

Meeting report

Towards a virtual *Arabidopsis* plant

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Published: 31 July 2001

Genome Biology 2001, **2(8)**:reports4019.1-4019.4

The electronic version of this article is the complete one and can be found online at <http://genomebiology.com/2001/2/8/reports/4019>

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A report on the Royal Society Discussion Meeting "The plant cell: between genome and plant", London, UK, 13-14 June, 2001.

Now that most of the *Arabidopsis* genome is sequenced, plant biologists are left with the task of assigning functions to the large number of newly identified genes. Speakers at this Royal Society Discussion Meeting presented data clarifying the cellular and developmental functions of a variety of plant genes.

The *Arabidopsis* genome project

Mike Bevan (John Innes Centre, Norwich, UK) presented an overview of the current status of the *Arabidopsis* genome project. The sequence of ten contigs covering the arms of all five *Arabidopsis* chromosomes, as well as of a large proportion of the centromeric heterochromatin, has been determined, thoroughly annotated and organized in public databases. The sequenced part of the *Arabidopsis* genome contains about 25,000 predicted genes, 70% of which show significant homology to known genes of other organisms. Interestingly, the *Arabidopsis* genome contains many more genes than the *Drosophila* genome (14,000 genes), although a similar number of distinct categories of proteins are thought to be present in both organisms (11-12,000). This indicates the presence in the *Arabidopsis* genome of large families of genes that encode similar proteins.

The challenge now confronting the plant research community, as seen by Bevan, is to generate an integrated functional map of the *Arabidopsis* genome or, in more popular terms, a "virtual *Arabidopsis* plant". He envisages the virtual plant as an extensive database that contains functional information collected under various experimental conditions for each identified gene. Information to be incorporated into this database would include description of gene-expression

patterns and knockout phenotypes as well as of biochemical properties, intracellular localization and interaction partners of gene products. An organized collection of 100,000 *Arabidopsis* T-DNA or transposon-insertion lines, together with the sequences of the genomic regions flanking the insertion sites, is expected to be available by 2005 and will allow easy identification of knock-out lines for almost every *Arabidopsis* gene.

All the other speakers at the meeting presented data aimed at elucidating cellular and/or developmental functions of specific plant genes, and thus contributing to the functional map of the *Arabidopsis* genome that Bevan proposed. Key topics addressed were the regulatory processes and molecular mechanisms involved in pattern formation, cell division and cell differentiation. Several speakers - in particular Keith Roberts (John Innes Centre, Norwich, UK) - have emphasized the tremendous importance of analyzing gene functions at the cellular level if we are to gain a comprehensive understanding of how genes control plant development.

Genes involved in pattern formation

Jim Haseloff (University of Cambridge, UK) is interested in the signaling between different tissues that is required for the organization of functional root tips. He presented fascinating imaging technology based on confocal microscopy and advanced three-dimensional reconstruction methods that allows detailed observation of the cellular structure of living root tips. Various *Arabidopsis* enhancer-trap lines have been established in his laboratory, each with a specific pattern of expression of the yeast GAL4 transcription factor (marked by GAL4-induced expression of green fluorescent protein (GFP)) in different root tissues, including the lateral root cap. GAL4-dependent expression of genes that stimulate cell division or induce cell death specifically in the lateral root cap was shown to have dramatic effects on the extension of this cell layer and on other tissues in the root tip. A dominant genetic screen based on the random insertion of

GAL4-responsive promoter elements into the genome of the enhancer-trap line expressing GAL4 in the lateral root cap has been initiated to identify genes involved in the signaling between the lateral root cap and other root tissues.

Liam Dolan (John Innes Centre, Norwich, UK) has shown that as early as late *Arabidopsis* embryogenesis, the expression of the *CAPRICE* (*CPC*) gene, which encodes a Myb-type transcriptional regulator without an activator domain, is restricted to cells that will later form root hairs, and also that *CPC* expression inhibits the activity of the *GLABRA-2* (*GL-2*) gene. In addition, he presented data indicating that *GL-2* activity inhibits another transcriptional regulator encoded by the *VEET* (*VET*) gene, which stimulates the expression of different genes required for root-hair formation, including *KOJAK* (*KJK*, which encodes a cellulose synthase) and *ROOT HAIR DEFECTIVE 2* (*RHD-2*, which encodes an NADPH oxidase that maybe required for the activation of Ca²⁺ channels at the tip of elongating root hairs).

