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P3/C1–PLANT CELL ARCHITECTURE

Organised by J.-P. Verbelen and A.H. Kingston-Smith for the Cell Section and S. Turner for the Plant Section

P3/C1.1 Molecular Mechanisms that Regulate Stem Cell Activity in Arabidopsis Shoot and Floral Meristems

J.C. Fletcher, U.C. Berkeley

Higher plants have the unique ability to produce organs continuously from actively growing tips called apical meristems. The shoot apical meristem of higher plants acts as a self-renewing source of uncommitted, pluripotent stem cells whose descendents acquire different fates as they become incorporated into organ and tissue primordia. Stem cell maintenance is an active process whereby the different regions of the shoot apical meristem constantly communicate with one another to coordinate the loss of stem cells through differentiation with their replacement through cell division. In *Arabidopsis thaliana*, signaling through the CLV-WUS pathway results in the generation of a spatial feedback loop, involving both positive and negative interactions, that stabilizes the size of the stem cell reservoir. Termination of stem cell activity during flower development is achieved by a temporal feedback loop involving both WUS and phase-specific flower patterning genes. We have isolated loss-of-function mutations in the Arabidopsis *ULTRAPETALA* (*ULT*) gene that result in the excess accumulation of shoot and floral meristem cell throughout development, and in reduced floral meristem determinacy. Our studies show that *ULT* encodes a small, novel protein that regulates the size of the *WUS*-expressing cell population in shoot and floral meristems via a CLV-independent mechanism. In addition, we find that *ULT* is a key component of the WUS-dependent stem cell termination pathway in floral meristems. These experiments reveal additional layers of complexity in plant stem cell maintenance networks.

P3/C1.2 Regulation of phyllotaxis via polar auxin transport

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Leaves and flowers are formed at the shoot apical meristem according to specific patterns, called phyllotaxis. The beauty and regularity of phyllotaxis have for centuries attracted the attention of philosophers, mathematicians and natural scientists, but experimental approaches have been few. Previous studies have shown that the plant hormone auxin triggers organ formation. Auxin undergoes specific polar transport, via cellular influx and efflux carrier proteins. I will discuss our experiments that show how these auxin carriers regulate phyllotaxis. Our data indicate that auxin is acropetally transported into the meristem through the epidermis and the meristem L_1 layer. In the meristem, auxin becomes redistributed by the primordia, which function as sinks for auxin. Hence, the pre-existing primordia dictate the position of future primordia by allowing auxin accumulation in the meristem only at certain minimal distances. The emerging model for phyllotaxis accounts for its reiterative nature, as well as for its regularity and stability.

P3/C1.3 *In vivo* actin cytoskeleton dynamics in the transition zone cells of *Arabidopsis* roots

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The dynamic actin cytoskeleton has several different functions during cell growth in plants. Dividing plant cells have F-actin meshworks densely underlying the plasma membrane and encircling nuclei while elongating and mature cells show longitudinal bundles of F-actin. It is not clear how these bundles are formed from perinuclear meshworks. Our previous immunofluorescence study, using Steedman's wax sections from maize root apices, identified that unique reorganization of F-actin is accomplished within cells of the transition zone.

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Using GFP-fimbrin construct, here we report similar reorganisation of F-actin in living transition zone cells of *Arabidopsis* roots. In conclusion, root cells of the transition zone are characterized with the dramatic rearrangements of F-actin arrays which switch from the dense perinuclear meshworks into the longitudinal bundles characteristic for all elongating and mature root cells.

P3/C1.4 The root transition zone: postmitotic cells between meristem and elongation

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Traditionally, two major growth zones are recognized in root apices: the primary apical meristem where all cells are formed and the elongation region where cells accomplish rapid, highly polarized cell elongation allowing an equally rapid root growth. Our detailed cytological and anatomical analyses of maize and Arabidopsis root apices document the existence of a specialized transition zone intercalated between the basal end of the apical meristem and the apical end of the elongation region. Cells of the transition zone dramatically reorganize their cytoskeletal elements, especially the actin filaments. The transition zone acts like a cell supply center: the apical part serves as a reservoir of potentially meristematic cells while the basal part provides a reservoir optimised for rapid onset of cell elongation. The unique sensory properties of cells passing through the transition zone then allows a finely tuned coordination of cellular development. Transition zone cells are obviously specialized for effective monitoring of several endogenous cues and diverse environmental parameters. The integrated output from their signal perception and processing is then transferred to adjacent cells as well as used autonomously for decisions about the further fates of these developmentally plastic cells.

P3/C1.5 Is the oxygen sensor located in the transition zone of the root apex?

S. Mancuso and S. Mugnai, University of Florence; D. Volkmann and F. Baluška, University of Bonn

Oxygen sensing is a fundamental physiological process representing the first step of the cellular response of plants to hypoxia. Given that the root apex is the first part to come in contact with new regions of the soil, the tip therefore represents the most probable site for oxygen sensing. On the basis of morphological, cytological and physiological characteristics, the growing root apex of maize can be divided into three different regions: the meristem (M), the transition zone (TZ) and the elongation region (ER). A number of data suggest that the TZ acts as a sensory zone, enabling the growing apex to continuously monitor various environmental parameters and to effect appropriate responses. Testing this hypothesis, we have evaluated responses of the different regions of the root apex to short episodes of hypoxia. Results obtained enabled us to demonstrate that the production of nitric oxide, which is required for the acclimation of roots to hypoxia, is specifically associated with the TZ of the root apex. Away from the TZ, only small effluxes of NO (1/10 lower than those produced in the TZ) were detectable. Importantly, restricting hypoxia treatment to the TZ increased dramatically the concentration of ADH and PDC all along the roots. In contrast, localised hypoxia treatments of the M or ER failed to increase the concentration of these enzymes throughout the growth regions. These results support the hypothesis that the TZ is the site of the oxygen sensing enabling the advancing root apices to 'navigate' their growth towards environmentally more hospitable areas in the soil.

P3/C1.6 The role of *ADL2a* in plant mitochondrial dynamics and morphology

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Dynamin-like proteins (high molecular-weight GTPases) are involved in the maintenance of mitochondrial morphology in eukaryotes through their role as part of the mitochondrial division apparatus. In most eukaryotic species studied mitochondrial division involves a single dynamin-like protein. However, in *Arabidopsis thaliana* four dynamin-like proteins have been implicated in the mitochondrial division process. We identified a T-DNA knockout line for one of these *Arabidopsis* proteins, ADL2a, and genetically transformed a population of plants segregating for the T-DNA insertion with a reporter construct to tag the mitochondria with GFP. *In vivo* visualisation of mitochondria showed that homozygous disruption of *ADL2a* led to altered mitochondrial morphology. Mitochondria in the mutant exhibited an elongated, tubular morphology with several constrictions along their length. In addition, many of these mitochondria had long, narrow protuberances, that we name 'matrixules', which extended for many micrometres in length. These results will be used as a basis to discuss the role of *ADL2a* in the control of mitochondrial morphology in higher plants.

