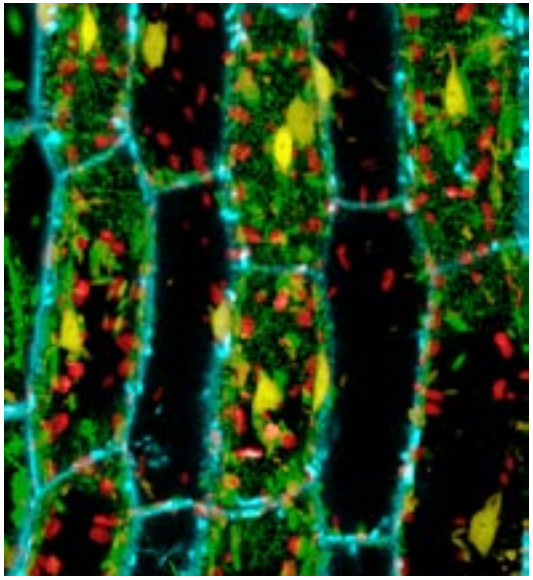
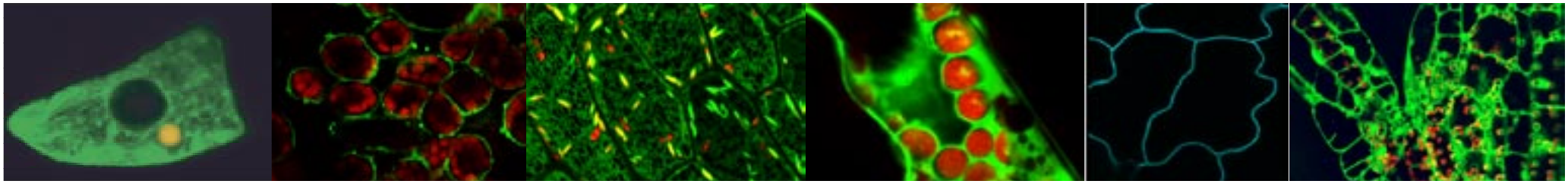
Fluorescent protein markers in Arabidopsis plants.

Over past years, we have developed a set of new genetic and optical techniques which allow us to visualise and manipulate cells within living plants. Soon after the cloning of the *Aequoria victoria* jellyfish green fluorescent protein (GFP) by Douglas Prasher, we obtained the cDNA sequence and proceeded to adapt the gene for use as a bright marker in transgenic plants. Unfortunately, the wild-type GFP cDNA is not expressed in *Arabidopsis*. We identified and removed a cryptic intron from within the *gfp* gene, and introduced modifications that confer improved folding and spectral properties and to alter the subcellular localisation of the protein. All of these alterations were incorporated into a single modified form of the gene (*mgfp5-ER*) which we now routinely use for monitoring gene expression and marking cells in live transgenic plants (Siemering *et al.*, Current Biology 6:1653-1663, 1996; Haseloff *et al.*, PNAS 94:2122-2127,1997).

We have developed fluorescence microscopy techniques for high resolution observation of living cells. The expression of GFP within an organism produces an intrinsic fluorescence that colours normal cellular processes, and high resolution optical techniques can be used non-invasively to monitor the dynamic activities of these living cells. Using coverslip-based culture vessels, specialised microscope objectives and the optical sectioning properties of the confocal microscope, it is possible to monitor simply and precisely both the arrangement of living cells within a meristem, and their behaviour through long time-lapse observations. In order to produce different coloured fluorescent proteins that are expressed well in plants, we have produced the variants mYFP (S65G, S72A, V163A, I167T, S175G, T203Y) and mCFP, (Y66W, V163A, S175G). The mCFP and mYFP variants have properties comparable to mGFP5, but possess distinct spectral properties. Although the emission spectra of the fluorescent proteins overlap to a large degree, laser scanning confocal microscopy allows the use of monochromatic light for selective excitation of the GFP variants. In addition, we are using derivatives of fluorescent proteins from coral to provide longer wavelength signals.



We have generated a range of GFP protein fusions that have provided useful subcellular markers in *Arabidopsis*. For example, we have produced histone2B and extensin fusions that are useful for following nuclear dynamics and visualising cell wall substructure, respectively.