Using *in situ* hybridization techniques and promoter::GFP fusion constructs, Jan Traas (Institut National de la Recherche Agronomique, Versailles, France) has analyzed gene expression in flower meristems of *Arabidopsis pin-1* mutants (which are defective in auxin transport) and of wild-type *Arabidopsis* plants treated with an inhibitor of auxin transport (naphthylphthalamic acid, NPA). These meristems are impaired in the generation of organ primordia, which are formed by normal *Arabidopsis* flower meristems and later develop into flower organs such as petals and sepals. In normal flower meristems, the *LEAFY* and *AINTEGUMENTA* genes are expressed in young organ primordia, whereas the *CUC1* and *CUC2* genes are expressed in the regions between these primordia. Traas has demonstrated that in *pin-1* and in NPA-treated meristems, all these genes show overlapping expression patterns in rings around the meristem. Local application of auxin was shown to cause outgrowth of primordia from these rings of gene expression in mutant or NPA-treated meristems. These observations establish that auxin regulates domain-specific gene expression in flower meristems and is essential for outgrowth of primordia.

As discussed by Jane Langdale (University of Oxford, UK), the cloning of *Selaginella* (lycopod) homologs of the maize genes *rough sheath2* (*rs2*) and *knotted1* (*kn1*), which encode developmental regulators with key functions in the switch from meristem identity to leaf primordium identity in the shoot apical meristem, indicates that early processes involved in the formation of leaves of the microphyllous type (*Selaginella*) and of the macrophyllous type (maize) are similar. Langdale also reported the identification of a second maize mutant, *corkscrew* (*cs*), which shows a similar phenotype to the *rs2* mutant. In addition, her lab has cloned two maize genes that appear to act downstream of *rs2*, *kn1* and *cs* and to be required for the development of normal chloroplasts either in C₃-type bundle-sheath and mesophyll cells

(*Golden2*) or in C₄-type mesophyll cells (*Golden2-like1*). Bundle-sheath and mesophyll cells are leaf cells that carry out different forms (C₃ or C₄ type) of photosynthesis. Homologs of *Golden2* and of *Golden2-like1* were cloned from rice as well as from *Arabidopsis* and were found to be specifically expressed in developing mesophyll cells. *Arabidopsis* plants with both genes knocked out were shown to be pale green and unable to form normal chloroplasts.

Genes involved in cell division

The investigation of cell-cycle regulation in plants has long been hampered by the lack of *Arabidopsis* cell suspension cultures that can be synchronized. Jim Murray (University of Cambridge, UK) has established such cultures and demonstrated specific expression or activation of cell-cycle regulators, including isoforms of CYCLIN B and CYCLIN D as well as of CYCLIN-DEPENDENT KINASES A and B, in these cultures at different stages of the cell cycle after synchronization by aphidicolin treatment or sucrose starvation. In addition, he presented data indicating that constitutive overexpression of CYCLIN D₃ in transgenic *Arabidopsis* plants stimulates cell division and tends to inhibit cell differentiation, which is consistent with a function for this protein as a regulator of the *Arabidopsis* homolog of the retinoblastoma (RB) protein. Interestingly, overexpression of CYCLIN D₂ in the *Arabidopsis shoot meristemless* mutant partially restored normal organogenesis, whereas overexpression of CYCLIN D₃ did not, which demonstrates functional differences between these two proteins and a role for CYCLIN D₂ in the organization of the shoot apical meristem in *Arabidopsis*.

Yasunori Machida (Nagoya University, Japan) described the characterization of a mitogen-activated protein (MAP) kinase cascade that is required for the expansion of the phragmoplast in tobacco cells undergoing cytokinesis. The phragmoplast is a structure exclusively found in dividing plant cells, which contains cytoskeletal elements as well as secretory vesicles and generates a new cell wall between newly formed daughter cells. Machida has demonstrated that NPK1, a MAP kinase kinase kinase, is activated by NACK1 (NPK1-activating kinesin-like protein) specifically during the M phase (mitosis) of the cell cycle. Putative downstream elements of the cascade (MAP kinase kinase and MAP kinase homologs) have also been identified. Using GFP tagging and immunofluorescence techniques, Machida has shown that both NPK1 and NACK1 are localized to the edge of expanding phragmoplasts in dividing tobacco BY-2 suspension culture cells. Consistent with these data, expression of a dominant negative mutant form of NPK1 (with a disrupted kinase domain) in BY-2 cells resulted in the formation of multinucleate cells with incomplete cell walls.