P3/C1.7 New insights on microtubule function and cell wall properties in expanding cells

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The dominant hypothesis for plant cell axial expansion expounds a mechanical role for cortical microtubules in orienting cellulose microfibrils. The discovery of mutants with defects in cytoskeletal organization and wall synthesis has introduced a new approach to unravelling the mysteries of cell morphogenesis. We identified a series of temperature-sensitive morphological mutants in *Arabidopsis thaliana*, including ones that reduce cellulose synthesis and others that disrupt cortical microtubules. We have used both types of mutants to assess the relationship between microtubule organization and cellulose microfibril orientation. Temperature-sensitive mutants allow normal development to proceed until the time of perturbation so that defects can be assessed at any developmental stage.

In *mor1* mutants at restrictive temperature, cortical microtubule arrays are quickly disrupted. At the same restrictive temperature, cellulose production is reduced in the *rsw1-1* allele of the cellulose synthase CesA1. Double mutant analysis showed that the *mor1rsw1* radial swelling phenotype is additive, predicting that cellulose deposition is independent of microtubule orientation. We confirmed this prediction by analyzing cell wall texture by field emission scanning electron microscopy in cells of known growth status. Our results demonstrate that: (1) parallel alignment of cellulose microfibrils is largely dependent of the rate of cellulose synthesis, supporting a self-ordering mechanism; (2) microtubule disruption causes radial swelling without altering parallel cellulose microfibril deposition; (3) cellulose microfibril alignment can recover from drug-induced disruption even when microtubules are disorganized. A new model will be presented to resolve the deficiencies of the cellulose synthase constraint hypothesis.

P3/C1.8 Coordination between cell wall synthesis and cell elongation in plant cells

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A long-standing question in plant biology is how cell growth is coordinated with the synthesis and deposition of cell wall polymers. We have been addressing this question using the Arabidopsis hypocotyl as a model system. This organ has a simple anatomy, shows a massive elongation when grown in the dark, which is entirely the result of cell elongation. We first showed that cell elongation in this organ was highly synchronized. Cell elongation was initiated in all hypocotyl cells 24 h after imbibition of the seeds and proceeded slowly until 48 h. Around 48 h, cell elongation accelerated at the hypocotyl basis. This acceleration was accompanied by massive changes in gene expression, as shown on protein gels and CATMA microarrays. Transmission Electron Microscopy showed that slowly growing cells had accumulated a thick and regularly layered cell wall. After the growth acceleration, the walls underwent extensive remodeling and thinning, consistent with the multi-net growth model. Interestingly, the cellulose-inhibiting herbicide isoxaben administered before 48 h inhibited subsequent acceleration, but when added after the growth acceleration had no influence on cell elongation. This change in sensitivity to the herbicide, suggests either the uncoupling of the regulation of growth from the synthesis of cellulose or alternatively a change in the cellulose synthesis machinery. To identify the components of the cellulose synthesis machinery we have been studying mutants with reduced hypocotyl growth. A group of cellulose-deficient mutants was identified among those using FT-IR microspectroscopy. This group contains mutants for 7 distinct genes. These genes encode three isoforms of the cellulose synthase catalytic subunit (CESA1, 3 and 6), a membrane-bound endo-β-1,4-endoglucanase (KOR1), a type II plasma membrane protein (KOB1), a secreted protein similar to basic endo-chitinases (POM1) and a GPI-anchored protein (COB). Our current view on the role of these proteins in the synthesis of cellulose and its coordination with cell elongation will be discussed.

P3/C1.9 Regulation of cellulose synthesis during secondary cell wall formation in Arabidopsis

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Cellulose is central to plant development and is an economically important target. Despite this it is still unclear how cellulose synthesis is regulated. Significant advances have recently been made in the cloning of genes involved in cellulose synthesis. Three different cellulose synthase catalytic subunits (CesAs) are essential for cellulose synthesis in the secondary cell wall. These three proteins all interact within the same protein complex.

The presence of all three catalytic subunits, but not their catalytic activity, is required for the correct interactions between these subunits and for the subsequent targeting of the cellulose synthase complex to the plasma membrane. The three secondary cell wall CesAs (IRX1, IRX3 and IRX5) are expressed in identical patterns, as shown by promoter reporter gene constructs and by immunodetection using specific antibodies. This suggests that the three genes are regulated using common mechanisms.

The complexity and size of the cellulose synthase complex is likely to mean that cellulose synthesis is also regulated post-translationally. This could occur at the level of assembly of the individual components into an intact complex, or once the complex has been inserted into the plasma membrane. Work in my laboratory is concentrating on the identification of these regulatory mechanisms using a number of strategies. The results of these experiments will be discussed.

The identification of regulatory mechanisms controlling cellulose synthesis will allow a unique opportunity to manipulate the content and composition of cell walls, a developmentally and economically important target for biotechnology.

P3/C1.10 Cell-wall structure and anisotropy in the cellulose synthase mutant *procuste* of *Arabidopsis thaliana*

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It is widely accepted that cellulose helps to shape plant cells and hence whole plants, because cellulose microfibrils constrain the direction in which each growing cell can expand. At a higher level of detail the control mechanisms are obscure. In the Arabidopsis mutant *procuste-1*, a defect in the CESA6 gene encoding a cellulose synthase reduces cellulose synthesis in the affected cells and severely inhibits elongation growth.

Using solid-state NMR and deuteration-FTIR spectroscopy of hypocotyl cell walls, we showed that no major change in microfibril structure or crystallinity accompanied the reduction in cellulose content. While the cellulose percentage in the cell walls was reduced, there was insufficient reduction in the total cell-wall content per hypocotyl for the dwarfed phenotype to be explained simply by reduced cell-wall synthesis. The cells became wider and their walls became thicker.

