Real-time studies of plasmodesmal gating in developing leaf cells

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Introduction

During development, leaves undergo a transition from net carbon import to export called the sink-source transition. We have previously shown that epidermal cells on sink leaves allow free diffusion of molecules up to 50kDa through their plasmodesmata whereas source leaf cells do not (Oparka *et al.*, 1999). This is correlated with a change in plasmodesmal structure from a simple to branched architecture during tissue maturation (Roberts *et al.*, 2001). However, it remains to be determined whether the reduction in communication between maturing cells is a gradual or abrupt process. The microprojectile bombardment technique used for the above study is random and unpredictable, and observations of GFP movement are not 'real-time' as the cells only become visible several hours after bombardment (Figure 1a-c). The recent development of PA-GFP, a photoactivatable form of GFP (Patterson and Lippincot-Schwartz, 2002) has enabled us to commence a real-time, non-invasive study of cell-to-cell communication in maturing leaves. PA-GFP is a variant of EGFP (enhanced GFP, Clontech) that is barely visible until exposure to ~400 nm light, at which point fluorescence increases up to 100 fold. PA-GFP has been used to great effect in animal cells and we now report its utility in plants (see also poster by Wright *et al.*). We have expressed PA-GFP transiently in *Nicotiana* species and constitutively in transgenic *Arabidopsis*.

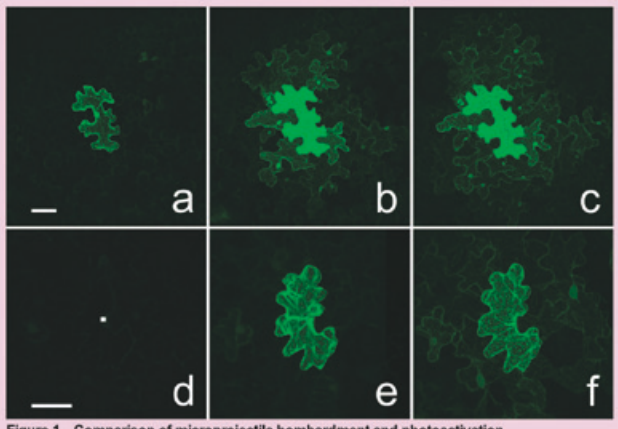


Figure 1. Comparison of microprojectile bombardment and photoactivation
N. benthamiana cells bombarded with pRTL2.GFP took several hours to accumulate detectable GFP; **(a)** is 6 hours post-bombardment. The fluorescence of the bombarded cell increased with time; **(b)** shows the cell at 21 hours. Movement of GFP into surrounding cells was detectable within one day **(b)** and more cells became visible as GFP production increased; **(c)** is 48 hours post bombardment. *N. tabacum* cells transiently expressing PA-GFP were barely visible before activation **(d)**. After activation in a small area over the nucleus (small square in **(d)**), the fluorescence spread throughout the cell within minutes **(e)**. If the activated PA-GFP moved it became visible in surrounding cells after one or a few hours and the fluorescence of the activated cell decreased; **(f)** is 16 hours post-activation. Scale bars represent 50 µm. Images were taken with a Leica SP2 confocal microscope.

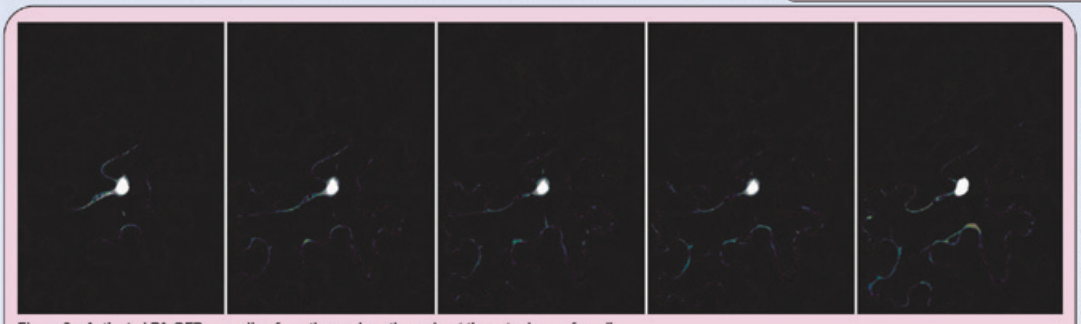


Figure 2. Activated PA-GFP spreading from the nucleus throughout the cytoplasm of a cell
These images are from a series of 20 taken over two minutes immediately following the activation of PA-GFP in the nucleus of a *N. benthamiana* cell. A false-colour look-up-table (LUT) was applied to make the changes in fluorescence intensity more obvious.