One of the few 'true cell biologists' at the meeting, Peter Hepler (University of Massachusetts, Amherst, USA) has studied the role of cytoskeletal motor proteins during cell

division. He has shown that microinjection into *Tradescantia* stamen hair cells of an antibody that activates a kinesin-like calmodulin-binding protein (KCBP) by binding to its calcium-sensitive domain speeds up early processes during cell division, such as breakdown of the nuclear envelope and metaphase plate formation, but prevents phragmoplast formation and inhibits cytokinesis. Artificial activation of KCBP, a minus-end-directed microtubule motor protein that appears to function as a microtubule-bundling protein during cell division, may prevent the microtubule cytoskeleton from undergoing essential reorganization at the onset of cytokinesis. Hepler also demonstrated that drugs that affect the activity of myosins (such as BDM and ML-7) specifically inhibit expansion and positioning (but not formation) of the phragmoplast. These findings confirm earlier observations indicating an essential role of the acto-myosin system in the growth and positioning of new cell walls during cytokinesis in plant cells.

Genes involved in cell differentiation

Expression of the *Antirrhinum majus MIXTA* gene, which encodes a Myb-type transcriptional regulator, causes epidermal petal cells to develop a conical shape. Cathie Martin (John Innes Centre, Norwich, UK) has demonstrated that the protein encoded by the *A. majus DEFICIENS* gene controls *MIXTA* expression and binds, in a complex with other transcriptional regulators, to elements in the *MIXTA* promoter. *DEFICIENS* specifies petal and stamen identity in the developing flower and is thus called a 'B-function' homeotic gene. Constitutive overexpression of *MIXTA* in tobacco and *A. majus* causes epidermal cells in various tissues to assume a conical shape and induces formation of trichomes (hair-like, often branched appendages extending from the epidermis of aerial plant organs). The latter observation indicates that *MIXTA* homologs may be involved in the initiation of trichome formation. Martin has identified different *MIXTA*-related genes in *A. majus* as well as in *Arabidopsis*. She showed that an *Arabidopsis MIXTA* homolog is not essential for the ability of epidermal petal cells to assume conical shapes but rather stimulates the elongation of conical cells. Using differential RNA display, 160 genes have been identified whose expression is up- or down-regulated in *A. majus* plants that overexpress *MIXTA*. Interestingly, Martin showed that one of these genes encodes a receptor-like kinase that appears to localize to the cell cortex at the tip of elongating conical epidermal cells.

In *Arabidopsis*, each trichome consists of a single cell that has endoreduplicated its DNA several times. Martin Hülskamp (Cologne University, Germany) has observed a correlation between branching and endoreduplication in single-cell *Arabidopsis* trichomes. Mutant trichomes with reduced branching also show reduced DNA content, and *vice versa*. He proposed that *Arabidopsis* trichomes may have evolved from multicellular ancestors - trichomes formed by

many plant species, for instance *Nicotiana tabacum*, consist of several cells - to single-cell structures by omitting cytokinesis but not DNA synthesis. Consistent with this hypothesis, overexpression of *CYCLIN B1-2* in *Arabidopsis* was shown to result in the formation of multicellular trichomes. Hülskamp could not detect genetic interactions between the *CYCLIN B1-2* transgene and the *sim* mutation, which also induces *Arabidopsis* trichomes to become multicellular, indicating that *CYCLIN B1-2* and *SIM* may act in parallel pathways. Genes disrupted in several *Arabidopsis* trichome-branching mutants have been cloned in Hülskamp's lab: *ZWICHEL* and *REDUCED BRANCHING* encode microtubule- or tubulin-binding proteins (*KCBP2* and tubulin-folding cofactor 2, respectively), whereas *AUGUSTIFOLIA* and *STICHEL* do not show homology to characterized genes. Hülskamp also reported the interesting observation that trichomes formed by double mutants of *zwichel* and *distorted* (the *distorted* phenotype is abnormal shape and filamentous (F) actin organization in trichomes) have rounded tips instead of the normal pointed tips.

Keith Roberts described the observation that *Zinnia* mesophyll and palisade leaf cells can be induced to differentiate *in vitro* into tracheary elements (xylem cells) by a 1 hour treatment with auxin and cytokinin exactly 47 hours after isolating them from leaves. His group have performed a cDNA amplified fragment length polymorphism (AFLP) screen - a method to identify differentially expressed genes - resulting in the identification of 620 genes whose expression levels are altered at different times after the hormone treatment. Many of these genes were reported to encode proteins with putative functions in cell-cycle control, cell-wall synthesis, metabolism and apoptosis. High-throughput *in situ* hybridization analysis has shown that most of the identified genes are expressed in different regions of the xylem in intact plants. *Zinnia* tracheary elements developing *in vitro* eventually 'punch' a single hole into the cell wall at one end of the cell when they undergo apoptosis. To identify factors involved in this process, Roberts and colleagues have initiated screens for mRNAs that show polar distribution in the cytoplasm during this developmental stage.