Microfibril orientation was disrupted in *procuste-1* as shown both by FE-SEM imaging of individual microfibrils at the inner face of the cell wall, and by polarised FTIR microscopy. It is remarkable that a CESA mutation should disrupt the orientation, but not the structure, of the cellulose synthesised. Quantitative modelling of the effects on growth anisotropy showed that the ordered microfibril orientations at the inner face of the cell wall were consistent with the degree of dwarfing, while the broad distribution of orientations throughout the cell wall was not. This implies that the inner wall layers, containing newly synthesised cellulose, are crucial for the controlled expansion of plant cells.

P3/C1.11 Protein-mediated and protein-independent mechanisms of plant cell wall modification *in vivo*

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Many proteins thought to be present in the primary cell wall (PCW) appear relevant to wall assembly, loosening and tightening. They include proteins which, assayed *in vitro*, have the following activities: xyloglucan endotransglucosylase (XET), polysaccharide endohydrolases, polysaccharide exohydrolases, esterases, pectate lyase, peroxidase, laccase, expansins and yieldin. The existence of these proteins in the PCW is known, or inferred, from studies of the occurrence of their mRNAs, the proteins themselves (as antigens), and their activities (assayed *in vitro*). However, there is an important distinction between activity and action: an active protein does not necessarily act, *in vivo*. In this paper, I review evidence as to whether these proteins do act, in the walls of living plant cells. It is concluded that there is clear evidence for *in-vivo* action of XETs, hydrolases including esterases, and peroxidases/laccases. Detection of XET action *in vivo* by suitable dual-labelling ($^{13}C/^{3}H$) experiments will be emphasised. However, there is currently no assay that can unequivocally demonstrate the action of expansin or yieldin *in vivo*. Likewise, peroxidase action cannot be distinguished from laccase action. Attention is also given to the possibility that polysaccharide scission reactions observed in the PCW *in vivo* are not directly enzymic, but involve the action of hydroxyl radicals. I will present novel methods for detecting hydroxyl radical action on polysaccharides *in vivo*.

P3/C1.12 Division of roles among four Arabidopsis XTH genes, XTH17, -18, -19 and -20 in the cell wall dynamics in roots

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In cell walls of most flowering plants, cellulose microfibrils are cross-linked by xyloglucans to form a network structure, in which xyloglucans function as tension-bearing bridges. Xyloglucan endotransglucosylase/hydrolases (XTHs) are enzymes that cut and rejoin xyloglucan bridges, and that are considered essential for cell wall dynamics. Roles include the construction, modification, and maintenance of the cell wall architecture. In Arabidopsis thaliana, the enzymes are encoded by 33 members of the XTH gene family, suggesting a division of roles among the family members in the cell wall dynamics.
To gain insight into their functional diversity, we analyzed the expression profiles of four Arabidopsis XTH genes, AtXTH17, 18, 19 and 20, that are preferentially expressed in roots and that share a striking resemblance in their amino acid sequences. Promoter analyses using transgenic plants expressing various lengths of XTH promoter::GUS fusion genes showed that the conserved 5′-flanking sequences were responsible for the root specific gene expression. Whereas the four genes were preferentially expressed in roots, they exhibited distinct tissue specific expression profiles. The results clearly point to a division of their physiological roles in the dynamics of the cellulose/xyloglucan framework during growth of Arabidopsis roots.

P3/C1.13 Regulation of cell size and elongation rate in the *Arabidopsis* root: a complex matter of apoplastic and symplastic events

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The *Arabidopsis* root is used as a model system to study the molecular nature of the growth cessation process. In general, it is assumed that the stop in elongation is associated with changes in the structure and composition of wall material, a reduction in wall loosening and an increase in cross-linking events in the cell wall. We investigated the fast stop in cell elongation imposed by the precursor of ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC). ACC very quickly sets a limit to cell elongation, beyond which cells cannot expand. A similar type of response can be induced by plant own and environmental cues. Very probably the cross-linking of cell wall structural proteins HRGPs is a crucial player to limit or stop elongation. Furthermore, the accumulation of reactive oxygen species (ROS) in the apoplast of cells in the elongation zone seems indispensable to stop elongation. Removing ROS decreases the effect of ACC. The response is accompanied with an induction of callose synthesis and with an up-regulation of myosin VIII expression in cells blocked in their elongation. To identify genes that are crucial for the elongation stop, we are currently using an approach by which the cell content of trichoblasts is aspirated for mRNA isolation. Gene expression profiling is then executed by applying the samples to a microarray.
P3/C1.14Abstract not supplied

P3/C1.15 Identification of novel root-hair genes in *Arabidopsis thaliana* using transcriptomics data from wild-type and a root-hair mutant

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Root-hair cells are an excellent system for studying polarised plant cell growth. To date around 40 *Arabidopsis* genes have been identified that have roles in one or more stages of root-hair development. As many of these genes were originally isolated from classical genetic screens, the majority still await identification at the molecular level. We took a transcriptomic approach to identify further genes involved in root-hair development. We isolated total RNA from dissected primary root elongation and differentiation zones from wild-type roots and from roots of the mutant *rhd2-1*, which lacks normal tip-growing hairs. Through the GARNet service we used the Affymetrix GeneChip® whole genome microarray to examine the transcriptome in this region of the plant. From the expression data obtained we derived a list of candidate root-hair genes that were highly differentially expressed in wild-type relative to *rhd2-1*. We selected and screened 172 T-DNA insertion lines from a publicly-available knockout collection (http://signal.salk.edu/cgi-bin/tdnaexpress) and identified several lines with heritable root-hair abnormalities. We confirmed the position of these insertions by PCR and have successfully screened for further independent T-DNA insertions in the same genes.

P3/C1.16 Regulation of Ripening in Fleshy Fruits

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The aim of our work is to identify genes involved in the developmental regulation of ripening in fleshy fruits and particularly those that modulate cell adhesion and fruit texture. Cell adhesion is the single most important factor determining food mechanical properties. The proteins that control the degree of cell adhesion and softening in fleshy fruits, such as those regulating the relevant hydrolytic enzymes, have so far eluded identification.
In tomato a wide variety of single gene mutations have been described, including a very small number which have pleiotropic effects resulting in the reduction or almost complete abolition of the ripening. These pleiotropic ripening mutants such as ripening-inhibitor (rin), non-ripening (nor) and colourless non-ripening (Cnr) provide key tools for investigating the developmental regulation of ripening. The Cnr mutation in tomato results in ripe fruit where the pericarp cells remain colourless and show altered cell adhesion resulting in firm, but mealy fruits (Thompson et al, 1999, Plant Physiology 120, 383–389).The changes in cell adhesion reflect significant alterations in cell wall properties in the mutant (Orfila et al, 2001; Plant Physiology 126, 210–221 2002, Planta 215, 440–447). The presentation will discuss progress on identifying the gene at the Cnr locus and its role in the developmental regulation of fruit texture and ripening.

