

Meeting report

Mile-high view of plant biology

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A report on Plant Biology 2002, the annual meeting of the American Society of Plant Biologists, Denver, USA, 3-7 August 2002.

Plant Biology 2002, held in Denver, the 'mile-high city', covered diverse topics in plant biology, from molecular biology and genomics, through cell and developmental biology, to whole plant physiology. By necessity, this article is limited in its coverage: I focus on two of the major symposia about high-throughput biology and the cytoskeleton (although I also include details from other related talks).

High-throughput biology and plant 'omics'

The *Arabidopsis* genome sequence was published in 2000 but, as described by Brian Haas (The Institute for Genomic Research (TIGR), Rockville, USA), continuing research has led to improved accuracy and uniformity in the reannotation of the genome, and to the verification of hypothetical proteins through the identification of full-length cDNA sequences. Such work forms the foundations for large-scale analyses of the *Arabidopsis* genome, and for the development of large collections of *Arabidopsis* mutant lines that are now available from different sources, as described by Ken Feldmann (Ceres Inc, Malibu, USA). Many researchers are now taking advantage of these resources, which include overexpression lines and 'T-DNA knockouts' in which transferred-DNA from *Agrobacterium tumefaciens* acts as an insertional mutagen.

The symposium on 'High-throughput biology in the post-genomic era' featured talks on genomics, proteomics, metabolomics and phenomics and reflects a paradigm shift from focused analysis to broad-range screening. Large-scale screening projects require large-scale funding. In their talks, however, Jeff Woessner (Paradigm Genetics, Research Triangle Park, USA), John Yates (Torrey Mesa Research

Institute, San Diego, USA), Richard Trethewey (Metanomics GmbH, Berlin, Germany) and Feldmann each stressed that higher throughput will not necessarily lead to greater understanding unless experiments are properly designed and the resulting data managed efficiently. Feldmann described the phenotype-screening 'phenomics' program of soil- and agar-grown knockout lines of *Arabidopsis* at Ceres that should nearly saturate the genome by the end of this year. To date, approximately 15% of T-DNA insertion lines give visible phenotypes, with 25% of these being putative gametophytic lethals. Interestingly, recovered insertional events are not completely random, occurring more frequently just outside coding regions in the promoter and untranslated sequence. As described by Trethewey, Metanomics is also screening a large collection of *Arabidopsis* knockout and overexpression lines. With rigorously controlled growth and extraction conditions, and gas or liquid chromatography followed by mass spectroscopy, Metanomics can detect from 350 to 600 small metabolites in cell extracts, including a wide range of amino and organic acids, sugars and sugar derivatives. Although it has proved difficult to predict changes in metabolite distributions from changes in RNA levels, approximately 5 to 10% of the *Arabidopsis* lines show changed levels or patterns of metabolites, which vary from pathway-specific to pleiotropic.

The recent completion of the rice genome sequence, and its use for proteome analysis, formed the basis of the talk by Yates. His work has shown that a combination of matrix-assisted laser desorption/ionization - time of flight mass spectrometry (MALDI-TOF MS) and multi-dimensional liquid chromatography can be used to identify upwards of 2,500 individual proteins from extracts of leaves, roots and seeds. Analysis of expression patterns for different families of proteins raises numerous biological questions. For example, although more than 50 different peroxidases were identified, with some proteins expressed throughout leaves, roots and seeds, the functions of the 27 root-specific peroxidases remain unknown.

In a related symposium, Brad Till (Fred Hutchinson Cancer Research Center, Seattle, USA) discussed targeting induced local lesions in genomics (TILLING), a PCR-based method for identifying point mutations in chemically mutagenized organisms. Because this approach can isolate a spectrum of alleles resulting from single-base changes within target genes, it is a powerful complement to gene-knockout technology as it can be applied to essential genes in which knockouts would prove lethal. To date, the *Arabidopsis* TILLING Project has TILled more than 100 genes, identifying a large number of point mutations in them, the majority of which are missense and silent.