The work presented by Takashi Hashimoto (Nara Institute of Science and Technology, Japan) has identified and functionally characterized *Arabidopsis* genes that are required for normal microtubule organization and organ growth. Two such mutants are *spiral-1 (spr-1)* and *spiral-2 (spr-2)*, neither of which show sequence homology to previously characterized genes. In the epidermis of elongating *spr-1* and *spr-2* organs, cortical microtubules are arranged in left-handed helices rather than transversely as in wild-type organs. In subepidermal *spr* cells, the cortical microtubule cytoskeleton is completely disrupted, which results in anisotropic growth of these cells. Differences in the elongation rate of epidermal and subepidermal cells, caused by differential effects of the *spr* mutations on microtubule

organization in these two groups of cells, causes *spr* organs (such as roots, hypocotyls, petioles and petals) to develop right-handed helical files (columns) of epidermal cells and a right-handed twisted morphology. When grown on hard agar surfaces, the right-handed twisted epidermis of *spr* roots causes them to grow slanted to the right, unlike wild-type roots, which grow vertically towards the center of gravity. Hashimoto's laboratory has also identified two *Arabidopsis* mutants, *lefty-1* and *lefty-2*, whose roots grow slanted to the left under comparable conditions and which form left-handed epidermal cell files as well as left-handed twisted organs. Interestingly, these mutants show right-handed microtubule helices in epidermal cells and disrupted microtubule organization as well as anisotropic cell growth in subepidermal tissues. The two *lefty* mutants were found to contain point mutations in the genes encoding α -tubulin 6 and 4, respectively, which result in a single amino-acid exchange (S180F). Constitutive overexpression of the S180F mutant of α -tubulin 6 in *Arabidopsis* was shown to phenocopy the *lefty* mutants.

Nam-Hai Chua's laboratory at the Institute of Molecular Agrobiolgy, National University of Singapore, has found that moderately reduced α -tubulin protein levels in transgenic *Arabidopsis* plants - caused by the presence of an anti-sense α -tubulin expression construct in the genome - specifically affected the development of roots that were elongating at a maximal rate, five days after germination. Such roots expanded radially at the tip and eventually stopped elongating. These defects were shown to be caused by a disruption of microtubule organization in root-tip cells, which resulted in anisotropic cell expansion and defects in cell division. Reduced α -tubulin expression was also reported to cause ectopic formation of root hairs, abnormal root hair development, and reduced gravitropic responses, which were observed even before root elongation was affected. Overexpression in *Arabidopsis* plants of cDNA sequences encoding the actin-regulatory proteins profilin or actin depolymerizing factor (ADF) altered expansion of cells and organs, but did not affect cell division. Reduced ADF expression or increased profilin expression stimulated growth, whereas increased ADF expression or reduced profilin expression had the opposite effect. ADF expression levels were found to correlate inversely with the number of F-actin cables present in cells of the transgenic plants. Altered profilin levels did not detectably change F-actin organization but may have influenced the dynamic properties of the actin cytoskeleton. These results essentially confirm that profilin and ADF proteins function *in vivo* in the same way as predicted from analysis of their activities *in vitro*. Surprisingly, reduced profilin or ADF expression caused early or late flowering, respectively, indicating an involvement of the actin cytoskeleton in the regulation of flowering time.

Patrick Hussey (University of Durham, UK) demonstrated functional differences between ADF isoforms that are

expressed either in vegetative tissue or in generative cells (pollen), in both maize and lily plants. The actin-severing and actin-depolymerizing activities of vegetative ADFs were determined to be much higher than those of pollen ADF isoforms. Also, Hussey has shown that the activity of vegetative ADFs is regulated by phosphorylation and pH conditions, whereas the activity of pollen ADFs is not. Consistent with these results, pollen ADFs, but not vegetative ADFs, were found to associate with intact F-actin structures in living cells. In addition, Hussey's lab has used yeast two-hybrid protein-interaction screens to identify ADF-interacting proteins. One such protein, which contains a WD40 domain and binds to actin filaments, was proposed to increase the twist of actin filaments and to act as an ADF cofactor. Hussey also described the identification of a plant-specific class of microtubule-associated proteins, MAP65s, in tobacco and *Arabidopsis*. These proteins were shown to bind to microtubules *in vitro* and to a subset of the microtubules present in living cells. Interestingly, MAP65s were observed to be released from microtubules in living cells upon fungal attack.

Outlook

The availability of the *Arabidopsis* genome sequence, of growing collections of knock-out lines and of new tools such as DNA chips, enhancer-trap lines, GFP and confocal/multi-photon microscopy has opened up a wide range of new opportunities for plant research, of which scientists are making excellent use, as work presented at this exciting meeting has shown. The coming years will most certainly see tremendous progress in our understanding of how genes function in plant cells and during plant development.