P3/C1.17 Cell walls, cell growth and cell size

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A special feature of plant growth and development is the relatively massive increase in cell size following the cell's exit from the cell cycle. Surprisingly, we know relatively little about precise controls on expansion growth or the mechanisms that determine final cell size. I will discuss the intimate relationship between cell expansion and the production of new cell wall material and its deposition. These spatially controlled processes are also influenced by cell position, local cell/cell interactions, and local wall thickness. Many plants, and in particular Arabidopsis, show some sort of correlation between cell size and cellular ploidy. Cells that undergo DNA endoreduplication replicate their chromosomes without an intervening mitosis and the resulting larger, higher ploidy nucleus is often associated with an increase in cell size. I will describe genetic experiments that suggest that there is a key relationship between the amount of DNA in a cell and the volume of cytoplasm that can be sustained. A key requirement for in endoreduplication is a topoisomerase VI complex that we are currently characterising. We suggest that cell growth, ie increase in the cytoplasmic macromolecular mass, and cell expansion, ie increase in cell volume through vacuolation, both contribute independently to cell size determination in plants.

P3/C1.18 Xylem water transport failure by cell wall implosion, air-seeding, and membrane rupture in tall Douglas-fir trees

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The cohesion–tension model of water transport states that water is held together by strong internal forces and that it is pulled through a tree in tension. This tension could cause transport failure in at least three ways: breakage of the tracheid walls (implosion), interruption of the water column with an air bubble namely air-seeding trough pit membrane (margo) pores, or interruption of the water column when a safety features fails—in this case, air seeding when the membrane of an aspirated pit membrane breaks, permitting a gas bubble to spread to another tracheid. Using Douglas-fir trees of two age classes and ranging from 4 to 40 m in height, we asked if there was a constant safety factor with height for any of these three failure modes: implosion, air-seeding, and margo breakage. We calculated the safety factor using physiological measurements of vulnerability to embolism, and anatomical measurements of tracheid lumen diameter, cell wall thickness, and size of pores and thicknesses of strands in the margos of pit membranes. We showed that on an anatomical basis, as predicted by the 'air-seeding' hypothesis, hydraulic functions are directly linked to bordered pit functioning. This research aimed to elucidate the functional significance of variation in earlywood structure with position in a tree. Increasing tracheid cell wall resistance to implosion by increasing cell wall thickness in earlywood was at the cost of a decreasing resistance to embolism because the torus had to deflect more to be fully aspirated.

P3/C1.19 Transcript profiling during altered spatial and temporal leaf cell development: the role of carbon and water

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We have used the model tree, *Populus* to investigate the spatial and temporal control of leaf cell expansion and

production and determined how two abiotic variables – carbon and water – may influence these processes. Growth analysis has been complemented with gene expression data from a *Populus* microarray. When leaves were exposed to additional atmospheric carbon dioxide, the spatial control of leaf growth was altered such that leaf shape was changed. Transcript profiling has revealed a number of key genes that may be active in determining this response, particularly cyclins affecting the spatial production of cells and genes associated with cell wall loosening determined temporally. Data for leaf development has revealed differences in the spatial and temporal control of leaf size and shape for two contrasting *Populus* genotypes that are the parents of a mapping population, with *P. deltoides* investing in the production of cells to a greater extent than *P. trichocarpa*, as suggested earlier and for which putative QTL are already available. These contrasting responses have been linked to patterns of global gene expression and latest findings from this analysis will be presented. Common and species-specific transcripts have been identified in response to drought and the strategy for linking these to QTL in the F2 progeny will also be described.

P3/C1.20 Inside the condemned cell — heredity, environment, remission and rehabilitation

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Although senescence usually ends in death, the photosynthetic tissues of senescing leaves are not dying: they retain the metabolic and developmental capacities of viable cells. The most prominent architectural modification to which the senescing cell is subject is the transdifferentiation of chloroplasts into gerontoplasts. Recent advances in our understanding of the cell and molecular biology of the chloroplast-to-gerontoplast transition, and in particular the fate of photosynthetic pigments, have given new insights into how the process is regulated by specific genes and environmental interactions. A characteristic of the transition is that it can be reversed. The reassembly of a chloroplast from a gerontoplast has some features in common with the greening of proplastids or etioplasts, but also some fundamental differences which have wider implications for the network of plastid developmental pathways that underlies the differentiation of plant cell types.

P3/C1.21 Mechanisms contributing to the senescence of pea nodules

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Nodule development and senescence were studied in commercial pea (*Pisum sativum* cv Phoenix) plants inoculated with *Rhizobium leguminosarum* biovar *viciae*. Plants were grown for 12 weeks until both nodules and leaves had largely senesced. Nodule nitrogen fixation capacity started to decrease after 3 weeks. Nodule total ascorbate and glutathione content showed a strong positive correlation with the decrease in nitrogenase activity. Similarly, nodule dehydroascorbate reductase activity showed a positive relationship with nitrogenase activity. In contrast, no correlation was observed between the rate of nodule N fixation capacity and any of the other activities of antioxidant enzymes measured. To characterise the nodule senescence process in more detail the abundance of transcripts of putative molecular markers was determined. A strong decline in the total amounts of extractable RNA encoding leghemoglobin mRNA, 16S and 18S rRNA was observed after 9 weeks. In contrast, other putative markers such as cysteine proteinase and ascorbate peroxidase transcripts were fairly constant during development. Using specific substrates for the Cathepsin group of cysteine proteinases, we found that the activities of these enzymes were relatively constant during nodule development with highest activities catalysed by Cathepsin B, followed by lower Cathepsin L activities and only relatively low activities for Cathepsin H. Of these only Cathepsin L showed a transient change during development with an increase at 7 weeks. Evidence that pea nodules exhibit a form of programmed cell death during senescence will be discussed.

