Instituto Juan March
de Estudios e Investigaciones

72 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Plant Morphogenesis

Organized by

M. Van Montagu and J. L. Micol

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INTRODUCTION

J.L. Micol and M.Van Montagu

The present and future of the study of plant morphogenesis

José Luis Micol and Marc Van Montagu

The architecture of pluricellular organisms is the result of complex cell mechanisms which obey genetic controls operating and interacting in specific spatial and temporal programs. To dissect such morphogenetic processes in animals genetic approaches have proven extremely helpful, although this cannot yet be said for plant developmental biology, a field which has only recently gone from descriptive approaches to causal analyses emphasizing in genetic and molecular methods. As an example of this, a relatively well known textbook on Plant Development by Lyndon (1990) states in its first paragraph "... three-dimensional form is specified in some way by the genes, *since there are genes for leaf shape, for example*, ...". Most, if not all biologists, would accept such an assertion, which appears to be common sense. However, available published data do not match common sense in this case, since the question of how a leaf is made remains largely unanswered at the genetic level. Nevertheless, the analysis of plant development is rapidly approaching the level of that of animals. In the past ten years, wide screens for mutations followed by epistasis analysis and molecular studies have been carried out and regulation models have been succesfully constructed particularly for flower development, a topic covered twice, in 1991 and 1995, in activities sponsored by the Fundación Juan March. In addition, information is becoming available on other aspects of plant development, since

several research teams have focused their efforts on the genetic and molecular analysis of embryo and root development, meristem structure and functions, leaf organogenesis and pattern formation, the morphogenesis of some speciallized cells and cell organelles, the role of signalling mechanisms in plant-pathogen interactions, and the elements and paths of intercellular communication.

The study of plant development is in our days especially exciting, and hs yielded much to help in our understanding of the molecular mechanisms that regulate pattern formation, the specification of cell fate and cell differentiation. Molecular genetics and biochemical approaches are defining an emerging picture on morphogenetic pathways and their interplay in space (organogenesis) and time (phase change). It is becoming clear that complex signalling networks are involved in the control of plant development and that cell to cell communication has consequences in the differentiation of plant cells.

It is not difficult to predict that new paradigms will arise in the years ahead from advances made in the study of mechanisms that determine pattern formation and those that regulate cellular differentiation in plants. The panoramic view on the present of plant developmental biology provided by the workshop covered in this book unequivocally points to a bright future for the field. Soon it will not be possible, as it is now, to maintain that the study of animal development is more advanced than that of plants.

Session 1: Molecular mechanisms that regulate pattern formation

Chairperson: José Luis Micol

GENES WITH ESSENTIAL FUNCTIONS DURING EMBRYOGENESIS IN ARABIDOPSIS.

David W. Meinke.
Department of Botany, Oklahoma State University, Stillwater, OK 74078 USA

One objective of the Multinational Coordinated Arabidopsis thaliana Genome Research Project is to identify every gene with an essential function during plant growth and development. Many of these genes are likely to be expressed during seed development because this is such a critical stage in the plant life cycle. The purpose of my research program has been to identify genes with essential functions during embryo development through the isolation and characterization of embryo-defective (emb) mutants of Arabidopsis. In this talk I will present an overview of the wide range of mutant phenotypes and target genes identified to date and how information gained from the analysis of these mutants is being used to address important questions in plant cellular and developmental biology. One practical application of this research is the identification of mutants defective in basic metabolic processes. Two different biotin auxotrophs identified among our mutant collection illustrate how the analysis of emb mutants can facilitate the genetic dissection of essential plant functions. Another practical application is the identification of genes (e.g. LEC, TWN, TTN) with important regulatory roles in maintaining cotyledon, suspensor, and endosperm identity during seed development. Molecular characterization of these genes promises to provide a foundation for a general model of the genetic regulation of embryo and endosperm development in Arabidopsis. Research in my laboratory has been supported by grants from the National Science Foundation (Developmental Biology and EPSCoR Programs) and the S.R. Noble Foundation (Ardmore, OK).

PATTERN FORMATION AND CYTOKINESIS IN THE *ARABIDOPSIS* EMBRYO

G. Jürgens. Lehrstuhl für Entwicklungsgenetik, Universität Tübingen, Spemannstr. 37-39, 72076 Tübingen, FRG. geju@mpib-tuebingen.mpg.de

Embryonic pattern formation generates the basic body organisation of flowering plants, as expressed in the seedling (Jürgens, 1995, Cell 81, 467-470). The seedling harbors, at opposite ends of its axis of polarity, the root and shoot meristems which will produce additional organs during post-embryonic development. The meristems themselves originate during embryogenesis as part of the apical-basal pattern which also includes the cotyledons, hypocotyl and root. Perpendicular to the apical-basal axis, a radial pattern consists of concentric tissue layers. Due to the nearly invariant cell division pattern in the *Arabidopsis* young embryo, the origins of seedling structures have been traced back to very early stages of embryogenesis. A few genes have been shown to be expressed in specific locations, for example in the outer cell layer or at the top of a globular embryo, suggesting that developmental cues have already segregated cell fates in a position-dependent manner (Laux & Jürgens, 1997, Plant Cell 9, 989-1000). In order to analyse mechanisms underlying pattern formation in the embryo, a genetic approach has been taken in *Arabidopsis*. Our current studies focus on the analysis of a few genes involved in apical-basal development, such as *GNOM* and *FACKEL*. The *GNOM* gene is required before the first cell division and appears to be necessary for firmly establishing the apical-basal axis of the embryo (Mayer et al., 1993, Development 117, 149-162; Vroemen et al., 1996, Plant Cell 8, 783-791). The *GNOM* gene encodes a 163 kDa protein of unknown function (Busch et al., 1996, Molec. Gen. Genet. 250, 681-691) which shows sequence similarity to yeast proteins involved in vesicle trafficking (Peyroche et al, 1996, Nature 384, 479-484). The *FACKEL* gene has a more restricted effect on the apical-basal pattern, being required for hypocotol development. In conventional mutagenesis screens, developmental genes are identified on the basis of their distinct phenotypes. To circumvent this limitation, we have taken a phenotype-independent approach that is based on GUS reporter gene expression patterns resulting from insertions near an appropriate enhancer sequence ("enhancer trap") or into developmentally regulated genes ("gene trap"). Screening for region- and tissue-specific expression in the developing embryo serves two purposes, isolation of molecular markers that can be used to characterise pattern mutants, and isolation of developmental genes.

Cytokinesis partitions the cytoplasm of the dividing cell. This basic biological process serves multiple purposes in plant development (Jürgens, 1996, Sem. Cell Dev. Biol. 7, 867-872). For example, cell fates can segregate during the division of a polarised cell, and cell divisions that are regulated in time or space contribute to morphogenesis since the wall-bounded cells cannot change their positions relative to their neighbors. In more general terms, the newly-formed plasma membrane and cell wall may act as a barrier to help establish separate microenvironments for the daughter nuclei. Plant cytokinesis starts in the center of the dividing cell where a specific cytoskeletal array, the phragmoplast, forms between the daughter nuclei. Golgi-derived vesicles are transported along the phragmoplast to the plane of division where they fuse with one another to form the cell plate, a membrane-bounded incipient cell wall (Staehelin and Hepler, 1996, Cell 84, 821-824). As the phragmoplast is being displaced centrifugally, the disc-shaped cell plate expands by the continuous incorporation of new vesicles and eventually fuses with the parental cell wall. We have taken a genetic approach to analyse mechanisms that underlie cytokinesis. One of several genes identified by mutation, *KNOLLE*, is transcribed in a cell-cycle dependent manner and encodes a syntaxin-related protein (Lukowitz et al., 1996, Cell 84, 61-71). Syntaxins are members of a family of vesicle-docking proteins, and KNOLLE protein may thus play a specific role in cytokinetic vesicle trafficking. As shown by indirect immuno-fluorescence and electron microscopy, KNOLLE protein is made during the M phase of the cell cycle and accumulates in the plane of cell

division during cytokinesis, mediating the formation of the cell plate by vesicle fusion (Lauber et al., submitted). Mutations in another gene, *KEULE*, also cause defects in cytokinesis, and the mutant phenotype resembles that of *knolle* embryos (Assaad et al., 1996, Molec. Gen. Genet. 253, 267-277). Whereas the single mutants are seedling-lethal, the *knolle keule* embryo develops into a huge single cell with many nuclei, suggesting that the two genes have partially overlapping functions in cytokinetic vesicle trafficking (Waizenegger et al., in prep.).

GENETIC ANALYSIS OF ROOT INITIATION AND PLANT CELL AXIALIZATION IN *ARABIDOPSIS THALIANA*

Christian S. Hardtke, Gerhard K.H. Przemeck,
Jim Mattsson, Margarete Strasser, Thomas Berleth

Genetics Institute, Maria-Ward-Str. 1A, D80638 Munich, FRG; e-mail: uj44205@sunmail.lrz-muenchen.de

Regulated cell expansion is one of the key determinants of plant morphology. The embryonic development of *Arabidopsis thaliana* is characterized by an almost invariant sequence of cell expansions and divisions enabling screening for mutants impaired in certain aspects of cell expansion.

We have analyzed cell axialization, the coordinated elongation of cells in the shoot-root axis, recognizable as oriented cell expansions during hypocotyl initiation as well as vascular strand formation. We have isolated mutations in at least three genes that result in cell axialization defects. The mutant *monopteros (mp)* represents the most dramatic phenotype. The *MP* gene is required for the formation of the hypocotyl and the radicle in the *Arabidopsis* embryo. The deletion of these structures in mutant seedlings reflects the failure of basal cells in the early embryo to elongate along the apical-basal axis. Post-embryonic defects in the formation of vascular strands and a reduced polar transport of auxin suggest a role of the *MP* gene in cell axialization throughout the *Arabidopsis* life-cycle. We have recently isolated the *MP* gene from a chromosome walk. The *MP* gene encodes a transcriptional regulator that is expressed in early embryos and emerging organs. During embryo and organ maturation *MP* expression becomes gradually confined to axialized regions and is finally restricted to the vascular tissues. Details of the molecular analysis of the *MP* product and its expression pattern will be discussed.