Emerging patterns in cytoskeletal organization and control

Plant cells contain dynamic arrays of actin and microtubules that regulate growth polarity and morphogenesis and which vary between cells and tissues and in response to external stimuli. Until recently, however, there has been little progress in determining the factors regulating this dynamism and organization. The speakers covering ‘The dynamic cytoskeleton in plant cell biology’ demonstrated that cytoskeletal regulatory mechanisms can be dissected in plant cells that show polarized growth. Model systems discussed included trichomes (leaf hairs), rapidly growing pollen tubes, and the epidermal pavement cells of leaves that interlock like pieces in a jigsaw puzzle and are produced by cell expansion in lobes and growth inhibition in neck regions (Figure 1). Chris Staiger (Purdue University, West Lafayette, USA) spoke on his group’s biochemical characterization of the actin-binding protein profilin. Measurements of intracellular actin and profilin concentrations in growing maize pollen tubes indicate that many of the differences in actin organization and dynamics towards the tube tip can be explained, at least in part, by the steep, tip-high gradient of calcium and profilin’s calcium-dependent buffering of the pool of unpolymerized actin. In collaboration with Noni Franklin-Tong (University of Birmingham, UK), Staiger has also investigated the self-incompatibility (SI) response of poppy pollen. In this system, pollen-tube growth rapidly ceases following recognition of allele-specific S proteins, with this recognition triggering a large calcium influx and a sustained depolymerization of actin filaments (F-actin). Quantitative analysis of total actin and profilin levels indicate that other factors must also be involved during depolymerization of F-actin in the SI response.

Zhenbiao Yang (University of California, Riverside, USA) presented elegant data showing that ROPs, a plant-specific family of Rho GTPases, are master switches that control multiple downstream pathways regulating the cytoskeleton in *Arabidopsis*. Activated ROPs regulate actin dynamics through RICs, a group of proteins containing the CRIB domain, a Cdc42/Rac-interactive binding motif. In pollen-tube tips, locally activated, pollen-specific ROPs bind to and

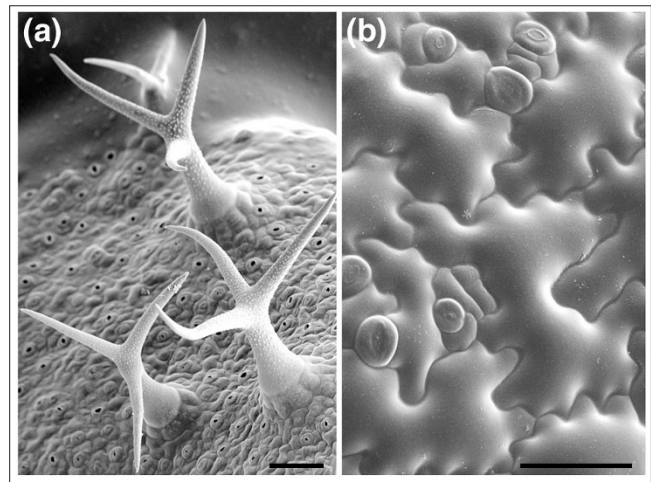


Figure 1

Arabidopsis thaliana trichomes and epidermal pavement cells provide model systems in which the cytoskeleton’s role in plant cell development is investigated. **(a)** Unicellular, multi-branched trichomes on the leaf epidermis of a 6-day-old wild-type plant. **(b)** Interlocked pavement cells form the epidermis of a 6-day-old cotyledon. Pavement cell development requires the activity of a ROP/RIC mediated pathway, while mutants such as *spike1* lack pavement-cell lobing, and also have defective trichome morphology. Scale bars represent 50 μm . Scanning electron micrographs kindly provided by Geoff Wasteneys, Australian National University.

activate two functionally distinct RICs, with one promoting actin assembly and another promoting actin disassembly, probably through the formation of the calcium gradient and profilin activation. It is this ROP/RIC-mediated interplay between actin assembly and disassembly that generates the dynamic array of diffuse actin at the tube tip that is essential for localized exocytosis and continued polarized growth (Figure 2). The formation of lobed pavement cells is also ROP/RIC-dependent. In growing lobes activated ROPs activate RICs, promoting the formation of diffuse cortical actin, but in non-growing necks inactivated ROPs do not bind RICs. Instead, one of these inactivated RICs binds to and stabilizes transverse microtubule arrays. It remains to be determined how neighboring cells coordinate their growth such that the growing lobe of one cell interlocks with the non-growing neck of the adjacent cell.

Other regulators of the cytoskeleton are also emerging, and these may also be parts of ROP signaling pathways. Dan Szymanski (Purdue University, West Lafayette, USA) focused on the *spike1* mutant of *Arabidopsis*. This mutant has defects in trichome morphology and lacks lobing of pavement cells, as a result of failure to form localized patches of diffuse cortical actin during lobe initiation. The 207 kDa Spike protein has some carboxy-terminal homology to the CDM proteins that in animal cells locally recruit and activate Rac GTPases, modulating actin organization. Thus, Szymanski suggests that Spike1 locally activates ROPs during the development of pavement-cell lobes, and raised the possibility that it might