P3/C1.22 UV-induced programmed cell death

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Plants, animals and several branches of unicellular eukaryotes use Programmed Cell Death (PCD) for defence or development mechanisms. This argues for a common ancestral apoptotic system in eukaryotes. However, at the molecular level, very few regulatory proteins or protein domains have been identified as conserved across all eukaryotic PCD forms. A very important goal is to determine which molecular components may be used in the execution of PCD in plants, which have been conserved during evolution and which are plant specific. Using *A. thaliana* we have shown that UV radiation can induce apoptotic-like changes at the cellular level and

that an UV experimental system was relevant to the study of PCD in plants. UV induction of PCD requires light and a protease cleaving the caspase substrate Asp-Glu-Val-Asp (DEVDase activity) is induced within 30 minutes and peaks at one hour. This DEVDase appears related to animal caspases at the biochemical level, being insensitive to broad-range cysteine protease inhibitors. In addition, caspase1, caspase-3 inhibitors and the pancaspase inhibitor *p35* were able to suppress DNA fragmentation and cell death. These results suggest that a YVADase (Tyr-Val-Ala-Asp) activity and an inducible DEVDase activity are possibly mediating DNA fragmentation during plant PCD induced by UV overexposure. Progress is being made toward the biochemical characterisation of the proteases involved.

P3/C1.23 Structural changes in the cuticle of *Arabidopsis* lead to post-genital organ fusions and a strong resistance to *Botrytis*

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The cuticle is part of the extracellular matrix of the epidermal cells of plants and is deposited at the outside of the cell wall. A major structural component of the cuticle is cutin. The functional analysis of cutin *in vivo* has been limited because no mutants specifically affected in cutin biosynthesis have been characterized until recently. Therefore, transgenic *Arabidopsis* that express and secrete the cutinase from *Fusarium solani* f. sp. *pisi* were generated.
Cutinase-expressing *Arabidopsis* plants show an altered ultrastructure and an enhanced permeability of the cuticle to solutes, e.g. leading to an enhanced herbicide sensitivity. These differences coincide with strong postgenital organ fusions. The junctions of the fusions are composed of cell wall material, in particular pectin with a low esterification grad. As fused organs grow apart from each other, organ deformations and protrusions composed of epidermal cells develop at positions with high mechanical stress. These results demonstrate that an intact cutin layer is not only important for the protection against the abiotic environment of the plant, but is also necessary for normal development and organ formation.
Furthermore, the interaction of cutinase-expressing plants with different fungal and oomycetes pathogens was investigated. Surprisingly, cutinase-expressing plants are strongly resistant to the attack by the necrotrophic fungus *Botrytis cinerea*. This resistance is neither due to constitutive or conditioned expression of typical pathogenesis-related proteins, e.g. PR-1 and PDF1.2, nor due to sensitivity of the fungus to cutinase or to the intercellular fluid diffusing out of the plant. A new resistance mechanism based on the degradation of the cuticle and/or compositional changes in the cell wall is proposed.

P3/C1.24 Nitric oxide contributes towards papilla-based resistance and the hypersensitive response in barley attacked by *Blumeria graminis*

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In plants, nitric oxide (NO) is implicated as an intra- and inter-cellular signal controlling various vital processes. In model plant-pathogen systems (such as suspension cultures), NO acts synergistically with reactive oxygen intermediates to orchestrate the hypersensitive response (HR). However, there is need for analyses of its role in economically important crop/pathogen interactions. We have therefore examined the barley/powdery mildew interaction over the period 6–24 h after inoculation, when success of plant defence responses is determined. We used an NO-specific fluorescent stain (DAF-2DA) and confocal microscopy to visualise NO generation in susceptible barley (Pallas) and an isoline (Pallas-01; P-01) carrying the *Mla1* gene conditioning HR. In addition, we used sodium nitroprusside (SNP) as a light-activated NO donor, carboxy-PTIO as a scavenger, and L-NAME as a nitric oxide synthase (NOS) inhibitor, and monitored their effects by histological analyses.
Staining revealed very early (10–12 h), but transient, localised NO generation coinciding with initiation of defensive papillae that prevent penetration of living plant cells. This was several hours earlier than H_2O_2 and autofluorogens accumulate. Furthermore, massive whole-cell generation of NO (13 h) preceded cell death in the HR. Our evidence also suggests that suppressing NO accumulation in papillae suppresses penetration resistance of living host cells, whilst the HR is accelerated by the presence of NO. Overall, the data indicate that NO influences the expression of both papilla-based, race non-specific resistance and HR due to race-specific *R*gene-*avr*gene interaction.

P3/C1.25 Rapid hormonal and solute response to salinity of growing leaf cells

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We are interested in the possible limitation of extension growth of leaf cells by the rate of solute accumulation and water transport and its regulation through hormones. The rate of solute accumulation becomes particularly important when plants are exposed to salt stress, and cells have to osmotically adjust. When 100 mM NaCl was added to the root medium of barley plants, elongation of the growing leaf three ceased rapidly, and it took about 20–30 min for growth to resume. The first significant accumulation of solutes was detected after growth recovery, at 1h of stress. This early solute accumulation was confined to the proximal portion of the leaf elongation zone. From here, solute accumulation continued towards the tip of the leaf and by 8 h was large enough to account for the entire growth recovery. ABA accumulated within 10 minutes in leaf tissue. The pattern of accumulation differed between growing and emerged zone of the developing leaf three. A parallel decrease in stomatal conductance and transpiration suggests that ABA mediated growth recovery through increase in xylem water potential and re-establishment of a gradient in water potential between xylem and peripheral growing cells that favoured water uptake into the latter.

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P3/C1.26 Cell-wall matrix assembly: nascent pectin and xyloglucan are linked together in the Golgi apparatus during synthesis

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Cell-wall matrix polysaccharides are synthesised in the Golgi apparatus and then transported to the cell wall. We have investigated the properties of nascent pectin formed by the biosynthetic system *in vitro*. Microsomes were prepared from etiolated pea epicotyls (Baydoun et al, 2001, J. Plant Physiol 158:145–150) and used for *in vitro* synthesis of 1,4-β-[U-^{14}C]galactan using UDP-[U-^{14}C]galactose. The enzyme products were characterised by selective enzymic degradation, size-exclusion chromatography and anion-exchange chromatography. Evidence was obtained for the formation of 1,4-β-galactan chains attached to a pectic backbone containing both polygalacturonic acid and rhamnogalacturonan I (Abdel-Massih et al, 2003, Planta 216: 502–511). This radioactive pectin was partially degraded by digestion either with endo-1,4-β-D-glucanase or with xyloglucan-specific endoglucanase, indicating that part or all of this nascent pectin was present as a complex with xyloglucan. The complex bound strongly to paper, confirming the presence of xyloglucan.

Acidic cell-wall polysaccharides prepared from the same tissue also contained both pectin and xyloglucan, indicating that the linkage between the two persists in the cell wall. However, the pectin-xyloglucan complex required alkaline conditions in order for it to be extracted from the cell wall, probably due to hydrogen-bonding to cellulose.