Characterisation of genes involved in early embryogenesis in maize.

Virginia Stiefel, Matilde José, Ramon Roca, Enrique Graziano, Eduardo López Becerra, Miriam Bastida and Pere Puigdomènech.
Departamento de Genética Molecular. CID-CSIC. Jordi Girona, 18. 08034 Barcelona. Spain.

Two different approaches have been developed in our group to identify genes involved in maize early embryogenesis. One is the search for genes having characteristic patterns of expression in the maize early embryo, including genes specifically expressed at these initial stages of development. The second one is the cloning of genes important for maize embryogenesis by transposon tagging of embryo mutants.

Results will be presented on the patterns of expression of genes during early embryogenesis. In particular the features of the expression of genes coding for proline-rich proteins, genes related to cell proliferation and genes having a consitutive expression in the embryo. Also a number of genes specific for the embryo have been identified, including a gene coding for a malic enzyme specific for the embryo root epidermis.

Two mutants produced by the Ac transposon have been analyzed at the molecular level. The mutations belong to the *defective kernel* (*dek*) group of mutants. Two of these mutations (called *dekA* and *dekB*) have been shown to segregate with a DNA band hybridizing with the Ac transposon. The *dekB* mutation blocks embryogenesis at the transition stage, the *dekA* mutation produces a general disorganization of the embryo from the coleoptilar stage. The *dekB* gene codes for a new type of transmembrane protein while the *dekA* gene codes for a protein with no homology to any sequence in the data base.

Development and pattern of stomata in Arabidopsis

Laura SERNA and Carmen FENOLL

We have defined three types stomatal complexes Arabidopsis leaves, depending on the number of subsidiary cells which flank the guard cells. Since stomatal development in Arabidopsis associates with leaf expansion, with mature complexes at the tip and complexes in increasingly early developmental phases towards the leaf base, in a single expanding leaf the complete developmental sequence of complexes can be followed. By serial anatomical observations we have deducted the pattern of cell divisions that lead to a stomatal complex from a single protodermal cell. To unequivocally demonstrate the clonal origin of the complexes, we have performed a clonal analysis, by following the excision of the *Ac* element in transgenic plants containing a 35S*GUS*::Ac fusion. *GUS* expression in the cells that integrate complexes placed over the borders of leaf sectors generated by *Ac* transposition, fully supports this clonal origin. Stomatal complexes in leaves show the maximum degree of aggregation, with most complexes immediately adjacent to each other. This pattern could be explained solely by their cell lineage, which obviously imposes a minimum distance of two epidermal cells between stomata. two epidermal cells. However, other processes are needed to explain our observation that, much like mature stomata, stomatal precursor cells never appear in direct contact early during leaf expansion or in the basal part of the developing leaf. This fact can be better explained by the existence of inhibitory factors arising from the stomatal precursors. Therefore, stomata pattern will arise from a combination of lateral inhibition from stomatal precursors and the cell division history of the stomatal complexes. This pattern is under genetic and physiological control. The spacing is altered in *ctr1*, a mutant displaying constitutive activation of ethylene responses, and can be disturbed in wild type plants by the ethylene precursor ACC. Inhibitors of ethylene synthesis and perception blocked ACC-dependent cluster formation in wt plants, but did not affect stomata spacing in *ctr1*. Clonal analysis by transposon excision showed that ACC-induced clusters have a clonal origin. This sustains our previous findings based on the expression analysis of transgenic Arabidopsis lines that contain markers for cell division and cell identity of epidermal cells. The behavior of such lines indicate that ethylene induced changes in the fate of a subpopulation of epidermal cells, which experienced unscheduled division and acquired a new identity, producing stomatal clusters as a result of the combination of both events. Taken together, all these results provide the first evidence that ethylene modulates stomata patterning in Arabidopsis, and that it probably does so by acting as a morphogen on specific epidermal cells whose fate is shifted by the hormone, thus interfering with pre-established differentiation pathways in the leaf epidermis. We will present additional data on the behavior of other mutants altered in ethylene biosynthesis and responses and in other developmental processes with respect to stomata development.

Session 2: Specification of cell fate

Chairperson: Sarah Hake

Organogenesis in the Arabidopsis root: the epidermis

Liam Dolan1, Fred Berger1,2, Catherine Kidner1, Stamatis Rigas1,3 and Polydefkis Hatzopoulos3
1.Department of Cell Biology, John Innes Centre, Norwich NR4 7UH, UK.
2.UMR CNRS/INRA/ENS, Ecole Normale Superieure de Lyon, 46 allee d'Italie, 69364 Lyon, France.
3.Laboratory Of Molecular Biology, Agricultural University of Athens, Iera Odos 75,Athens 11855, Greece.

Organogenesis is the process by which a group of founder cells becomes patterned, proliferates and expands during the formation of plant structures. For the purpose of this communication we will focus on three distinct activities that constitute part of the organogenetic program in roots. These involve 1) the examination of the pattern of cell division using clonal analysis which identifies a role for positional information 2) the further exploration of positional information using laser ablation techniques 3) genetic and molecular analysis of the mechanism of cell expansion in hair cells, a model system for the study of cell growth.

Roots are indeterminate structures initiated from the basal end of the dicot embryo. During the development of the root system, lateral roots are formed from a subset of pericycle cells which reiterate the organogenetic program. The seedling primary root (which is formed in the embryo) is composed of a distal set of initials surrounding a tier of four relatively quiescent cells (Dolan et al., 1993). Above this is a zone of meristematic activity which gives way to a zone where growth continues in the absence of cell division, the so called elongation zone. When cells cease elongating they develop secondary walls and differentiate. In contrast, the shoot system of plants is characterised by the serial development of lateral appendages - leaves and floral organs. The identity of these appendages is specified by a battery of genes defined by homeotic mutations while little is known about the molecular mechanism underpinning the organogenesis of such structures.

We are using the root to investigate the molecular mechanism of cellular morphogenesis in plants. We have focused on the development of the root epidermis specifically because the epidermis is composed of a small number of cells (16-22) (Dolan et al., 1994; Galway et al., 1994). Epidermal cells have either of two fates: they differentiate as root hair cells if located over anticlinal walls of underlying cortical cells or they differentiate as non-hair epidermal cells if located over periclinal cortical cell walls. The regular pattern of differentiation facilitates the identification of mutations affecting the development of the epidermis. Such an analysis has allowed the isolation of genes required for this process. Furthermore, by virtue of its simple anatomy and small diameter the root is amenable to laser-based surgical analysis.

To determine the nature of the cues directing cell fate in the root it is necessary to know the life history of the constituent cells. For example, if lineage-restricted decisions were important in fate specification, it might be expected that cell lineage would be invariant. If on the other hand lineage were not of prime importance in the establishment of patterned cell differentiation, it might be expected to be more variable.

To gain mechanistic insights into these processes we induced marked clones in root meristems of three day old seedlings using a modified maize Ac/Ds transposon system. The clones revealed variation in the patterns of cell division that might otherwise be overlooked. The displacement of a subset of clones induced in initial cells indicated that initials are continuously being replaced - there is no permanent population of initials. This indicates that final decisions regarding the fate of cells is not made in the initial zone.

Examination of clones induced in the meristem identified a small number of clones that included cells in two adjacent tissues. The abundance of this class of clone suggests that occasionally cell divisions occur in a tissue layer resulting in one of the daughter cells being located in a different layer from the parent. Similar clones have been identified in chimeric tobacco shoots in which genetically green cells were found to "invade" underlying albino tissue (Stewart and Burk, 1970). Our observations substantiate these earlier observations indicating that cells differentiate late according to their position and not their lineage.
the examination of rare longitudinal divisions in the root epidermis. The majority of divisions in the future epidermis are transverse anticlinal in orientation and increases the number of cells per file. Occasionally meristematic cells in the "hair cell" location undergo longitudinal anticlinal divisions. Such divisions result in the development of two cell files where previously there was one. In almost all cases so far examined, daughter cells overlying the cortical anticlinal wall differentiate as hair cells while those cells overlying the periclinal cortical walls develop as non-hair epidermal cells. This suggests that cells are being exposed to external signals that specify their fate in a position-dependent manner. If a cell produces a daughter cell in a new spatial context it, in turn, is subjected to a different set of cues that specify its fate.

These observations suggest that positional cues direct cell fate in the root epidermis. To further characterise the nature of these signals we made use of laser microsurgical analysis. It was found that cells could be isolated from neighbouring epidermal cells without altering their eventual fate. Furthermore, isolation of epidermal cells from underlying cortical cells had no affect on fate, indicating that living cells in the root meristem were not required for fate specification. These observations indicate that there is either an organ-wide set of spatial cues that does not depend on individual cells for its existence (e.g. a hormone gradient) or alternatively that a cell specification activity resides in the extracellular matrix.

Cell expansion plays a central role in the establishment of organ shape during organogenesis. While expansion most likely does not play a determinative role in the development of organs it is a process that is tightly regulated by positional and temporal cues. To gain insights into the molecular process of cell expansion we have focused our attention on the cellular morphogenesis of the specialised root hair cell.

Root hairs are tip-growing projections that emanate from the basal ends of specialised epidermal cells, trichoblasts. The earliest stage in the process is a localised outgrowth from the cell's basal end. Growth is initially slow and appears to involve local thinning of the cell wall. Growth accelerates to approximately

2?m.min-1 when the outgrowth is approximately 20?m in length, coinciding with the assembly of a tip-growing cytoplasmic organisation characterised by local accumulation of Golgi-derived vesicles, axialisation of the microtubule cytoskeleton and movement of the nucleus into the hair.