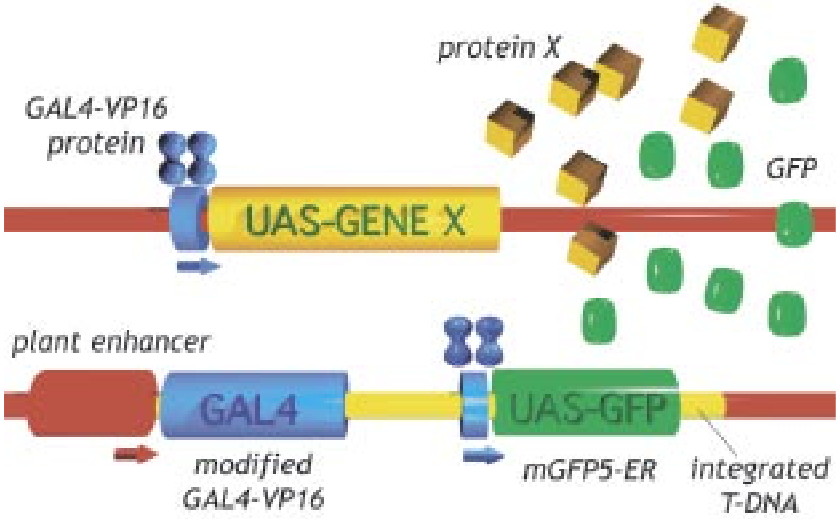


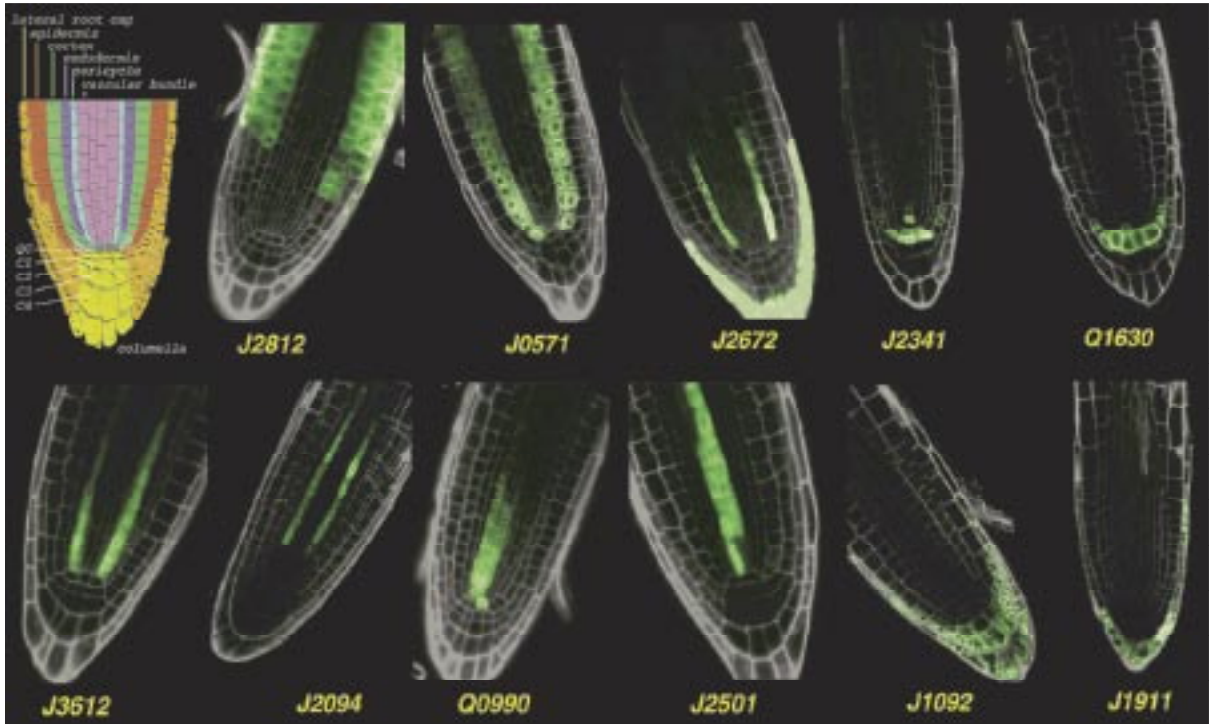
GAL4 targeted expression.

In order to genetically manipulate cells during meristem development, we have devised a scheme for targeted gene expression, which is based on a method widely used in *Drosophila* (Brand and Perrimon, Development 118:401-415, 1993). We have used an "enhancer-trap" strategy to generate many transgenic plants which express different patterns of a yeast transcription activator, GAL4. A chosen target gene can then be placed under the control of GAL4 upstream activation sequences (UAS), transformed into plants, and maintained silently in the absence of GAL4. Genetic crosses between this single line and any of the library of GAL4-containing lines specifically activates the target gene in a particular tissue or cell type. The phenotypic consequences of mis-expression, including those deleterious to the organism, can be conveniently studied.