This pectin-xyloglucan complex may be similar to pectin-xyloglucan complexes found in the cell wall in rose suspension cells (Thompson and Fry, 2000; Planta 211:275-28) and cauliflower stem (Femenia et al. 1999, Carbohydrate Pol 39:151–164). Current studies aim to clarify the changes in pectin size and structure that occur during the various stages of synthesis and deposition in the wall.

P3/C1.27 Infection of Chinese cabbage by *Plasmodiophora brassicae* leads to a stimulation of plant growth: studying cell wall metabolism and hormone balances

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The clubroot infection of Brassica hosts with the biotrophic pathogen *Plasmodiophora brassicae* can have devastating effects on crop yield. The spores induce cell elongation and cell division leading to gall formation on infected roots. We noticed a transient growth promotion during the first two weeks of clubroot infection in Chinese cabbage and *Arabidopsis thaliana* Col-0. This growth promotion is in contrast to what might normally be expected in plants infected with a pathogen (i.e. under stress conditions). Concomitant with the initial growth response, xyloglucan endotransglucosylase/hydrolase (XTH) action is located in cell clusters in the epidermal layer of infected roots starting from 4 DAI. XTH action is correlated with cell wall loosening during

cell elongation, indicating the presence of expanding epidermal cells in infected tissue. Because the effect of growth promotion and altered cell elongation indicate changes in plant hormone balances, endogenous hormone balances were analysed and compared between infected and non-infected Chinese cabbage and *A. thaliana* roots. The results presented suggest an important link between the effects of clubroot infection and plant hormone balances in infected plants.

P3/C1.28 Evolutionary study of the XTH enzymes involved in cell wall elongation

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The plant cell wall is a solid, yet dynamic structure with a fibrous skeleton composed of cellulose microfibrils that are coated and linked by xyloglucans. During cell elongation the cooperation of a set of enzyme families enables the wall to grow without loosing its strength. Xyloglucan endotransglucosylase/hydrolases (XTHs) are believed to be involved in 'wall loosening'. They break and rejoin xyloglucan chains, allowing the cellulose microfibrils to move apart driven by protoplasmic pressure.
Using a fluorescent *in vivo* assay (Vissenberg *et al*, 2000; Plant Cell, 12, 1129–1137) we have located XTH-action in the elongation zone of different vascular plants, from the spore plant *Selaginella* to *Zea mays* (Vissenberg *et al*., 2003; J. Exp. Bot., 54, 335–344). The presence and complexity of the XTH-gene family in primitive vascular plants as well as its role in vascular plant elongation in general is therefore a topic of current interest in our laboratory. In contrast to expansins (other important wall modifying enzymes), evolutionary information on XTHs is still lacking.
With diverse PCR techniques based on conserved domains in known XTH-genes, *Selaginella*-specific XTH-sequences have been picked up and are being analysed *in silico*. From the results up to date it seems that there is at least one XTH gene present in the *Selaginella* genome, which shows the highest similarity with XTH9 from *Arabidopsis*. Subsequently this XTH and possibly others will be heterologously expressed in *Pichia pastoris* and functionally analysed (Fry, 1997; The Plant J., 11, 1141–1150). This research will add an evolutive aspect to the knowledge on the role of XTHs in growth and elongation of vascular plants.

P3/C1.29 Filipin-reactive structural sterols are important for the tip growth of A*rabidopsis* root hairs

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Structural sterols were shown in the plasma membrane of root epidermal cells of *Arabidopsis* using filipin as a fluorescent probe (Grebe et al. (2003), Curr. Biol. 13: 1378–1387). We visualized structural sterols in root hairs of *Arabidopsis* by filipin. Our results show that filipin-sterol complexes are abundant in the plasma membrane of root hairs and are internalized into endosomal compartments. Irreversible binding of filipin to the structural sterols causes rapid decrease of the tip-growth rate in a concentration-dependent manner, suggesting the involvement of structural sterols in the tip growth of root hairs. To strengthen this assumption, we used methyl-β-cyclodextrin as an alternative agent to deplete structural sterols (Ilangumaran and Hoessli, (1998), Biochem J. 335: 433–440) and we obtained similar results.
We correlated the growth of root hairs after filipin treatment with the architecture of the cytoplasm by analysing living root hairs with computer-assisted high-resolution light microscopy. Filipin treatment causes severe changes of root hair tips. The vesicle-rich region becomes invaded by large organelles and the vacuole, and the dynamic behaviour of vesicles and organelles is affected considerably. Formation of the filipin-structural sterol complexes compromises the integrity of the clear zone and, in due course, inhibits the tip-growth of root hairs. From our results we conclude that recycling structural sterols of the plasma membrane are essentially involved in the tip growth of root hairs of *Arabidopsis*.
This work was supported by a grant for the research trainings network TIPNET (HPRN-CT-2002-00265) from the European Community.

P3/C1.30 A putative palmitoyl transferase in *Arabidopsis* is encoded by *TIP1*

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Palmitoylation is known to be involved in protein targeting and trafficking in all cells. Disrupted polar tip growth and poor cell expansion are phenotypes associated with protein palmitoylation defects. Root hairs and pollen tubes are both examples of tip growing cells in

Arabidopsis and would be expected to require efficient protein palmitoylation for correct growth and function. The *tip1* mutants of *Arabidopsis*, which are smaller than wild type, display these symptoms with mature plants possessing short, branched root hairs and defective pollen. TIP1 shows high homology to a known palmitoyl transferase from yeast, AKR1. *TIP1* is capable of rescuing the morphological and temperature sensitive defects of *akr1* mutants indicating that TIP1 is a functional palmitoyl transferase. Inhibition of palmitoyl transferase activity in *Arabidopsis* root hairs phenocopies *tip1*. TIP1 belongs to a large family of proteins conserved across eukaryotes that have been implicated in a diverse range of processes and diseases including Huntingdons disease, neuronal growth, cell division and small GTPase regulation. TIP1 is the first palmitoyl transferase to be identified in a higher eukaryote and may be useful in the study of growth polarity and protein targeting in multicellular eukaryotes.