Genetic analysis of root hair growth has identified a large number of genes required for the specification of the site of hair emergence, initiation of local cell expansion, transition to tip growth and the subsequent execution of tip growth. The TINY ROOT HAIR1 gene is defined by a T-DNA-induced complete loss of function allele. trh1-1 mutants initiate root hairs and undergo the process of local cell expansion but fail to make the transition to tip growth. TRH1 encodes a protein similar to a class of high affinity potassium transporters form fungi and bacteria. This suggests that the establishment of tip growth requires a high affinity K+ translocator. It is likely that TRH1 plays a role in the maintenance of cytoplasmic ionic balance in the rapidly growing root hair. That K+ uptake is not compensated by previously identified K+ translocators and channels in conditions of high external potassium is intriguing. At this stage we cannot rule out the possibility that the translocator acts directly in intracellular compartments.

This analysis has examined the role of three processes that occur during organogenesis in roots: patterning, cell division and cell expansion. The results indicate that pattern is probably established during embryogenesis and is propagated through the active meristem perhaps via an imprinted extracellular matrix. That cells respond to the pattern imprinted on the matrix is revealed by clonal analysis. Once cells have been specified they undergo a position-dependent cell expansion. The TRH1 gene product has implicated a high affinity K+ transporter in this process. Future research will identify further molecules involved and bring these three apparently disparate activities together in a general mechanism of organogenesis in plants.

Dolan et al., 1993 Development 119, 71-84.
Dolan et al., 1994 Development 120, 2465-2474.
Galway et al., 1994 Developmental Biology 166,740-754.
Stewart and Burk, 1970 American Journal of Botany 57, 1010-1016.

- John Schiefelbein

Cell Fate Specification in the Root Epidermis of Arabidopsis

Multicellular organisms possess diverse cell types that are organized in particular patterns. A general problem in developmental biology is to understand how cells acquire distinct identities and form appropriate patterns of cell types. The long-term objective of our research is to use root epidermis formation in Arabidopsis as a model for understanding the molecular mechanisms controlling cell fate in plants.

The development of the root epidermis in Arabidopsis provides a simple system for studies of plant cell fate, because there are only two cell types (root hair cells and hairless cells) and cell fates are specified in a position-dependent manner. Epidermal cells located in the crevice between underlying cortical cells (in contact with two cortical cells) differentiate into root hair cells, whereas cells located directly outside a cortical cell (in contact with a single cortical cell) differentiate into hairless cells (Dolan et al., 1994; Galway et al., 1994). Furthermore, the precursors to the root hair and hairless cells can be accurately identified prior to cell maturity. The developing root hair cells (trichoblasts) possess more densely-staining cytoplasm, reduced vacuolation, and reduced length, relative to the developing hairless cells (atrichoblasts).

To define the molecular basis of the position-dependent cell type differentiation in the Arabidopsis root epidermis, we have employed genetic studies to identify loci that influence the normal pattern of epidermal cell types (Masucci and Schiefelbein, 1994; Galway et al., 1994; Masucci et al., 1996). The analysis of two of these loci, TRANSPARENT TESTA GLABRA (TTG) and GLABRA2 (GL2), suggest that they encode negative regulators of root hair cell differentiation or, alternatively, positive regulators of hairless cell differentiation. The recessive ttg mutations cause nearly every epidermal cell to adopt a root hair cell fate, and the expression of the maize R gene in Arabidopsis (which suppresses ttg defects in other plant parts) leads to excessive hairless cell formation. The recessive gl2 mutations also cause essentially every epidermal cell to form a root hair, though these ectopic root hair cells retain the secondary cellular characteristics of hairless epidermal cells. The GL2 gene encodes a homeodomain-containing protein and is expressed preferentially in the atrichoblast cells, which implies that GL2 acts as a cell-position-dependent transcriptional regulator to repress hair formation (Masucci et al., 1996). In addition to negative regulators of root hair formation, genes required for root hair cell differentiation (e.g. RHD6) have been identified.

In this presentation, the nature of the relationship between the genes controlling root epidermal cell fate will be explored. In addition, the role of these genes in regulating epidermal processes in other tissues of the Arabidopsis plant will be discussed. The analysis of these gene pathways is expected to lead to a greater understanding of the complex regulatory circuits that have evolved to ensure appropriate cell

type differentiation during plant development.

REFERENCES:

Dolan, L., Duckett, C., Grierson, C., Linstead, P., Schneider, K. Lawson, E., Dean, C., Poethig, S., and Roberts, K. (1994). Clonal relations and patterning in the root epidermis of Arabidopsis. Development 120, 2465-2474.

Galway, M., Masucci, J., Lloyd, A., Walbot, V., Davis, R., and Schiefelbein, J. (1994). The TTG gene is required to specify epidermal cell fate and cell patterning in the Arabidopsis root. Dev. Biol. 166, 740-754.

Masucci, J., and Schiefelbein, J. (1994). The rhd6 mutation of Arabidopsis thaliana alters root hair initiation through an auxin- and ethylene-associated process. Plant Physiol. 106, 1335-1346.

Masucci, J., Rerie, W., Foreman, D., Zhang, M., Galway, M., Marks, M., and Schiefelbein, J. (1996). The homeobox gene GLABRA2 is required for position-dependent cell differentiation in the root epidermis of Arabidopsis thaliana. Development 122, 1253-1260.

Direct transdifferentiation of mesophyll cells into xylem cells in culture

Hiroo Fukuda (Botanical Gardens, University of Tokyo, JAPAN)

Differentiation in plant cells is plastic. Differentiated cells can transdifferentiate to other types of cells in response to external stimuli. Xylem cell differentiation *in vitro* is an excellent example of transdifferentiation occurring at the cellular level in higher plants. We have established an *in vitro* experimental system as a useful model for the study of transdifferentiation, in which single mesophyll cells isolated from *Zinnia* leaves can transdifferentiate directly into single tracheary elements (TEs) without cell division, synchronously and at high frequency by the initiation of wounding and a combination of auxin and cytokinin. Recent increase in our knowledge on the induction and progression of this transdifferentiation has indicated the presence of multi-step determination during the transdifferentiation. In this paper, I will show the dissection of the process of the transdifferentiation and discuss *in vitro* cell differentiation in plants.

Various lines of our results have indicated that the process of the transdifferentiation is divided into three stages, Stage I, II, and III (Fukuda, 1996, 1997). Stage I, which immediately follows the induction of differentiation, corresponds to the functional dedifferentiation process during which isolated mesophyll cells lose their ability to carry out photosynthesis. This dedifferentiation process also involves the expression of wound-induced genes and the acquisition of the cell ability to grow and differentiate in a new environment. However, it should be emphasized that this dedifferentiation can proceed without accompanied by cell division. A typical example of functional dedifferentiation during Stage I is the change in the organization of the reticulate arrays of actin filaments, which are thought to anchor chloroplasts to the plasma membrane (Kobayashi et al., 1987). These arrays are reorganized into a three-dimensional network over the entire length of the cell, which causes chloroplasts to leave the vicinity of the plasma membrane and the mesophyll cells to lose their photosynthetic function. We have isolated cDNA clones corresponding to 12 genes that are induced during Stage I. These genes have been categorized into three groups. The transcription of all of these genes are induced by wounding but not by phytohormones. However, phytohormones do affect the changes in the level of their transcripts during Stage II and Stage III of TE differentiation. These results suggest that the dedifferentiation process involves wound-induced events which are regulated by phytohormones at a specific later stage.

To analyze Stage II, we isolated, through differential screening of a cDNA library, three clones that contained cDNA inserts whose corresponding mRNAs were expressed differentiation-specifically in Stage II, and their corresponding genes were designated *TED2* to *TED4* respectively. Based on the analysis of these genes, we have presented a hypothesis that during stage II, the ability of differentiation in cells that have dedifferentiated from mesophyll cells are restricted from pluripotency for differentiating into immature xylem and/or phloem cells to a single potency for differentiating into TEs, that is TE precursor cells (Demura and Fukuda, 1994). This process seems to correspond to the *in vivo* process that meristematic cells change to procambial cells, and then to TE precursor cells.

The transition from Stage II to Stage III seems to be a critical check point in TE differentiation. The entry into Stage III is also regulated by some specific factors. Iwasaki and Shibaoka (1991) have shown that the inhibition of brassinosteroid biosynthesis by uniconazol prevents TE differentiation from *Zinnia* mesophyll cells. This conclusion has been substantiated by our recent investigations of the inhibitory effect of uniconazol on the expression of genes involved in TE differentiation. Uniconazol does not inhibit the expression of genes induced during Stage I or Stage II including *TED*s, but does prevent the expression of Stage III-specific genes, including those encoding enzymes involved in the secondary wall thickenings and enzymes involved in cell death (Yamamoto et al., 1997). By contrast, brassinolide and several intermediates of brassinosteroid biosynthesis can circumvent the uniconazol-induced inhibition of both TE differentiation and gene expression. These data imply that endogenous brassinosteroids may induce the entry into Stage III.

Stage III involves TE-specific events such as secondary wall formation and cell death process, and occur both *in vitro* and *in situ*. The cell death process that takes place during Stage III of TE differentiation is coupled tightly with the formation of secondary walls. Indeed, it has not been possible to experimentally separate the cell death from secondary wall formation in TE differentiation. TEs reach maturity following the loss of cell contents, including the nucleus, and the partial digestion of primary walls. This cell death process is different from apoptosis. The cell death process involves the induction of genes for cysteine proteases and a new type of DNase, the accumulation of these enzymes in the vacuole, and the disruption of the vacuole to permit these enzymes to attack organelles in the cytoplasm. Genes for these hydrolytic enzymes are expressed in a very similar pattern, suggesting that these genes are regulated via a common mechanism that relies on the same *cis* and *trans* activation factors.

