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 CFP in the endoplasmic reticulum (ER), Golgi bodies or linked to -tubulin for MT, antibody labelling of -tubulin, and Alexa-phallloidin staining of actin to see the various cell components. The leaf tissue was treated with 10µg/ml BFA for 1h or 100µg/ml BFA, 200µM cytochalasin B, 25µM latrunculin, 20µg/ml oryzalin, 500µM colchicine or 0.02% sodium azide for 2h prior to imaging. Control tissue was infiltrated with water.

Control
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

Brefeldin A
Disrupts the endomembrane system - affecting the Golgi or ER but having no apparent effect on actin or MT.
ER BFA10µg/ml
ER completely disrupted
Reabsorbed into disrupted ER
No apparent effect of high BFA
MT and possible tubulin at sites of disrupted ER

Cytochalasin
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

Latrunculin
Depolymerises actin - therefore also stops the movement of ER & Golgi, & changes tubulin distribution.
ER Golgi
Actin Filaments disappeared

Colchicine
Fragments microtubules - but has no apparent effect on ER, Golgi or actin.
ER Golgi
MT fragmented

Oryzalin
Disrupts microtubules - but has no apparent effect on ER, Golgi or actin.
ER Golgi
MT MT disrupted

Azide
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

Control
At lesion edge MP not associated with MT
MT & TMV, MP not protecting MT so latter disrupted

+Colchicine