of a TMV.MP-GFP infection MP accumulates at the vertices of the ER (right). These clusters (white) are stationary at the ER vertices and surrounded by freely flowing ER membranes (green).
Photobleaching of these clusters reveals that this phase of infection involves MP flux to ER-associated clusters (below).

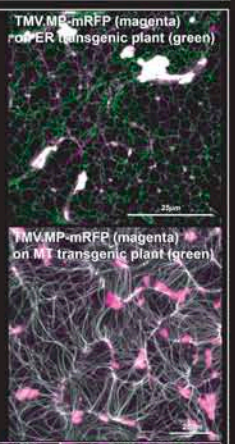




FRAP of MT

Further behind the infection front MP is transferred from the ER vertices (upper right image) to the MT (lower right image).

FRAP of this MT-associated MP (below) shows that MP does not move along the MT, is not incorporated into new MT by treadmilling, and accumulates evenly along the bleached area. There is also some indication of MP appearing in ER-clusters following beaching (darts).



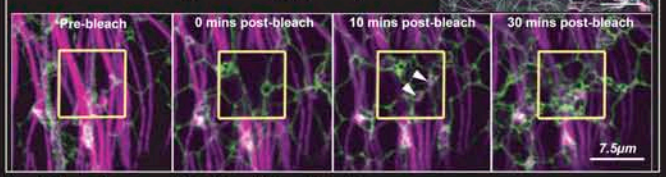
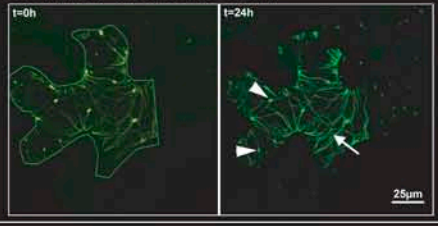


Photo-switchable CFP.

By converting PS-CFP-tagged MP to the green fluorescent form we have been able to show that MP can remain associated with MT in excess of 24h.



Conclusions

Using TMV-MP tagged with a number of fluorescent proteins, in combination with inhibitors, we have demonstrated that targeting of MP to PD involves the actin/ER network. Later on in the infection cycle, the association of MP with MT is consistent with a sequestration role prior to degradation.