References:

1. Fukuda, H. : Xylogenesis: Initiation, progression and cell death. Ann. Rev. Plant Physiol. Plant Mol. Biol. 47: 299-325, 1996.
2. Fukuda, H.: Tracheary element differentiation. Plant Cell 9, in press, 1997.
3. Kobayashi, H., Fukuda, H. and Shibaoka, H.: Reorganization of actin filaments associated with the differentiation of tracheary elements in *Zinnia* mesophyll cells. Protoplasma 138, 69-71, 1987.
4. Demura, T. and Fukuda, H.: Novel vascular cell-specific genes whose expression is regulated temporally and spatially during vascular system development. Plant Cell 6, 967-981, 1994
5. Iwasaki T, Shibaoka: Brassinosteroids act as regulators of tracheary-element differentiation in isolated *Zinnia* mesophyll cells. Plant Cell Physiol. 32, 1007-14, 1991.
6. Yamamoto, R., Demura, T., and Fukuda, H.: Brassinosteroids induce entry into the final stage of tracheary element differentiation in cultured *Zinnia* cells. Plant Cell Physiol. 38, in press.

The role of GL1 and other genes in Arabidopsis trichome development

M. David Marks
Department of Genetics and Cell Biology, University of Minnesota
St. Paul, MN 55108

The development of trichomes in Arabidopsis provides a convenient model to study the control of cell fate in plants. Our analyses suggest that trichome initiation is not preceded by an asymmetric cell division as is often in seen during the initiation of root hairs or of trichomes in monocots. In addition, there is no evidence for a specialized cell lineage that produces trichomes as is seen for the development of Arabidopsis guard cells. As occurs during bristle development in Drosophila, it appears that trichome initials are chosen from fields of cells having equivalent competency. We have found that genes that are involved in the early development of trichomes are expressed throughout these fields of cells. The commitment of a cell to the trichome pathway coincides with a up regulation of these genes concomitant with an apparent down regulation in neighboring non trichome cells. Since the spacing of trichomes is not random, a lateral inhibition mechanism is likely acting to prevent neighboring cells from entering the trichome pathway. We have evidence that both the GL1 and TTG genes, which are genes required for trichome initiation, are needed for efficient lateral inhibition. The TRY gene also has been reported to be involved in controlling lateral inhibition. Evidence will be presented that TRY also may have a role in preventing mesophyll cells from becoming trichomes. In addition, a new gene that may have a role in preventing trichome support cells from becoming trichomes will be described.

Session 3: Morphogenetic pathways and phase change

Chairperson: Ton Bisseling

Shoot apical meristem function in Arabidopsis: the cellular basis

Jan Traas, Patrick Laufs, Claudia Jonak, Jan Dockx

INRA, Laboratoire de Biologie cellulaire
Route de Saint Cyr
78026 Versailles cedex
FRANCE
e-mail traas@versailles.inra.fr

All aerial parts of the plant are generated by groups of undifferentiated, proliferating cells called meristems. These structures determine organ identity, position and numbers and at least partially establish organ structure and size.
Careful histological as well as clonal analyses have led to a model which postulates that meristems are divided into morphologically and functionally distinct zones. According to this model organs are initiated in the peripheral zone. This part surrounds the central zone which is supposed to be important for meristem maintenance. The rib zone, at the basis of the meristem, will give rise to the inner parts of the stem. Genetic and molecular analysis have allowed to the cloning of some of the genes involved in meristem structure, maintenance and primordium initiation in Arabidopsis. Thus, the SHOOTMERISTEMLESS homeobox gene is required for meristem maintenance, whereas the CLAVATA1 gene encodes a putative receptor kinase, which could regulate meristem size. Although the isolation of these genes has opened important new perspectives, many questions regarding meristem function remain unanswered.
One of our main research interests is to study the way the behaviour of individual cells is coordinated within the meristem of Arabidopsis thaliana. For this purpose we are using two different approaches. First, we are studying the spacial control of cell division and growth in the shoot apex. Second, we are identifying genes that are necessary for the maintenance of correct proliferation patterns using a genetic approach.
A detailed analysis has allowed us to determine the mitotic index, cell size and expression patterns of cell proliferation genes in the growing meristem and young primordia of wild type inflorecences. This has shown that the mitotic index and cell size are not homogeneous in the meristem. For instance, as has been described for other species, cells in the central part of meristem divide slower than cells at the periphery. The L1 layer of the young inflorescence meristem in the WS ecotype can be presented as a disc with a diameter of 60 - 65 microns. Cells lying within 12 microns from the center of the disk have a mitotic index of 2 - 2.4 %, whereas the cell population further away divides at a rate of 4 - 4.3 %.

This central part, which contains about 20 cells, could represent the central zone described above. The model or ÇmapÈ of the wild type shoot apex is now used to classify the division patterns in a range of mutants. Some of these, such as clavata or fasciata have been described by others, but we have also identified new mutants wich show abnormal meristem structure and/or produce abnormal organ numbers. One set of mutants combines a reduced capacity to initiate organs with dwarfism. Interestingly, the size of the individual, fully differentiated cells in mutant leaves is not altered. This implies that the smaller size of these plants is essentially due to a reduction of the cell number. The genes therefore are necessary for the coordination of cell proliferation at the shoot apex. This set of mutants is being characterized further, and a phenotypical and genetic analysis will be presented. Four of the mutants were obtained by transformation with T-DNA. Since they are most likely tagged we have isolated the sequences that flank the T-DNA. So far this has lead to the identification of three candidate genes, that could be involved in coordinating the growth of mitotic cells.

Feiler, H., Desprez, T., Santoni, V., Kronenberger, M., Caboche, M., Traas, J. (1996) The higher plant A. thaliana encodes a functional CDC48 homologue which is highly expressed in dividing and expanding cells. EMBO J.: 14 (22): 5626-5637.

Traas, J.A., Laufs, P. (1997) Cell cycle mutants in plants, a phenotypical overview. In: Plant Cell Division (Francis, D, Dudits, D., Inz, D. eds). Portland Press.

MERISTEM FUNCTIONS: THE FINE LINE BETWEEN DETERMINATE AND INDETERMINATE FATES

Sarah Hake, George Chuck, Naomi Ori, David Jackson, Erik Vollbrecht, Debbie Laudencia-chingcuanco. Plant Gene Expression Center, USDA-ARS and the University of California-Berkeley, 800 Buchanan St., Albany, CA. 94710.

Our laboratory is using the knotted1 homeobox gene as a tool to study meristems in maize and other species. Knotted1 (kn1) encodes a homeodomain protein that is abundantly expressed in shoot meristems and is absent in leaves. The down-regulation of kn1 in the meristem appears to predict where the next leaf will form. kn1 is expressed during embryogenesis at the site of the initiating meristem. Expression persists into the inflorescence, branch and floral meristems, but disappears as carpels initiate. Loss of function phenotypes in maize reveal that KN1 is required to initiate branch meristems and to repress extra carpels and leaves. We have shown that overexpression of kn1 in Arabidopsis causes a lobed leaf phenotype. Meristems initiate in the sinus of the lobes. We are using the inflorescence defect in maize and the lobed leaf phenotype in Arabidopsis to obtain downstream and interacting proteins. Other experiments take advantage of the anti-KN1 antibody to examine meristems in other species.

Chuck, G., Lincoln, C. and Hake, S. (1996) KNAT1 induces lobed leaves with ectopic meristems when overexpressed in Arabidopsis. Plant Cell 8:1277-1289.

Hake, S. and Char, B. (1997) Cell-cell interactions during plant development. Genes & Development 11:1087-1097.

Kerstetter, R. and Hake, S. (1997) Shoot meristem formation in vegetative development. Plant Cell 9:1001-1010.

Kerstetter, R.A., Laudencia-Chingcuanco, D., Smith, L.G., and Hake, S. (1997) Loss of function mutations in the maize homeobox gene, knotted1, are defective in shoot meristem maintenance. Development [in press]

Sarah Hake
Plant Gene Expression Center
800 Buchanan St.,
Albany Calif. 94710

phone: 510 559 5907
fax: 510 559 5678

JANE A. LANGDALE
Department of Plant Sciences
University of Oxford
South Parks Road
OXFORD OX1 3RB (U.K.)

PATTERN FORMATION AND CELLULAR DIFFERENTIATION IN MAIZE LEAVES

Mature maize leaves exhibit a series of parallel veins that are surrounded by concentric rings of bundle sheath (BS) and mesophyll (M) cells. The development of this uniform pattern can be considered to occur in two stages - first cells become specified and then they differentiate. To identify and characterize genes that control these processes, we have screened transposon mutagenized populations of maize for two types of mutants. The first type disrupts the specification of cell types within the leaf such that overall leaf pattern is perturbed. The second type exhibit normal leaf shape but disrupt the differentiation of specific cell-types. Analysis of two of these mutants will be discussed.

The *twisted dwarf* (*twd*) mutant was identified in an *Spm* mutagenesis program and was found to be allelic to *rough sheath 2* (*rs2*). Mutant plants exhibit an overall twisted morphology in the shoot (roots are unaffected). Furthermore, leaf shape is aberrant in that patches of sheath-like tissue invade the blade. This aspect of the phenotype is reminiscent of that seen in dominant *Rough sheath 1* (*Rs1*) mutants which ectopically express the *rs1* homeobox-encoding gene in the leaf. We have shown that RS1 also accumulates ectopically in recessive *rs2* mutants and as such we propose that the *rs2* gene constrains *rs1* expression to the meristem during normal development. Using *Spm* as a tag, we have cloned *rs2* using the *rs2-twd* allele. The gene encodes a *myb*-like transcription factor with significant homology to the *PHANTASTICA* gene of *Antirrhinum*. The implications of these results will be discussed.

In *bundle sheath defective 1*(*bsd1*) mutant plants, BS cells fail to differentiate yet M and all other leaf cell-types develop normally. We have identified a number of *Spm*-induced alleles of *bsd1* and have used one of them (*bsd1-s1*) to clone the *Bsd1* gene. Gene sequence data revealed the existence of a bipartite nuclear localization signal in the first exon and we have shown that *Bsd1*-reporter gene fusions are targeted to the nucleus in onion epidermal cells. Further sequence analysis indicates the presence of a novel domain within the deduced protein sequence that shares some features with TEA DNA binding domains. As such, we propose that *Bsd1* acts as a novel transcriptional regulator of cellular differentiation in maize. A comparison of transcript accumulation patterns in C_4 and C_3 photosynthetic maize tissues suggests that BSD1 functions to regulate differentiation of all photosynthetic cells in C_3 tissue. However, in C_4 tissue the gene functions specifically to regulate bundle sheath cell differentiation.