Activation of PA-GFP in plants

PA-GFP-expressing cells were located using high gain (e.g. 700V) with 488 nm light. For imaging, the gain was reduced to between 350V and 500V to remove background fluorescence. PA-GFP was activated by single scans of regions of interest at maximal zoom (x32) with 405 nm light from a blue diode laser. PA-GFP was activated in nuclei of expressing cells in *Nicotiana* species 24-48 hours post agroinfiltration and also in transformed *Arabidopsis*. PA-GFP moved out of the nucleus into the surrounding cytoplasm almost instantaneously and then rapidly distributed throughout the cell (Figure 2).

Movement in *Nicotiana* species as a function of leaf age

Extensive movement of PA-GFP from activated cells on the smallest infiltratable leaves was observed (Figures **3a** and **b**) but it was restricted to the activated cell in the majority of mature source cells (Figures **3c** and **d**). Interestingly, as was observed with bombardment of pRTL2.GFP, some mature cells allowed movement of PA-GFP into one or two neighbouring cells (Figure **4a**). This suggests that at any one time the plasmodesmata linking individual mature cells may be gated to allow free exchange of information. Movement of PA-GFP on source leaves was most commonly observed into and out of subsidiary cells adjacent to stomata (Figure **4b**).

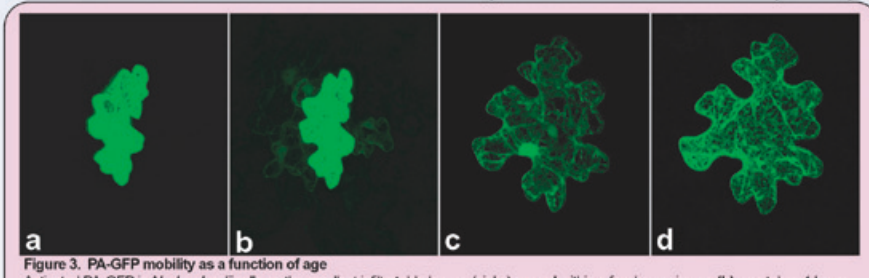


Figure 3. PA-GFP mobility as a function of age
Activated PA-GFP in *N. clevelandii* cells on the smallest infiltratable leaves (sinks) moved within a few hours; image **(b)** was taken 4 hours after image **(a)**. In contrast, the PA-GFP did not move from the majority of activated cells on large (source) leaves; figures **(c)** and **(d)**.

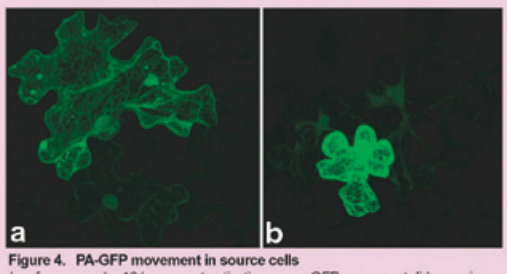
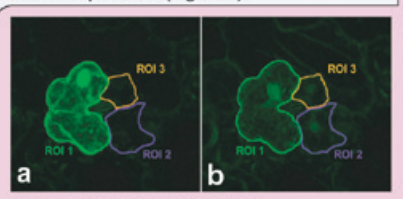


Figure 4. PA-GFP movement in source cells
In a few cases, by 16 hours post-activation, some GFP movement did occur in source tissue of N. tabacum **(a)** and *N. clevelandii* **(b)**.

Quantitation of movement

The activated PA-GFP is diluted as it diffuses from the initial cell and is more difficult to detect at greater distance from the activated cell, therefore the observed movement of the probe is partly dependent on the amount initially activated. The movement of the activated PA-GFP in transformed *Arabidopsis* was also quantified (Figure 5).



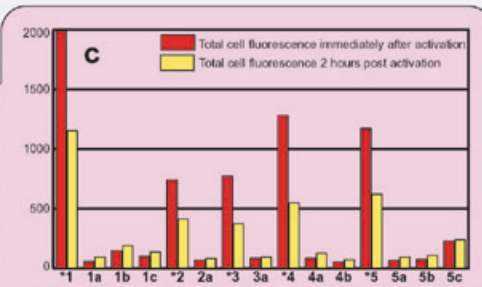


Figure 5. Quantitation of movement
The total fluorescence of the activated cell (ROI1 in **(a)** and **(b)**, and * in **(c)**) was determined immediately after activation **(a)** and 2 hours later **(b)**. The fluorescence of some surrounding cells was also measured (additional ROIs). The fluorescence of all activated cells decreased while it increased in non-activated cells. **(a)** and **(b)** show the ROIs for cell 4 in **(c)**; ROI 2 corresponds to 4a and ROI3 to 4b.

Summary

This work is preliminary but it demonstrates the enormous potential of PA-GFP for non-invasive, real-time studies of plasmodesmal gating. Although the correlation between the exact position of the sink-source transition and the extent of GFP diffusion out of photoactivated cells has not yet been determined, it is likely that even the youngest tissues examined here were well beyond the transition zone (*i.e.* source tissue). The data suggest that the down-regulation of the plasmodesmal SEL is gradual and occurs after the sink-source transition.

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References

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