

# What are you doing to me?

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

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### Introduction

During the course of our investigations into the interaction of Tobacco mosaic virus movement protein with plasmodesmata 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.

### Materials and methods

We have used transgenic plants expressing GFP in the endoplasmic reticulum (ER), Golgi bodies or linked to  $\alpha$ -tubulin for MT, antibody labelling of  $\beta$ -tubulin, and Alexa-phalloidin staining of actin to see the various cell components. The leaf tissue was treated with  $10\mu\text{gml}^{-1}$  BFA for 1h or  $100\mu\text{gml}^{-1}$  BFA,  $200\mu\text{M}$  cytochalasin B,  $25\mu\text{M}$  latrunculin,  $20\mu\text{gml}^{-1}$  oryzalin,  $500\mu\text{M}$  colchicine or 0.02% sodium azide for 2h prior to imaging. Control tissue was infiltrated with water.

Inhibitor	ER	Golgi	Actin	MT	$\beta$ -tubulin	ER & MT
<b>Control</b> Highly mobile reticulate network requiring actin skeleton for remodelling Bodies in rapid movement on ER, requiring actin Cytoskeleton required for ER & Golgi. Not yet imaged "live" MT network formed by treadmilling, gradually rearranges MT network, but fixed prior to antibody labelling ER (YFP-HDEL) in relation to MT						
<b>Brefeldin A</b> Disrupts the endomembrane system - affecting the Golgi or ER but having no apparent effect on actin or MT. At low conc. ER still shows a reticulate network Golgi is reabsorbed into the ER ER completely disrupted Reabsorbed into disrupted ER No apparent effect of high BFA MT and possible tubulin at sites of disrupted ER						
<b>Cytochalasin</b> Disrupts actin - therefore also stops the movement of ER & Golgi, & changes tubulin distribution. ER concentrated at vertices & not moving Bodies not moving & stuck at ER vertices Filaments fragmented MT + accumulations presumably of tubulin Tubulin accumulation appears to be at ER vertices						
<b>Latrunculin</b> Depolymerises actin - therefore also stops the movement of ER & Golgi, & changes tubulin distribution. Filaments disappeared						
<b>Colchicine</b> Fragments microtubules - but has no apparent effect on ER, Golgi or actin. No apparent effect No apparent effect No apparent effect MT fragmented MT fragmented <b>Control</b> At lesion edge MP not associated with MT						
<b>Oryzalin</b> Disrupts microtubules - but has no apparent effect on ER, Golgi or actin. No apparent effect No apparent effect No apparent effect MT fragmented MT disrupted MT disrupted <b>MT &amp; TMV.MP</b> MP not protecting MT so latter disrupted <b>+Colchicine</b>						
<b>Azide</b> Metabolic inhibitor, depletes ATP levels - stops the movement of ER & Golgi, disrupts actin & changes tubulin distribution. ER concentrated at vertices & not moving Bodies not moving Filaments fragmented Majority of MT intact but tubulin accumulations Tubulin at ER vertices						