Jane A. Langdale and Catherine A. Kidner (1994). *Bundle sheath defective*, a mutation that disrupts cellular differentiation in maize leaves. **Development,** *120* 673-681

Ronelle Roth, Lisa N. Hall, Tom P. Brutnell & Jane A. Langdale (1996) *Bundle sheath defective 2*, a mutation that disrupts the co-ordinated development of bundle sheath and mesophyll cells in the maize leaf. **The Plant Cell,** *8* 915-927

Lisa N. Hall & Jane A. Langdale (1996) Molecular genetics of cellular differentiation in leaves. Tansley Review #88. **The New Phytologist** *132* 533-553

A genetic approach to the dissection of leaf ontogeny in *Arabidopsis*

Bernâ, G., Robles, P., Candela, H., Serrano, J., Pérez-Pérez, J.M., Piqueras, P., Ponce, M.R., Vera, A., Martínez-Laborda, A., and Micol, J.L.
División de Genética. Universidad Miguel Hernández. Campus de San Juan. 03550 Alicante. Spain.

Mutations affecting leaf architecture have been described in several plant species, but many fundamental questions on the ontogeny of leaves remain unanswered. We have begun a genetic approach to the causal analysis of leaf development, using as a point of departure the isolation of *Arabidopsis thaliana* variants with abnormal venation patterns, marginal configurations, shape or size of their rosette leaves. Such variations in leaf architecture have been screened in ecotypes, in mutants from already existing collections, and in new mutants.

Strong variations in the marginal configuration of leaves, leaf shape, leaf size and rosette compactness have been found among 194 ecotypes studied. Crosses involving ecotypes showing extreme and opposite variations in such leaf architectural features have led us to conclude that the studied traits are in most cases polygenic.

Two of the 266 studied ecotypes showed remarkable variations in the arrangement of their vascular bundles. The venation pattern of one of them is extremely simple and inherited as a monogenic recessive trait, which we have named Hemivenata.

We have also studied the *Arabidopsis* Information Service collection of form mutants, stored at the Nottingham Arabidopsis Stock Centre, focusing our attention on mutants suspected to be affected in the coordination between the division and/or expansion of cells of the adaxial and abaxial surfaces of the leaf. The phenotype of such mutants, characterized by rolled up leaf margins, has been called Incurvata (Icu). We are studying nine *ICU* genes, five represented by already known mutants and the remaining four corresponding to new EMS and fast neutron induced mutants.

In a large scale screen for abnormally shaped or sized leaves, we have found 1926 putative mutants induced by EMS, only 333 of which were viable and fertile, exhibiting a fully heritable mutant phenotype. We studied 255 such new mutants, which have been assigned to 19 phenotypic classes. Their mutant phenotypes are inherited as monogenic traits, with complete penetrance and small variations in expressivity. They belong to 94 complementation groups.

In contrast to other *Arabidopsis* organs or tissues, the leaf has received little attention from a developmental point of view. In this context, our work provides the basis for further genetic analyses which will allow a more thorough study of the process of leaf morphogenesis. Our collection of new mutants will provide a suite of mapped genes affecting leaf architecture, which will be candidates for study so that their involvement in the control of leaf ontogeny can be ascertained.

Turning over a new leaf: the regulation of leaf identity in maize and Arabidopsis

All plants produce different kinds of leaves, or leaf-like structures, at different times in their development. In flowering plants there are generally four types of leaf-like organs: cotyledons, juvenile leaves, adult leaves and bracts. The number and distribution of these organs along the shoot axis differs between species and in different genotypes within a species. This pattern often parallels changes in many other aspects of shoot development (e.g. reproductive competence), and is therefore an excellent marker of the developmental phase of the shoot. Analyses of mutations that affect leaf identity in maize and Arabidopsis suggest that the pattern of leaf production during shoot development is regulated by two interacting processes--one which regulates the temporal expression of organ identity genes, and another that regulates the rate of leaf initiation. Evidence supporting this model, as well as recent work that addresses the function of leaf identity genes in Arabidopsis will be presented.

Scott Poethig
Department of Biology
University of Pennsylvania
Philadelphia, PA 19104-6018

Phone: 215-898-8915
FAX: 215-898-8780
Email: spoethig@sas.upenn.edu

Genetic control of leaf lamina growth in *Arabidopsis*.

Mieke Van Lijsebettens, Jonathan Clarke*, Rudy Vanderhaeghen, Martine De Bleeckere and Marc Van Montagu. Department of Genetics, affiliated to Flanders Interuniversity Institute for Biotechnology, University Gent, K.L. Ledeganckstraat 35, 9000 Gent, Belgium. * Department of Molecular Genetics, John Innes Centre, Colney Lane, Norwich, Norfolk NR4 7UH, United Kingdom.

Typical for leaf development in *Arabidopsis* is lateral growth resulting in lamina formation, determinate growth with no persistent meristem, and distinct processes of pattern formation resulting in a dorsiventral asymmetric architecture, a reticulate venation network and the regulated distribution of specialized cell types in the epidermis such as stomata and trichomes.

We present results from an *Ac/Ds* transposon insertion mutagenesis programme to tag genes that are critical for lamina formation. Three *Ds* transgenic lines with the streptomycine resistence gene as the excision marker and the hygromycine resistence gene as the transposition marker (Bancroft et al., 1992) were crossed with an *Ac* transgenic line with a p*35S-transposase* (Swinburne et al., 1992) to generate 1500 F2 families. Full green seedlings representing *Ds* transposition events were selected and occurred at a frequency of around 50%. Two leaf mutations were obtained from screening 377 F2 and 173 F3 populations.

One of them, *nrl1*, *narrow leaf 1*, consisted of rosette and cauline leaves with extended length and severely reduced width, short and swollen primary roots, and deformed, though fertile flowers. Morphological and anatomical analyses showed that cell expansion is affected, visible by a reduced growth of the hypocotyl, inflorescence stem and primary root and by radial swelling of cortex cells in hypocotyl and primary root. Cell division is also affected apparent by a reduced cell number in root apical meristem, shoot apical meristem and leaf primordia; a 50% reduction in cell number was observed in expanded first leaves.

Genetic and molecular analyses indicated a tight linkage between a transposed *Ds* (t*Ds*) and the *nrl1* mutation and the gene was cloned by inverse PCR. DNA sequencing of the insertion site revealed that the t*Ds* left border was deleted explaining why no reversion of the phenotype had been obtained. The wild type *NRL1* gene was cloned and it is homologous to the yeast KTI12 gene with a putative action on cell cycle. The *NRL1* gene is identical to the *DRL* gene of *Arabidopsis* (T. Dyer, personal communication). *nrl1* represents a weak allele with the *Ds* insertion towards the 3' end of the coding sequence disrupting a calmodulin-binding site, *drl* represents a strong allele with a *Ds* insertion at the 5' end of the coding sequence. The NRL1 amino acid sequence has features of the Ras-type GTP-binding proteins. Our mutant and molecular analyses indicate that NRL1 plays a role in the proper functioning of the cytoskeleton and this is further investigated. With the study of this mutant we might be able to distinguish whether lamina width is merely determined by the group of cells set aside by the shoot apical meristem, or rather depends on the lateral growth in young primordia.

References

Bancroft I., Bhatt A.M., Sjodin C., Scofield S., Jones J.D.G., and Dean C. (1992). Development of an efficient two-element transposon tagging system in *Arabidopsis thaliana*. Mol. Gen. Genet. 233, 449-461.

Swinburne J., Balcells L., Scofield S., Jones J.D.G. and Coupland G. (1992). Elevated levels of *Activator* transposase mRNA are associated with high frequencies of *Dissociation* excision in *Arabidopsis*. Plant Cell 4, 583-595.

Arabidopsis pathogen interactions : from signalling to morphogenesis

MARC VAN MONTAGU
Lab of Genetics, Flanders Inter University Institute for Biotechnology
K.L. Ledeganckstraat 35
9000 Gent
Belgium

Plant growth and development has traditionally been the study field of morphologists and plant histologists. Improved imaging techniques allow at present the precise description of the alterations occurring in mutant plants.
To understand however the role and function of the mutated gene and to fully exploit this knowledge it is essential to describe the alterations observed in molecular terms. Rapid progress in the molecular genetics of the modelplant Arabidopsis thaliana guarantees a boost in plant developmental biology.

Studying the interactions between nematodes and Arabidopsis or between Rhodococcus fascians, a gram positive bacterium (Actinomycete) demonstrates the power of linking morphological and molecular genetic studies.
The chemical signals secreted by the nematodes have profound influence on the DNA synthesis and structure of the cells that will differentiate into feeding cells. Unraveling this cascade of reactions brings a wealth of information on the cell biology of plants.(1)
In comparison a set of "secundary metabolites" synthesized by Rhodococcus fascians can induce meristem proliferation and induction of shoots.
The biosynthesis of these compounds is encoded mostly by genes locatel on a 180 Kb linear plasmid.(2)
The bacteria can infect plants without wounding, move intercellularly and stablish itself intracellularly in structures remeniscent of those enclosing the bacteroids in symbiotic nodules

(1) GHEYSEN G., DE ALMEIDA ENGLER J., and VAN MONTAGU M.
Cell cycle regulation in nematode feeding sites.
In *Cellular and Molecular Basis of Plant-Nematode Interactions*, F. Grundler, S. Ohl, and C. Fenoll (Eds.). Dordrecht, Kluwer Academic Publishers, pp. 120-132 (1997).

(2) Crespi M., Vereecke D., Temmerman W., Van Montagu M., and Desomer J., (1994). The *fas* operon of *Rhodococcus fascians* encodes new genes required for efficient fasciation of host plants. *J. Bacteriol.* 176, 2492-2501.