We found that GAL4 is not expressed in *Arabidopsis* due to a high A/T content, which can interfere with mRNA processing in plants. We have produced a modified form, mGAL4-VP16, that it is expressed efficiently in plants, and randomly inserted the modified gene into the *Arabidopsis* genome, using *Agrobacterium tumefaciens*-mediated transformation. The transformation vector was designed so that expression of the mGAL4-VP16 gene would be dependent upon the fortuitous proximity of an *Arabidopsis* enhancer element. The inserted DNA also contained a GAL4-responsive *mGFP5-ER* gene. Thus, interesting "enhancer-trap" patterns of GAL4 gene expression were immediately and directly visible, with each GAL4-expressing cell marked by bright green fluorescence. We have used in vivo detection of GFP to directly screen for GAL4-directed GFP expression in 8000 regenerated plantlets. We have documented a collection of over 250 *Arabidopsis* lines with distinct and stable patterns of mGAL4-VP16 and GFP expression in the root. This collection has been added to by a screen of an extra 5000 lines generated as part of a collaboration with Scott Poethig's laboratory. In addition, we have recently completed the generation of another 13,000 lines containing a HAP1-CFP enhancer trap vector. These libraries of transgenic lines provide several major benefits:

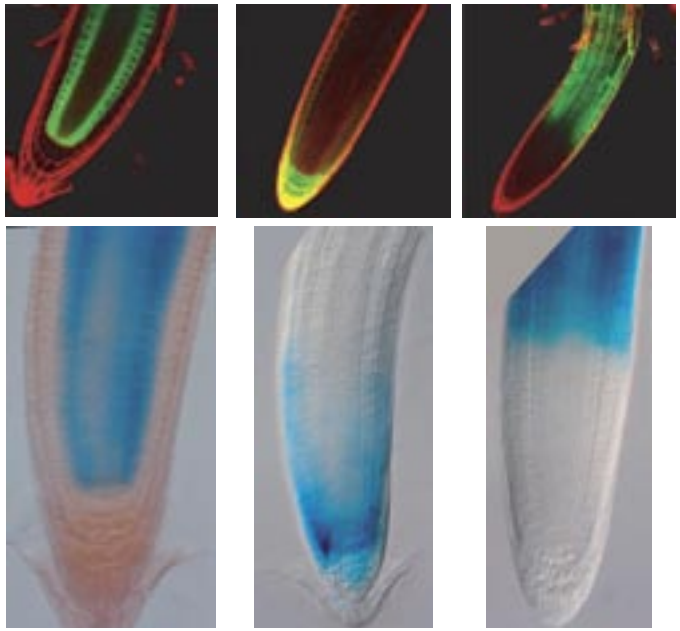
(i) a valuable set of markers, where particular cell types are tagged and can be visualised with unprecedented ease and clarity in living plants. These are





becoming widely used in developmental studies, e.g. Berger *et al*., Current Biology, 1998; Sabatini *et al*., 1999; Wysocka-Diller *et al*., 2000.

(ii) a means of triggering ectopic expression of a chosen gene at a particular time and place during *Arabidopsis* development. Regulatory proteins or toxins can be transformed and maintained in *Arabidopsis*, silent behind a GAL4-responsive promoter. We can now activate these genes in specific cells by crossing to a chosen mGAL4-VP16 expressing line. This is a powerful system for genetically perturbing and monitoring the behaviour of particular cells within a living plant.



(iii) a source of mutants. The collections contain transformed lines with mutant phenotypes, some likely T-DNA tagged. We have also used the GAL4 system as the basis for a gain-of-function screen in the root epidermis, and have islolated a number of mutants including a tagged allele of *tornado1*. (L. Laplaze, E. Truernit & J.H. unpublished).

(iv) a means of identifying important regulatory genes. Several GAL4 lines with bright and distinct GFP expression patterns have been mapped and found to identify important regulatory genes. For example, we have found that the GAL4 gene in line J0481 is inserted next to a WRKY transcription factor expressed in the root epidermis (L. Laplaze & J.H. unpublished), and similarly M0223 contains an insertion adjacent to the *Cup-Shaped Cotyledon 1* gene (Cary et al., 2002).