P3/C1.31 The rate of the slow time-dependent component of cell wall extensibility correlates with mean cellulose fibril orientation in the onion epidermis

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The mechanics of cell walls are determined by their structure which resembles that of composite materials. Semicrystalline cellulose microfibrils cross-linked by hemicelluloses are embedded in a gel-like matrix of pectins. Cellulose microfibrils have traditionally been considered as the main load-bearing component in the cell wall, their orientation determining the direction of wall extension. However there have been few direct confirmations on cell level that in seed plants a specific alignment of cellulose microfibrils confers mechanical anisotropy to thin non lignified cell walls.
The relation between wall mechanical properties and cellulose orientation was studied in the isolated adaxial epidermis of onion bulb scales. The mean or net cellulose orientation in the outer periclinal wall of these cells was parallel to their long axis. *In vitro* cell wall extensibility was much higher in the direction perpendicular to the net microfibril orientation than parallel to it, both in the initial deformation and in the time-dependent creep. The cellulose orientation determines as well the elasticity of the cell wall as the ratio between real plastic deformation and retarded elasticity. The net orientation of cellulose microfibrils thus confers mechanical anisotropy to the walls of higher plants in terms of elasticity and plasticity, especially the latter may be relevant for anisotropic cell growth.

P3/C1.32 The influence of ascorbate, glutathione and homoglutathione on the development of *Medicago sativa* leaf protoplasts

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Ascorbate (ASC), gluthatione (GSH) and (in Fabaceae) homoglutathione (hGSH) are the major low molecular weight antioxidants involved in the protection against oxidative stress (Noctor and Foyer 1998, Annu Rev Plant Physiol Plant Mol Biol 49, 249–279), and in the regulation of plant growth (Potters et al. 2002, Plant Physiol Biochem 40, 537–548). Now, stress (combined with a low auxin concentration) has been shown to instigate isolated leaf protoplasts to develop into somatic embryos, as would a high auxin concentration, whereas a low auxin concentration alone caused the cells to form microcalli (Pasternak et al. 2002, Plant Physiology, 129, 1807–1819). Therefore, we decided to observe how ASC, GSH and hGSH affect this developmental process. Apparently, cell development into somatic embryos is correlated with decline of ASC and hGSH pools, but an increase in GSH concentration. To further elucidate the role of these molecules, ASC, GSH or hGSH have been added to the culture medium. ASC was immediately oxidized to dehydroascorbate (DHA) which was then taken up and re-reduced internally, as observed in other tissues (Potters et al. 2000, Plant Physiol 124, 17–20). DHA afterwards forces the cell to form microcalli, even at high auxin levels. GSH apparently promotes the process of cell de-differentiation (also at low auxin levels). Interestingly, homoglutathione did not stimulate the leaf cells to dedifferentiate into somatic embryos, demonstrating for the first time a differential response of plant tissue to glutathione and homoglutathione.

P3/C1.33 Systematic determination of protein localisation in *Arabidopsis* cells

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Plant cells are highly compartmentalised, with most functions being restricted to specialised organelles. Cytologically recognisable features include membrane-bound organelles such as the nucleus, mitochondria and chloroplasts; some such as the nucleolus have no membrane boundary. Since most functions are spatially

restricted, we reasoned that the location of a given gene product can reveal useful clues as to its probable function. We have developed and applied a streamlined approach for studying intracellular localisation of GFP-fusion proteins in an *Arabidopsis* cell suspension culture. A method has been developed for efficient transient transformation of cells with constructs containing full length ORFs and translational N-terminal GFP fusions, delivered by a hypervirulent strain of *Agrobacterium*. Full-length ORF clones were obtained from the SSP consortium (http://signal.salk.edu/SSP/index.html) and converted to the Gateway™ system in a 96 well format.
Localisation patterns of expressed fusion proteins have been classified into five main categories: nucleus, nucleolus, cell wall, cytoplasm and sub-cytoplasmic endomembrane compartments. Using this approach we have ascribed a subcellular localisation to several proteins whose genes were annotated as 'unknown'. The localisation patterns were compared with bioinformatics (PSORT) predictions from amino acid sequence motifs. This pilot study has shown that the approach taken could easily be scaled up to cover the entire genome.

P3/C1.34 Does microfibril cross-linking strengthen or weaken the cell wall? – and other questions

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Recently, the cell wall of growing plant tissues has frequently been interpreted using a model of cell wall structure in which inextensible cellulose microfibrils are 'tethered' by hemicellulose polymers attached to the microfibril surface by hydrogen bonds. It is thought that some enzymes (such as glyosidases and transglycosidases) may cause growth by breaking these tethers and others (such as expansins) by disrupting non-covalent interactions at the microfibril surface).
Testable predictions of this model might include:
(1) The majority of work done during wall extension is involved in breaking non-covalent interactions between hemicellulose and cellulose. Were this not the case, then these interactions could not limit growth rate. However, modelling of the surface area available for hydrogen bonding suggests that relatively limited extension would 'consume' all hydrogen bonds in the wall.
(2) Cell walls should be weakened by competition for binding sites on the microfibril surface, but calcoflour has no detectable effect on creep of tomato fruit epidermis.
(3) Composites of cellulose with hemicelluloses should be stronger than pure cellulose. The work of Gidley's group using cellulose and cellulose composites produced using Acetobacter has shown that the opposite is true.
Additionally, calcium chelators closely mimic the biomechanical effects of expansins, an observation that is hard to reconcile with the 'tether' model.
It is therefore proposed that models of cell wall biophysics in which cell wall polymers act as 'scaffolds' to regulate the space available for microfibril movement are more likely to be correct.

P3/C1.35 Identification of novel genes involved in secondary cell wall formation in *Arabidopsis thaliana* using reverse genetics

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AtCesA7 and AtCesA8 (IRX3 and IRX1) encode cellulose synthases expressed specifically during secondary cell wall formation. These genes were used as markers in quantitative PCR (qPCR) to determine expression levels in various sections of the inflorescence stem, leaves and hypocotyls. The results indicate large sequential increases in AtCesA7 and AtCesA8 expression from the tip to the base of the stem. In addition, the hypocotyl shows significantly higher expression levels than the base of the stem and the leaves have the lowest expression levels of all samples.
Microarray analysis has revealed a similar pattern of AtCesA7/8 expressions confirming that these large changes are reproducible. Initial examination of the microarray data has indicated a number of potential genes that are likely to be upregulated during secondary cell wall formation. To facilitate the selection of genes for further study the software package MaxD was used to group genes that closely resembled the expression pattern of AtCesA7. T-DNA insertions in several members of this group exhibit the *irx* phenotype. This suggests that reverse genetics can be used to identify novel genes involved in cellulose synthesis or other aspects of secondary cell wall formation.