A genetic analysis of plastid development in higher plants

K.A.Pyke. School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey. TW20 OEX. United Kingdom

The development of the plastid cellular compartment is a vital component in cell functionality. Although various plastid types have been described, the development of the chloroplast in leaf mesophyll cells from proplastids in the cells of the shoot apical meristem has been the best studied plastid differentiation pathway. A major component of chloroplast development is chloroplast division whereby young chloroplasts divide within individual mesophyll cells producing large populations of chloroplasts. An analogous division process occurs in meristem cells in which proplastids divide to maintain plastid continuity through cell division. The number of chloroplasts in an individual mesophyll cell is dependent on several factors including cell volume, cell:cell surface attachment and chloroplast size but many plants contain 100-200 chloroplasts per cell. The molecular genetic control of this process was completely unknown until the characterisation of Arabidopsis arc mutants in which chloroplast number and size were drastically perturbed. These mutants have allowed identification of several genes whose products are involved in the chloroplast division process. Two particularly interesting mutants, arc6 and arc12 result in only two greatly enlarged chloroplasts per cell. Characterisation of these mutants has revealed insights into several aspects of plastid development. Plastid lineage through cell division is maintained, even in the arc6 mutant, suggesting that a system exists for plastid segregation even in the absence of a functional division mechanism. Abnormal chloroplast number and morphology does not affect chloroplasts redifferentiation into a different plastid type; as in leucoplast production in Arabidopsis petals. Other mutants with altered chloroplast number and morphology will also be discussed, including the arc10 mutant in which there is large heterogeneity of plastid size within one cell.

Some Relevant References

K. A. Pyke and R. M. Leech (1992). Chloroplast division and expansion is radically altered by nuclear mutations in Arabidopsis thaliana. Plant Physiology 99, 1005-1008

K. A. Pyke and R. M. Leech (1994). A genetic analysis of chloroplast division and expansion in Arabidopsis thaliana. Plant Physiology, 104, 201-207

K.A. Pyke (1994). Arabidopsis - Its use in the genetic and molecular analysis of plant morphogenesis. Tansley Review No. 75. New Phytologist 128, 19-37.

K.A. Pyke, S.M. Rutherford, E.J. Robertson and R.M. Leech (1994) arc6, a fertile mutant of Arabidopsis with only 2 mesophyll cell chloroplasts. Plant Physiology 106, 1169-1177

E.J. Robertson, K.A. Pyke and R.M. Leech. (1995) arc6, a radical chloroplast division mutant of Arabidopsis also alters proplastid proliferation and morphology in shoot and root apices. J. Cell Science 108, 2937-2944.

K.A. Pyke .(1997). The genetic control of plastid division in higher plants. American Journal of Botany Special Review (in press, expected August 1997).

Cell cycle control in *Arabidopsis*

DIRK INZÉ

Laboratorium voor Genetica, Departement Genetica,
Vlaams Interuniversitair Instituut voor Biotechnologie, and
Laboratoire Associé de l'Institut National de la Recherche Agronomique (France),
Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

In all eukaryotes, including plants, progression through the cell cycle is controlled by cyclin-dependent kinases (CDK). In *Arabidopsis* two, possibly three CDKs and at least 14 cyclins are probably involved in cell cycle progression. Interestingly, one class of plant CDKs (*e.g.*, Cdc2b in *Arabidopsis*) have some unique features not found in other organism, such as their G2/M-specific accumulation. It is speculated that this class of plant CDK plays a role in controlling plant-specific aspects of mitosis, such as the formation of the preprophase band and the phragmoplast.

In order to gain further insights into the mechanism controlling cell division in plants, the yeast two-hybrid system was used. With the Cdc2a as bait, we found a large number of interacting clones encoding D-type cyclins, CKS (a kind of docking factor), several substrates, of which some are novel, and inhibitors (resembling p27 and p21 in animals). An overview of the progress made in the molecular characterization will be given. Current evidence indicates that CKS has a role in endoreduplication, as an inhibitor of mitotic CDK/Cyclin activity. The expression of one of the inhibitors suggest a key role in cell cycle arrest. Experiments are in progress to test this hypothesis.

References

De Veylder, L., Segers, G., Glab, N., Casteels, P., Van Montagu, M., and Inzé, D. (1997). The *Arabidopsis* Cks1At protein binds to the cyclin-dependent kinases Cdc2aAt and Cdc2bAt. *FEBS Lett.* 412, 446-452.

Shaul, O., Mironov, V., Burssens, S., Van Montagu, M., and Inzé, D. (1996). Two *Arabidopsis* cyclin promoters mediate distinctive transcriptional oscillation in synchronized tobacco BY-2 cells. *Proc. Natl. Acad. Sci. USA* 93, 4868-4872.

Segers, G., Gadisseur, I., Bergounioux, C., de Almeida Engler, J., Jacqmard, A., Van Montagu, M., and Inzé, D. (1996). The *Arabidopsis* cyclin-dependent kinase gene *cdc2bAt* is preferentially expressed during S and G_2 phases of the cell cycle. *Plant J.* 10, 601-612.

Genetic Control of Apical and Floral Meristem Structure in *Arabidopsis*

Robert W. Williams and Elliot M. Meyerowitz
California Institute of Technology, 156-29, Pasadena, CA. 91125, USA

Post-embryonic growth and development in flowering plants are accomplished by meristems, groups of undifferentiated cells that persist as stem cells and initiate organs. The shoot apical meristem (SAM) contains slowly dividing cells in the center that are surrounded by more mitotically active cells that produce organ primordia (1). In *Arabidopsis*, the SAM is initiated during embryonic growth, then initiates leaves, secondary shoots and flowers. The SAM remains roughly the same size throughout the life span of the plant suggesting that the rates of cell proliferation in the center of the meristem is balanced with the incorporation of cells into organ primordia. Mutations in the *CLAVATA1* (*CLV1*) or *CLAVATA3* (*CLV3*) genes result in plants that have enlarged apical and floral meristems, leading to stem fasciation and extra floral organs, indicating an imbalance in proliferation versus differentiation in the SAM (2,3). On the basis of double mutant phenotypes and interactions of *clv1* and *clv3* alleles, the *CLV1* and *CLV3* gene products are thought to function in the same pathway (3). Thus, the *CLV* loci provide an entry point to study the regulation of meristem development.

CLV1 encodes a protein with a putative extracellular domain containing 21 tandem leucine-rich repeats (LRRs) followed by a cytoplasmic ser/thr kinase domain. The *CLV1* gene is expressed in the center of the SAM and young floral meristems but not in organ primordia (4), suggesting that CLV1 may perceive a signal that regulates cell proliferation in these meristematic regions. Furthermore, CLV1 RNA is not detected in the epidermal layers of the SAM, providing a molecular example of intercellular signaling within the SAM. *In vitro* experiments show that the CLV1 kinase domain autophosphorylates on serine residues then binds KAPP (kinase-associated protein phosphatase; 5). KAPP can dephosphorylate CLV1. *KAPP* has a broad expression domain, including all *CLV1* expressing cells. Plants containing a 35S::*KAPP* transgene have a phenotype similar to weak *clv1* loss-of-function alleles, and transgenic lines that reduce KAPP function partially suppress the *clv1-4* mutant phenotype. The KAPP protein likely functions in several pathways but appears to be a negative regulator of the CLV1 signaling pathway (6).

Many other LRR-kinases have been identified in the *Arabidopsis* genome. A gene of unknown function, called *CLavata1-Like4* (*CLL4*), is specifically expressed on the flanks of the SAM, in a pattern complementary to *CLV1*. The *ERECTA* LRR-kinase (7), which was defined by mutations that result in plants with short internodes and siliques, is expressed throughout the SAM and in expanding floral organs. Therefore, members of the LRR-kinase gene family may play important roles throughout plant development.

1. Steeves, T. A., and Sussex, I. M. (1989). Patterns in Plant Development. (New York: Cambridge University Press).

2. Clark, S. E., Running, M. P., and Meyerowitz, E. M. (1993). *CLAVATA1*, a regulator of meristem and flower development in *Arabidopsis*. Development *119*, 397-418.

3. Clark, S. E., Running, M. P., and Meyerowitz, E. M. (1995). *CLAVATA3* is a specific regulator of shoot and floral meristem development affecting the same processes as *CLAVATA1*. Development *121*, 2057-2067.

4. Clark, S. E., Williams, R. W., and Meyerowitz, E. M. (1997). The *CLAVATA1* gene encodes a putative receptor kinase that controls shoot and floral meristem size in *Arabidopsis*. Cell *89*, 575-585.

5. Stone, J. M., Collinge, M. A., Smith, R. D., Horn, M. A., and Walker, J. C. (1994). Interaction of a protein phosphatase with an Arabidopsis serine/threonine receptor kinase. Science *266*, 793-795.

6. Williams, R. W., Wilson, J. M., and Meyerowitz, E. M. (1997). A possible role for kinase-associated protein phosphatase in the *Arabidopsis* CLAVATA1 signaling pathway. PNAS 94, in press.

7. Torii, K. U., Mitsukawa, N., Oosumi, T., Matsuura, Y., Yokoyama, R., Whittier, R. F., and Komeda, Y. (1996). The Arabidopsis *ERECTA* gene encodes a putative receptor protein kinase with extracellular leucine-rich repeats. Plant Cell *8*, 735-746.

Session 4: Cell to cell communication and differentiation

Chairperson: Scott Poethig

CELL POLARIZATION: A KEY PROCESS FOR PATTERNED DIFFERENTIATION AND PLANT ORGANIZATION.

Tsvi Sachs, Department of Plant Sciences, The Hebrew University, Jerusalem 91904, Israel

What cellular traits could be responsible for organization of tissues and entire plants? A fundamental example might well be the polar transport of signals along one axis in preference to all others. The polar transport of auxin is, in fact, well known and there is probably a preferred axis of transport, with no set direction, of various other developmental signals. Knowledge of mutations that perturb various aspects of such polarities is accumulating rapidly. The purpose here will be to suggest a framework for understanding the effects of these mutations on the basis of responses to wounds, grafts and hormone applications. These treatments are complementary to mutations: rather than defining essential components they demonstrate developmental capacities, often unexpected, of a given genotype under varied conditions. Because the topic is too broad, the discussion will concentrate on one aspect: what happens when tissue polarity is reoriented by transverse cuts and local auxin applications.

