3D visualisation of cellular organisation during *Arabidopsis* meristem development

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The genetic control of plant development is mediated by cellular interactions, and we have developed new optical and genetic techniques for manipulating the fates of cells inside living meristems. The *Arabidopsis* root meristem provides an ideal model system for investigating cellular interactions. The root meristem possesses indeterminate growth and has a simple and transparent architecture. Lateral root meristems are derived from single pericycle cells within mature roots, and lateral meristem initiation and growth is directly accessible to microscopic, and can be triggered by auxin application. It has become crucial to gain a better understanding of the precise timing and arrangement of cell divisions within normal, and genetically perturbed meristems. Our aim has been to develop and apply better techniques for the three dimensional visualisation of cell proliferation and differentiation during initiation and growth of root meristems: (i) to adapt computational methods for 3D segmentation to mark the arrangements of particular cells within optically sectioned meristems, (ii) describe the arrangement and timing of cell divisions and cell shape changes that take place during initiation of secondary root meristems and (iii) adopt these same techniques to analyse meristems that have been genetically perturbed by GAL4 targeted cell ablation or misexpression.

We are using these techniques to dissect a cellular interaction that appears to be responsible for the control of cell division and elongation in the root meristem. Work from our laboratory suggests that positional information may be exchanged between the lateral root cap and epidermal cell layers to control this transition in the growing root. We are using confocal microscopy and 3D reconstruction techniques to map the precise arrangements of lateral root cap cells with respect to the cell types in the underlying epidermis layer.

We have generated transgenic *Arabidopsis* lines that contain a histone2b - yellow fluorescent protein fusion, which allows the direct observation of nuclear dynamics in living tissues. We are using this marker to describe the pattern of cell divisions around the epidermis – lateral root cap boundary. In addition, we have used this nuclear marker to construct a system for marking clonal sectors – to allow us to precisely map cell lineages in the root meristem. For example, we have precisely mapped the common lineage of lateral root cap and epidermal cells. These markers provide both dynamic and historical records of cell division in the meristem, and are a valuable complement to 3D segmentation for model building.

We are now using GAL4 targeted gene expression to kill cells and to trigger ectopic cell divisions in the lateral root cap and epidermal layers of the meristem. We have characterized the epidermis and root cap expression patterns of a number of GAL4-GFP *Arabidopsis* lines and used these to trigger cell death or ectopic cell division in the different layers. We see phenotypes due to the localized death or proliferation of cells which are consistent with the intercellular control of cell division and elongation. These experiments are the prototype of a general approach to dissecting meristem regulation: (i) establish whether there is a precise correlation between the 3D arrangement of cells and a transition in the meristem, (ii) use GFP markers to visualise behaviour at this transition, and (iii) directly test models for cellular interaction by perturbing cell division patterns within specific cells of the root meristem.