Generation of a fluorescent reporter protein for the plant vacuolar membrane

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Background

Tonoplast Intrinsic Proteins.

Vacuoles are the hallmark of plant cells. They can act as cellular dustbins (lytic vacuoles) or as receptacles for storage proteins (storage vacuoles). It has been claimed that both types of vacuole can be found in a single cell.

Different isoforms of tonoplast intrinsic proteins (TIPs) are classically used as markers for different vacuoles. For example, α-TIP is a marker for storage vacuoles whereas γ-TIP is a marker for lytic vacuoles. The localisation of a third isoform, δ-TIP, in living cells has not yet been systematically explored.

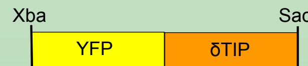
Yellow Fluorescent Protein.

Fluorescent proteins, originally purified from a jellyfish, are used as tools for studying subcellular localisation in vivo by fusion to a protein of interest and observation using confocal microscopy.

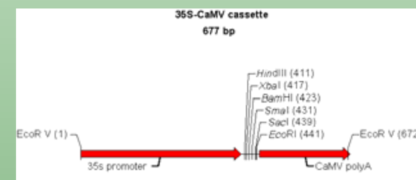
Aim

I wanted to establish whether tonoplast intrinsic protein (δTIP) localised to the lytic or the storage vacuole. My main objective was to clone and express delta TIP tagged with Yellow Fluorescent Protein (YFP) to determine its subcellular localisation in living tobacco cells using confocal microscopy.

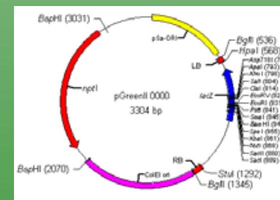
Methods



1. The fusion YFP-δTIP (above) was inserted into the multi cloning site of the 35S cassette cloning vector (below) at restriction sites XbaI and SacI. The vector was introduced into *Escherichia coli* cells.



2. The vector was cut at the EcoRV sites to isolate the YFP-deltaTIP expression cassette. This was inserted into the p-Green plant vector (below). The vector was then inserted in *Agrobacterium tumefaciens*.



3. A tobacco leaf was infiltrated with the Agrobacteria and left for three days for the protein to be expressed.

Results - Cloning

Cloning step 1. The asterisks indicate *E. coli* colonies containing the YFP-δTIP expression cassette (arrowhead)

Cloning step 2. Two *E. coli* colonies (asterisks) contain the finished cloning product (YFP-deltaTIP fusion into the p-Green plant expression vector).

Having successfully cloned the fluorescent protein reporter, I have expressed it in tobacco leaves and studied its localisation by confocal microscopy.

Results - Microscopy

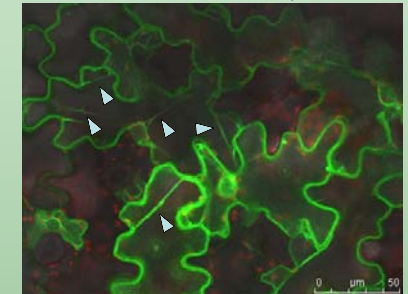


Figure 1. Tobacco leaf epidermal cells expressing YFP fused to Delta-TIP. Green, YFP; Red, chlorophyll autofluorescence.

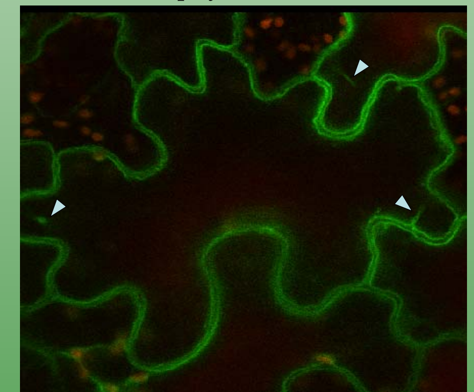


Figure 2. A higher magnification image clearly indicates the membranous nature of localisation. The presence of transvacuolar strands (arrowheads) is indicative of the tonoplast membrane.

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Conclusions

I successfully cloned and expressed the YFP-Delta TIP protein, and found that it localises to the tonoplast of the large central, lytic vacuole.