The following are some major results and concepts suggested by these experiments:

[a] The reorientation of the polarity of the cambium and its products involves a reorganization of an entire tissue, including varied cell types. The processes whose axis is changed include intrusive cell growth, cell divisions and both vessel and sieve tube differentiation. The changes are gradual and indicate a competition between cells for limited signals and a relatively long-term "search" for the best response. The observations also show that the information carried by auxin, and perhaps other signals, includes an essential directional component which is yet to be studied at the cellular level.

[b] Similar treatments of meristematic shoot axes lead to regeneration by different cellular processes. Here the cells grow in girth, apparently adjusting the cross section of the tissue to the requirements of signal flow, and this leads to axis regeneration. This is a response at right angles, rather than along, polar signal flow. These observations emphasize the importance of differences in tissue competence to respond to polar signals. Further, though the apical tissues have a preferred response axis, they have no pre-determined direction or polarity. This "axiality", rather than polarity, of the meristematic cells accounts for the differentiation of complex vascular networks, especially in leaves, and could well be one of the major evolutionary innovations of Angiosperms.

[c] It is only in a fairly narrow competence window that auxin application causes its so-called "typical" effect, parenchyma growth along the axis of auxin flow. At this stage the repeated application of a highly localized source of auxin has led to the formation of an artificial organized gall. Callus development can be understood as a response of cells to the flow with no consistent direction of auxin and other signals.

[d] On a much larger scale, the removal or changed local environment of individual branches leads to changes in tissue polarity in the major stem or trunk. This suggests that the polar induction of vascular differentiation plays a central role in the relations between branches. The branches, and even vascular strands leading to the same branch, compete with one another for the orientation of the tissues that could connect them with the rest of the plant.

Polarity and its changes could thus play a role at the level of the form of entire plants, not only the organization of tissues.

The general picture to emerge from these and many other observations is that the flow of signals, to which cells act as preferred channels, sources, and sinks, is an important determinant of tissue organization. The varied cellular responses and tissue complexity appear to be due to interactions between these signals and cell competence to respond, which reflects the previous developmental history of the cells. This contrasts with concepts of varied organizational signals and of detailed programs, directly determined at the gene level. Mutations are most likely to affect the transport or the competence of one cell type, rather than the master system. The expression of many mutations, furthermore, may well be masked by the regenerative processes of the plant.

References

Sachs, T. (1991). Cell polarity and tissue patterning in plants. Development 1991, Supplement 1, 83-93.

Sachs, T. (1991). Pattern Formation in Plant Tissues. Cambridge University Press, Cambridge.

Sachs, T. (1993). The specification of meristematic cell orientation by leaves and by auxin. Australian Journal of Plant Physiology, 20, 541-553.

Sachs, T. & Hassidim, M. (1996) Competition and mutual support of branches in damaged plants. Vegetatio, 127, 25-30.

The role of lipochitooligosaccharides in legume nodulation

Ton Bisseling
Dep of Molecular Biology, Agricultural University of Wageningen, The Netherlands

Soil bacteria belonging to the genera Rhizobium, Bradyrhizobium and Azorhizobium (collectively called rhizobia) are able to invade the roots of their leguminous host plants, where they trigger the formation of root nodules. In these nodules, the bacteria are able to reduce atmospheric nitrogen into ammonia.

In brief, root nodule development involves the following steps: Upon inoculation, root hairs deform, and the bacteria invade the plant using a tubular invagination known as an infection thread, which develops within the root hair and the cortex. Simultaneously, cortical cells are mitotically activated, giving rise to the nodule primordium. In temperate legumes like pea and clover, cell division is initiated within the inner cortex. In the outer cortex, cells also enter the cell cycle but arrest in G2, leading to a radial allignment of cytoplasmic bridges (named preinfection threads). Infection threads grow toward the primordium via the preinfection thread and the transported bacteria are released into the cytoplasm of the host cells. Subsequently, the nodule primordium develops into a nodule.

To redirect the fate of existing root cell, the Rhizobium bacteria secrete lipo chitin-oligosaccharides based signal molecules-the so-caled Nod factors- which were characterized a few years ago. Nod factors comprise a b-1,4-linked N-acetyl-D-glucosamine backbone varying in length between four and six sugar units. The non-reducing terminal sugar moiety is substituted on the C-2 position with a fatty acid group of variable structure. Additional substitutions on both terminal sugar residues are species-dependent and determine the host specificity of the Nod factors. The biosynthesis of Nod factors is controled by the nod genes which encode the enzymes involved in the biosynthesis of the backbone as well as those involved in adding specific substitutions at the terminal sugar residues The rhizobial Nod factors, play a key role in the early stages of the nodulation process. Nod factors are essential for and even sufficient to elicit many of the key events involved in symbiosis, including root hair deformation, pre-infection thread formation and nodule organogenesis. Therefore, Rhizobium Nod factors are potent plant signalling molecules, capable of eliciting a variety of morphogenic and mitogenic responses in the host legume.

Each stage of bacterial penetration and nodule development is accompanied by the expression of nodule specific host genes, the so-called nodulin genes. One of the first plant genes that rhizobia avtivate is ENOD40. This gene encodes a peptide that changes the response of plant cells to phytohormones. The following aspects of nodulation will be discussed:1. How do the Nod factor induced responses relate to other plant developmental processes? 2. The mode of action of the two signal molecules- Nod factors, ENOD40- that play a key role in Nodulation.

Mylona P et al. (1995) Plant Cell 7, 869-885.
Van De Sande et al. (1996) Science 273, 370-373

Brassinosteroids - essential regulators of plant growth and development

Thomas Altmann, Max-Planck-Insitut für molekilare Pflanzenphysiologie, Karl-Liebknecht-Str. 25, Haus 20, 14476 Golm, Germany

Brassinosteroids (BRs) comprise a class of more than 40 native compounds showing high structural similarity to animal steroid hormones. BRs were first identified in *Brassica napus* pollen extracts (Grove et al., 1979) and subsequently were shown to be ubiquitously present in plants (Adam et al., 1996; Fujioka and Sakurai, 1997a). A multitude of physiological responses is elicited upon exogeneous application of very low amounts of these compounds (Sasse, 1997), among which the promotion of cell elongation and cell division are the most prominent. These features strongly suggested a function of BRs as a new class of phytohormones. The recent identification of *Arabidopsis thaliana* and *Pisum sativum* mutants deficient for endogeneous BR-biosynthesis or for BR-sensitivity, provided further strong evidence for their essential role in the regulation of plant growth and development (Clouse et al., 1996; Kauschmann et al., 1996; Li et al., 1996; Szekeres et al., 1996; Nomura et al., 1997). Precursor feeding studies and/or the analysis of endogeneous compounds allowed to identify four different reactions in the proposed biosynthetic pathway (Fujioka and Sakurai, 1997b) to be (individually) affected by the mutations. Furthermore, the genes corresponding to the *Arabidopsis* mutants have been isolated which showed similarities to FAD-dependent oxidases (Takahashi et al., 1995; Mushegian and Koonin, 1995), cytochrome P450 steroid hydroxylases (Szekeres et al., 1996) and steroid-5α-reductase (Li et al., 1996). The phenotypic changes caused by the biosynthetic defects in these mutants included a dramatic reduction in cell elongation resulting in extreme dwarfism, a de-etiolated development in darkness, delayed flowering and leaf senescence, reduced apical dominance and male sterility. Identical mutant phenotypes were observed in the BR-insensitive *Arabidopsis*-mutants which characterized one locus. The corresponding genes also was isolated recently (Li and Chory, 1997) and shown to resemble leucine-rich repeat receptor kinases. According to the BR-insensitivity caused by the loss of function of this gene, the encoded protein may serve as the BR-receptor. The drastic reduction in cell elongation

caused by the lack of BR-synthesis or -sensitivity is reflected in a decrease in the expression of genes such as *TCH4*, *meri5* (Kauschmann et al., 1996), and *expansin* which (potentially) are involved in cell wall weakening und thus permit cell wall expansion (Fry, 1995; Cosgrove, 1997). These observations may point towards a role of brassinosteroids as positive regulators of genes encoding essential components of the cell elongation machinery.

References

Adam, G., Porzel, A., Schmidt, J., Schneider, B. and Voigt, B. 1996. New developments in brassinosteroid research. *In* Studies in Natural Products Chemistry (S. T. Atta-ur-Rahmann, ed.), vol. 18, pp. 495-549. Elsevier, Amsterdam. ISBN 0-444-82458-8.

Clouse, S.D., Langford, M. and McMorris, T.C. (1996) A brassinosteroid-insensitive mutant in *Arabidopsis thaliana* exhibits multiple defects in growth and development. *Plant Physiol.* **111**, 671-678.

Cosgrove, D.J. (1997) Relaxation in a high-stress environment: the molecular bases of extensible cell walls and cell enlargement. *Plant Cell* **9**, 1031-1041.

Fry, S.C. (1995) Polysaccharide-modifying enzymes in the plant cell wall. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46**, 497-520.

Fujioka, S. and Sakurai, A. 1997a. Brassinosteroids. *Nat. Prod. Reports* **14**, 1-10.

Fujioka, S. and Sakurai, A. 1997b. Biosynthesis and metabolism of brassinosteroids. *Physiol. Plant.* **100**, 710-715.

Grove, M.D., Spencer, G.F., Rohwedder, W.K., Mandava, N., Worley, J.F., Warthen, J.D. Jr., Steffens, G.L., Flippen-Anderson, J.L. and Cook, J.C. Jr. (1979). Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. *Nature* **281**, 216-217.

Kauschmann, A., Jessop. A., Koncz, C., Szekeres, M., Willmitzer, L. and Altmann, T. (1996). Genetic evidence for an essential role of brassinosteroids in plant development. *Plant J.* **9**, 701-713.

