Studying plasmodesmal targeting of TMV-MP using FRAP

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Tobacco mosaic virus (TMV) is frequently studied as a model system to identify the mechanisms involved in plant viral infections. The structure and functions of all its genes have been identified and of particular interest is the 30-kDa protein required for cell-to-cell movement. This movement protein (MP) is involved in the transport of viral RNA to and through plasmodesmata (PD). TMV-MP accumulates within PDs and increases the size-exclusion limit of the PDs to allow trafficking of viral RNA to the next cell in the form of a MP-complex.

In recent years it has been possible to produce TMV derivatives that express fully functional MP fused to green fluorescent protein (GFP) thus enabling visualisation of the MP. At the leading edge of an infection, and throughout the infection site, MP is located within PDs. Near the edge of an infection, MP is associated with vertices of the cortical endoplasmic reticulum (ER) whilst further in the MP can be seen aligning with the microtubules (MT). Some researchers have speculated that MT are involved in the targeting of TMV-MP to PDs. However, at SCRI we have shown that disruption of MT with pharmacological agents has no effect on lesion growth and we suggest that MT are instead involved in the degradation of MP later in the infection process.

We are therefore investigating the targeting of MP to PDs using a technique called fluorescence recovery after photobleaching (FRAP). Tobacco leaves are infected with TMV-MP-GFP virus and the edge of the infection site located using a confocal laser scanning microscope. Using the laser it is possible to bleach the fluorescent GFP attached to MP within a PD. Images are recorded and then measured as the fluorescence increases due to the movement of new MP-GFP into the PD. Over the course of 40 minutes, under control conditions, the fluorescence recovers to approximately 40% of the pre-bleached value (see figure 1). We have tested whether this fluorescence recovery is influenced by a range of inhibitors that target the action of different cell components.

Provisional results indicate that movement of MP to the PDs is energy dependent since it is severely reduced in the presence of azide, a metabolic inhibitor (see figure 1). BFA, which disrupts the ER network, BDM, an inhibitor of myosin motors, and both cytochalasin and latrunculin, which affect actin activity, also decrease fluorescence recovery. However, colchicine and oryzalin, which inhibit MT action, and cycloheximide, an inhibitor of protein synthesis do not appear to have any effect on the fluoresence recovery. This therefore supports the view that MT are not involved in the movement of MP to PDs. We therefore propose that a mechanism involving the ER and the actin network is reponsible for trafficking MP to the PDs.

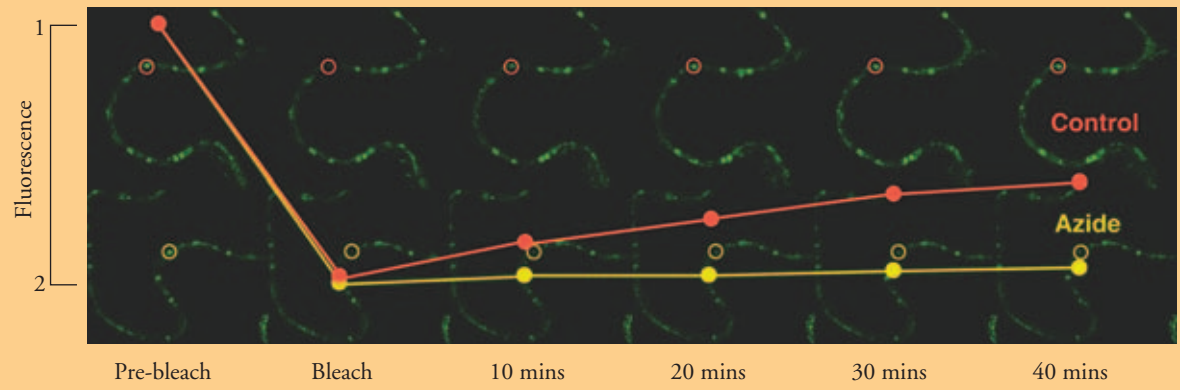

Figure 1 Images in the background illustrate the FRAP treatment of a PD under control conditions (see green PD within upper red circles) compared with the low level of recovery when treated with azide (green PD within lower yellow circles). The fluorescence intensity of these PDs was measured and is presented in the graph as a proportion of the pre-bleached value against time.

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