P3/C1.36 Regulation of cell division during primary vascular development in *Arabidopsis*

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Within plants the vascular system is comprised of two major types of conducting tissues, namely the xylem and phloem. The xylem acts primarily as a conduit for water and nutrients obtained from the soil, while the phloem is needed to transport organic materials from sites of production such as the leaves, to parts of the plant where it is required. Stem vascular tissue develops below the apical meristem where it continues to divide independently of other regions of the stem. The mechanisms that control the cell divisions in the vascular tissues are

unclear at present. The identification and characterisation of *Arabidopsis thaliana* mutants with altered vascular tissue will provide further insight into the control of vascular development. We have identified the recessive mutant *reduced vascular bundle* (*rvb*) in Arabidopsis. This mutant results in vascular bundles with reduced number of cells. Mutant plants possess few large mature metaxylem, resulting in a much less prominent vascular bundle. In wild type (WT) vascular bundles the phloem is composed of a contiguous group of cells, however in the mutant the number of phloem cells are smaller and often separated into discrete regions. It is considered that RVB is likely to be involved in regulation of cell division in vascular tissues. Mapping of the rvb gene, using a testcross population of ~1000 plants and markers of known position has shown that the gene maps to a 50 kb region located on chromosome 5. Sub-cloning of the 50 kb region is underway to unambiguously identify the rvb gene.

P3/C1.37 The genetic control of root hair morphogenesis in *Arabidopsis*

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ROP (Rho of Plants) GTPase signaling controls polar growth in tip-growing cells such as root hairs. Jones *et al* (*Plant Cell*, 14, 763–776, 2002) have shown over-expression of the ROP2 GTPase and constitutively active ROP2 (GTP-bound) led to additional and misplaced root hairs resulting from depolarized tip-growth, whilst dominant negative ROP2 (GDP-bound) showed a reduced number of root hair-forming sites. Loss-of-function mutations in the SCN1 (SUPERCENTIPEDE1) gene produce a root hair phenotype similar to those resulting from the over-expression of ROP2. SCN1 encodes a ROP Guanidine Nucleotide Dissociation Inhibitor (AtROPGDI1). In other systems RhoGDIs negatively regulate Rho GTPases by controlling their association with GDP and GTP as well as with cellular membranes (Hoffman *et al*, *Cell*, 100, 345–356, 2000). We are studying the molecular basis of the interaction between AtROPGDI1 and ROP2 using a variety of *in vitro* and *in vivo* methods.

P3/C1.38 Plant cells under stress- in animals

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We have identified changes in plant cell structure which occur during short-term exposure to the combined stresses of lack of oxygen and elevated temperature. Grazing cattle ingest living plant cells into the rumen (an anaerobic environment maintained at 39 °C) where they remain, perhaps for up to 24 h, while they are digested. Microbial enzymes degrade plant cell walls to release nutrients required for microbial growth, while plant proteases contribute to rapid post-ingestion proteolysis of leaf protein which is a major problem in livestock agriculture. We believe that proteolysis can be addressed by targeting plants to respond in an appropriate way to the rumen environment.

Clover leaves were incubated in an *in vitro* system simulating the rumen conditions but lacking a rumen microbial population. Light and fluorescence microscopy revealed that plant cell walls persisted during 12 h incubations but cell contents showed altered staining intensity accompanied by an apparent contraction of intra-plasmalemmal structures away from the cell walls. Individual chloroplasts could be identified after 8 h but not 12 h incubation. Discrete nuclei were observed by DAPI staining after 8 h incubation. Increased ion leakage was observed over the same period, suggesting vacuolar disruption. The 0–6 h period was also associated with extensive DNA degradation and around 60% decrease in leaf protein. The observed structural changes indicate that protein and DNA catabolism could be the result of intra-organellar events rather than being due to a widespread loss of compartmentation. Further work aims to identify if maintaining the subcellular localisation of key proteases is critical to proteolysis in the rumen.

P3/C1.39 Morphogenic effects of abiotic stress: reorientation of growth in *Arabidopsis thaliana* seedlings

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Abiotic stress responses include changes in physiological and biochemical processes as well as morphological and developmental patterns. It has remained an enigma what mechanisms are responsible for stress-induced morphogenesis. In this paper we clearly demonstrate that stress induced phenotypes comprise a re-orientation, rather than, a cessation of growth. Moreover, strong similarities between the phenotypes induced by excess copper, paraquat, salycilic acid and a hydrogen peroxide analogue, indicate that a common molecular-physiolog-

ical response system mediates these morphogenic stress responses. It is proposed that ROS play a key role in controlling the architectural changes in stressed *A. thaliana* seedlings.
We found that phenotypes of plants exposed to stress resemble, in terms of the redistribution of growth, plants altered in phytohormone metabolism. We also found that plants in which polar auxin transport is blocked (TIBA), strongly resemble, but are not identical to, plants exposed to abiotic-stress. Based on the stress-induced formation of lateral roots, we surmise that stress induces local auxin accumulation near the root pericycle.

P3/C1.40 Imaging *Festulolium* chromosomal introgressions by genomic *in situ* hybridisation as an aid to rapid plant breeding

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Although *Lolium* and *Festuca* are different species they are able to cross naturally and produce a *Festulolium* hybrid. This hybrid will contain traits derived from both species, allowing breeders to introgress desirable *Festuca* qualities into a *Lolium* background by repeated backcrossing. These plants are then screened to see whether the desired trait is stable and has been inherited. Usually this is done by subjecting the plants to the appropriate stresses, killing backcross plants lacking the introgressed region conferring resistance. However, while *Festuca* and *Lolium* species show close homology, they are sufficiently different structurally to be differentiated by genomic *in situ* hybridisation (GISH), in which fluorescent genomic *Festuca* DNA probes bind to *Festuca*-derived areas of the genome. Hence, GISH can be used to identify physical areas of the *Festuca* genome which have been introgressed into the backcross genotypes. By identifying which areas of introgression are common to all plants exhibiting the desired trait, GISH can be used to rapidly screen large populations for the trait and overcome the limitations of physiological or biochemical based screening techniques. The identified plants can be used as breeding stock for crop improvement. For instance, *Lolium* species provide highly palatable and digestible forage for grazing animals, however they lack the persistency of some closely related species. In contrast certain *Festuca* species are tolerant to abiotic stresses such as drought, thermal stress and anoxia, although *Festuca* is not particularly digestible. A forage grass which is both digestible and hardy would be of economical benefit for agricultural use.