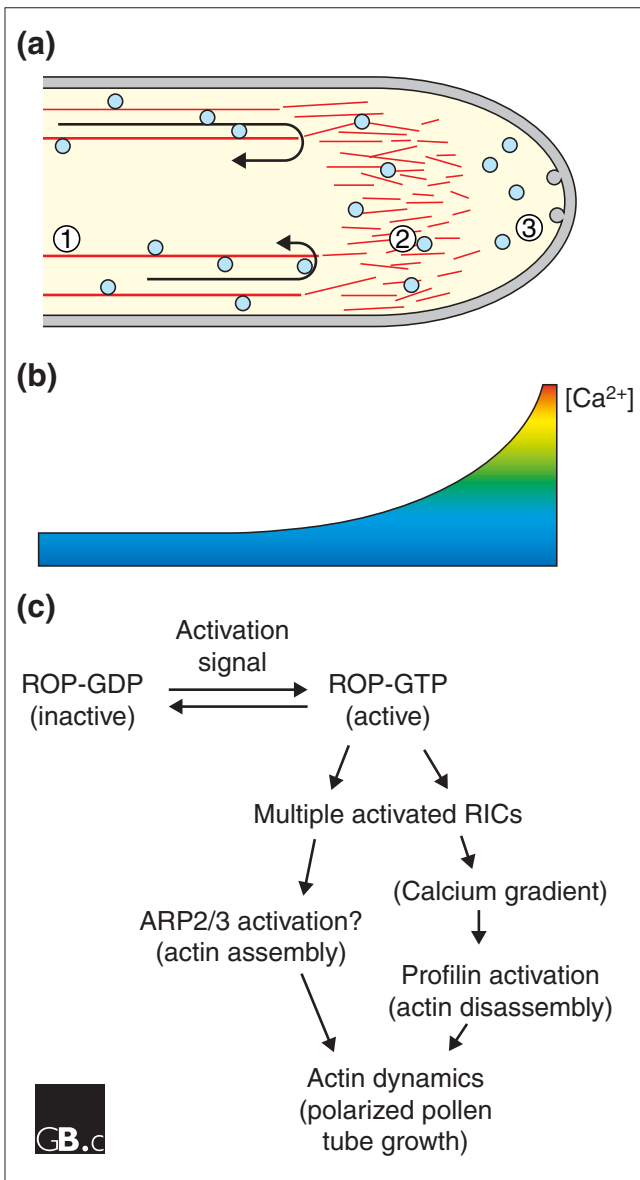


Figure 2
 ROP-mediated signaling controls the actin dynamics necessary for pollen tube growth. **(a)** Pollen tubes elongate through vesicle secretion at the tip of the tube. The delivery of vesicles (blue circles) requires cytoplasmic streaming (arrows) generated by stable actin bundles (1) and the movement of these vesicles through a meshwork of highly dynamic actin filaments (2) to an actin-free zone at the tube tip (3). Precise organization of actin is required for tube growth, as is a calcium gradient that increases towards the tip **(b)**. **(c)** As shown by Zhenbiao Yang, ROPs control actin organization in the pollen tube. An unknown signal activates multiple ROPs at the tip, forming the active, GTP-bound state. ROPs activate various RICs, which indirectly control actin organization either by promoting the calcium gradient which stimulates the effects of profilin on actin, or by promoting actin polymerization, possibly via the ARP2/3 complex.

epidermal-cell-pattern mutants. These mutants act at different sites in a common pathway and, like the *spike1* mutant, have defects that include abnormal pavement-cell shape and trichome morphology. Cell-shape defects associate with the failure to form localized cortical actin patches. The *Brk1* gene encodes an 8 kDa protein that is highly conserved in both plants and animals. In maize, this protein acts in a non-cell-autonomous manner, such that a wild-type *BRK1* cell will rescue adjacent *brk1* cells. Smith speculated that *Brk1* might be directly involved in the local regulation of actin polymerization, through interactions with the Arp2/3 complex. Consistent with this concept, work published by Marc Kirschner and colleagues since the meeting shows that the mammalian *Brk1* homolog, HSPC300, is a component of a protein complex that links Rac-GTPase signaling to actin polymerization via activation of the Arp2/3 complex.

Each of the symposia discussed here covered both new and published data and provided a broad perspective of two different fields, both of which are rapidly advancing. We can look forward to post-genomic technology providing tools for the further understanding of many aspects of plant cells, including the cytoskeleton. The abstracts for this meeting are freely available online through the American Society of Plant Biologists website [<http://abstracts.aspb.org/pb2002/public/>].

also function in cell-to-cell coordination. Laurie Smith (University of California, San Diego, USA) discussed three different *brick* mutants that were isolated in a screen for maize