Identification of cytoskeletal and plasmodesmatal proteins by screening of viral vector cDNA-GFP fusion libraries

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We have devised a functional genomics procedure to determine subcellular targeting of unknown plant proteins using viral vectors and fluorescent tagging.

Cloned transcripts are expressed *in planta* as GFP-fusion proteins

Fluorescent fusion proteins localizing to particular organelles can be identified by confocal microscopy.

Sequencing a clone from an individual lesion identifies the corresponding gene.

Method

Extract RNA from plant tissue (*Nicotiana benthaminia* roots or from *Arabidopsis* cell culture and leaf material).

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Select full length mRNA by CAP-dependent method.

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Synthesize cDNA and clone *en masse* into a tobacco mosaic virus vector for expression as GFP (or RFP) fusion proteins.

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Inoculate libraries onto *Nicotiana* leaves.

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Screen leaves for fluorescent lesions showing subcellular targeting. Each lesion expresses a single cDNA-GFP fusion.

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Excise lesions of interest.

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Extract RNA.

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RT-PCR.

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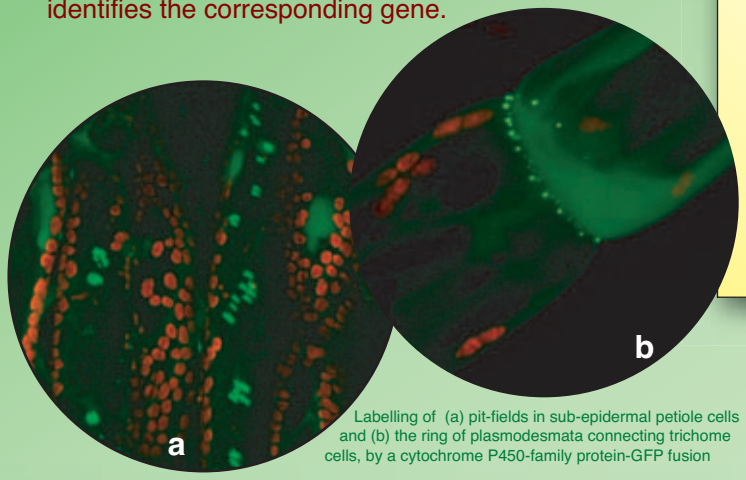
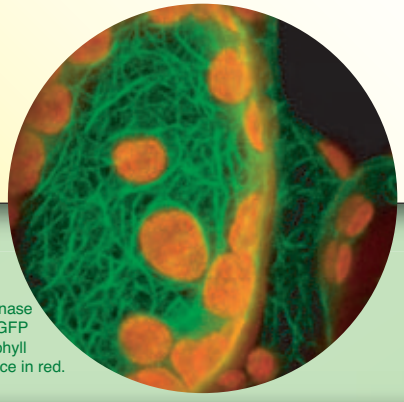
Sequence PCR product.



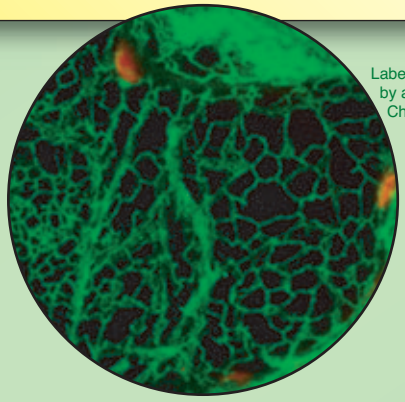
Labelling of (a) pit-fields in sub-epidermal petiole cells and (b) the ring of plasmodesmata connecting trichome cells, by a cytochrome P450-family protein-GFP fusion



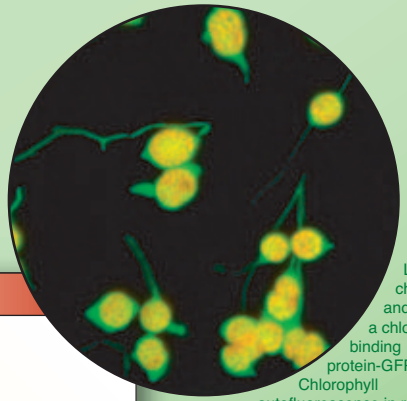
Labeling of cytoskeletal strands by a kinase family protein-GFP fusion. Chlorophyll autofluorescence in red.

Results

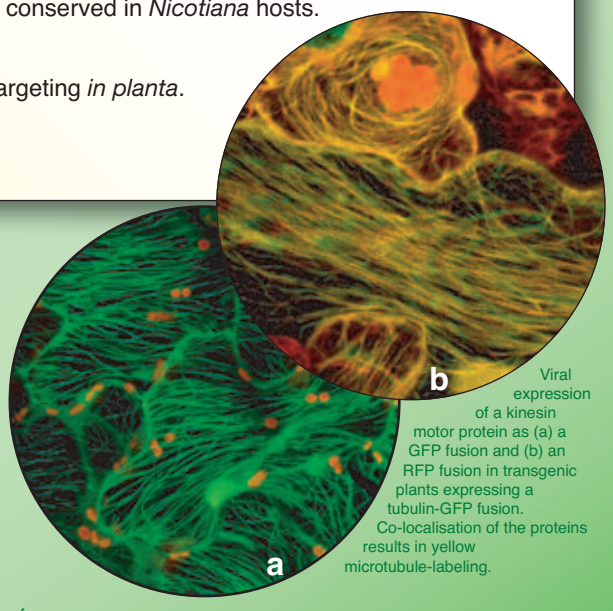
- Proteins that localize to numerous locations have been observed.
- For *Arabidopsis* cDNAs, the corresponding gene can readily be identified from the complete genome sequence.
- The fidelity of localization of characterized *Arabidopsis* proteins appears to be conserved in *Nicotiana* hosts.
- The localization of unknown proteins has provided clues as to their function.
- Some putative localizations predicted from the sequence tally with observed targeting *in planta*.
- Of particular interest are a number of lesions that show labeled cytoskeletal components or putative plasmodesmata-localised proteins.



Labeling of endoplasmic reticulum and nuclear envelope by a putative endoplasmic reticulum protein-GFP fusion. Chlorophyll autofluorescence in red.



Labeling of chloroplast stroma and stromules by a chlorophyll binding protein-GFP fusion. Chlorophyll autofluorescence in red.



Viral expression of a kinesin motor protein as (a) a GFP fusion and (b) an RFP fusion in transgenic plants expressing a tubulin-GFP fusion. Co-localisation of the proteins results in yellow microtubule-labeling.

Website

See high quality images of protein targeting and associated sequence information on our website (http://bioinf.scri.sari.ac.uk/protlocdb.html).

Acknowledgements
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References
Escobar NM, Haupt S, Thow G, Boevink P, Chapman S, Oparka K. (2003) High-throughput viral expression of cDNA-green fluorescent protein fusions reveals novel subcellular addresses and identifies unique proteins that interact with plasmodesmata. Plant Cell 15: 1507-23.