Li, J., Nagpal, P., Vitart, V., McMorris, T.C. and Chory, J. 1996. A role for brassinosteroids in light-dependent development in *Arabidopsis*. *Science* **272**, 398-401.

Li, J. and Chory, J. 1997. A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. *Cell* **90**, 929-938.

Mushegian, A.R. and Koonin, E.V. (1995). A putative FAD-binding domain in a distinct group of oxidases including a protein involved in plant development. *Protein Science* **4**, 1243-1244.

Nomura, T., Nakayama, M., Reid, J.B., Takeuchi, Y. and Yokota, T. (1997). Blockage of brassinosteroid biosynthesis and sensitivity causes dwarfism in garden pea. *Plant Physiol.* **113**, 31-37.

Sasse, J.M. (1997). Recent progress in brassinosteroid research. *Physiol. Plant.* **100**, 696-701.

Szekeres, M., Németh, K., Koncz-Kálmán, Z., Mathur, J., Rédei, G.P., Nagy, F., Schell, J. and Koncz, C. (1996). Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in *Arabidopsis*. *Cell* **85**, 171-182.

Takahashi, T., Gasch, A., Nishizawa, N. and Chua, N.-H. (1995). The *DIMINUTO* gene of *Arabidopsis* is involved in regulating cell elongation. *Genes Dev.* **9**, 97-107.

SYMPLASMIC NETWORKING AT THE SHOOT APICAL MERISTEM

CHRIS VAN DER SCHOOT[1] & PÄIVI RINNE[2]

[1]ATO-DLO, P.O. Box 17, NL-6700 AA Wageningen, The Netherlands; [2]Department of Virology, Agricultural University Wageningen, Binnenhaven 4, NL-6709 PD Wageningen, The Netherlands

Primary morphogenesis is organized at the shoot apical meristem (AM). Models describe the physiology and structure of the AM in terms of histological zonation and cell division patterns[1,2]. Two distinct histological zones are present in the AM, each of which comprises metabolically and cytokinetically uniform cells. These two populations reflect the different developmental tasks of the peripheral and central part of the AM. Independent of these histological zones, anticlinal divisions of initial cells and their derivatives give rise to one or more tunica layers superimposed on a corpus, where cells divide in all directions. How these structural and physiological differences within the AM are maintained, how they are integrated, and what functions they serve has remained largely unknown. Particularly relevant for the understanding of the AM, as contrasted with the floral meristem (FM), is to establish how this formative region is able to pattern and simultaneously sustain potentially infinite shoot development[3,4].

The AM resembles a *morphogenetic field* in developing animal systems, since it is a field of cells which cooperatively bring about a specific set of structures[5]. Morphogens participate in this pattern formation by creating gradient fields of *'positional information'*[5,6]. Gap junctions in animal systems provide convenient channels for the passage of diffusing morphogens[7,8]. In plants, plasmodesmata (pd) may serve similar functions, since they interconnect all cells of the meristem into a symplasmic unit[9]. In order for a symplasmic unit to serve the two major functions of the AM, i.e. patterning and sustaining indeterminate growth, the intrinsic signal network must be functionally subdivided. Such division can be achieved by subjecting the three-dimensional symplasm to a spatio-temporal control via opening and closing of pd at strategic locations. This would permit selective signal exchange for the coordination and control of cellular activities between shifting populations of AM cells during primary morphogenesis[3,4].

Investigating the possibility of such dynamic symplasmic networking, the AM symplasm was probed by microinjecting LYCH (Mw 457 Da) into single AM cells at

different locations[10, 11]. Since LYCH does not pass the cell membrane, its spread from cell to cell visualizes all symplasmic pathways available to small diffusing morphogens and metabolites up to 1 kDa. We found that AM cells in the tunica (birch, potato) were symplasmically coupled into two distinct *symplasmic fields*, which correspond in position to the peripheral and the central part of the AM[11]. These fields may harbour *gradient fields*[5] of diffusing morphogens. The membrane potentials (E_m), recorded from the individual cells within these fields, were field-specific and demonstrate that these fields also function as transient working units. The morphogenetic significance of these fields was demonstrated experimentally by their breakdown, induced environmentally, which blocked further development.

The results are discussed within the context of the supracellular organization of the AM and its self-regulatory behaviour.

1. Sachs, T. S. (1991) Pattern Formation in Plant Tissues. Cambridge University Press, Cambridge.
2. Steeves, T. A. and Sussex, I. M. (1989) Patterns in Plant Development. Cambridge University Press, Cambridge.
3. van der Schoot, C. (1996) In Plant Dormancy (ed Lang, G. A.), pp.59-81. CAB International, Wallingford, UK.
4. van der Schoot, C. and Rinne, P. (1996) In Proceedings 10ème Réunion Groupe d'Etude l'Arbe, Université d'Angers, pp. 36-55.
5. Müller, W. A. (1996) Developmental Biology. Springer, New York.
6. Wolpert, L. (1989) Development, Supplement, 3-12.
7. Wolpert, L. (1978) In Intercellular Junctions and Synapses: Receptors and Recognition (ed Fieldman, J., Gilula, N. B. and Pitts, J. D.), pp. 83-96. Chapman and Hall, London.
8. Pitts, J. D. (1990) In Parallels in Cell to Cell Junctions in Plants and Animals (ed Robards, A. W., Lucas, W. J., Pitts, J. D., Jongsma, H. J. and Sprey, D. C.), pp. 53-62. Springer, Berlin.
9. Bergmans, A., de Boer, D., Derksen, J. and van der Schoot, C. (1997) Planta (in press).
10. van der Schoot, C. and Lucas, W. J. (1995) In Methods in Plant Molecular Biology (ed Maliga, P., Klessing, D. F., Casmore, A. R., Gruisem, W. and Varner, J. E.), pp. 173-189. Cold Spring Harbor Laboratory Press, New York.
11. Rinne, P. and van der Schoot, C. (1997) submitted.

RETINOBLASTOMA PROTEINS AND CELL DIFFERENTIATION IN MAIZE

J.A.H. Murray
Institute of Biotechnology
University of Cambridge

In mammalian cells, the Rb protein plays a central role in cell division and differentiation. Rb is an inhibitor of cell division, which inactivates the transcription factor E2F required for DNA replication. For cell division to be initiated Rb must itself be inactivated by phosphorylation. This is carried out by cyclin-dependent kinases, particularly cyclin D-kinases.

A maize Rb homologue ZmRb-1 has recently been isolated (Xie et al., 1996). We have raised an antiserum against the C-terminal region of the ZmRb-1 protein, and show that multiple immunoreactive forms of ZmRb-1 are present in cells. These bands are estimated to be 130kDa, 112kDa and 60kDa in size. The 112kDa band consists of five closely spaced bands suggestive of alternative phospho-forms.

We have examined the abundance of these proteins during leaf morphogenesis. The 112kDa bands are absent in proliferating cells, but are abundant in differentiating cells at the tip of the leaf blade (leaf 2). A gradient of expression is seen both in time (following development of leaf 2) and spatially up the leaf blade. In a suspension culture of maize, the larger proteins are not detected, but the 60kDa band is abundant.

In order to understand in more detail the behaviour of ZmRb-1, we have examined the interaction of ZmRb-1 with plant cyclin D homologues (CycD). We find that CycD's of all classes bind ZmRb-1. We also find that ZmRb-1 binds both human and *Drosophila* E2F in yeast two-hybrid assays. Although a plant E2F homologue remains to be identified, these results suggest that plant Rb is likely to be important for cell differentiation, and that the cyclin D-Rb-E2F pathway is likely to be conserved throughout higher eukaryotes.

Cell signaling in *Arabidopsis* root development

Renze Heidstra, Claudia van den Berg, Viola Willemsen, Harald Wolkenfelt, Saskia Folmer, Dimitris Beis, Geert de Vrieze and Ben Scheres.
Department of Molecular Cell Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.

Plant organ development is initiated during embryogenesis and proceeds post-embryonically from distally positioned groups of dividing cells, the meristems[1]. The Arabidopsis root displays a simple and regular organisation of tissues, its ontogeny during and after embryogenesis is described at the cellular level[2,3], and despite rigid lineage relationships it exhibits the developmental flexibility that is characteristic for many aspects of plant development.

Laser ablation studies reveal that two types of signaling events contribute to the flexible mode of development[4,5].

First, many if not all cells within the root meristem can adopt novel fates according to new positions. Upon ablation of quiescent centre cells, cells originating from the vascular bundle were displaced toward the root tip. Promoter-marker gene fusions showed that these vascular derived cells now displayed columella-specific gene expression. Future vascular cells are separated from future columella cells already by the first division of the zygote. This apparently does not lead to a determined segregation of cell fates. Furthermore, upon ablation of cortical initial cells, cells derived from the pericycle but now within the cortical cell layer were capable of switching fate and formed both endodermis and cortex. Hence, the first zygotic division in the apical-basal plane, as well as the early embryonic divisions in the radial plane, do not restrict the developmental potential of the resulting daughter cells. By consequence, continuous signalling should maintain cell fate within the root. To determine the direction of signals, cortical initial cells were deprived from contacts with more mature cells within the cortex layer. As a result, the cortex initials no longer performed their specific asymmetric division pattern. This implies that cortex initial cells cannot differentiate autonomously, and they need reinforcement signals from more mature cortex cells.

Second, four central cells in the meristem appear to regulate the rate of cell differentiation by short-range interactions. These cells do not divide post-embryonically and together they are called the quiescent centre. Ablation of 2 quiescent centre cells results in cessation of cell division of only those columella initials which are in direct contact with the ablated cells. Starch granules normally found only in mature columella cells, now appear in the underlying columella initials after quiescent centre ablation. In addition, cortical initials directly contacting ablated quiescent centre cells behaved as cortical daughters and divided asymmetrically into cortex and endodermis cells. The differentiation status of columella initials in mutants lacking post-embryonic root meristematic cell division is also controlled by the quiescent centre, suggesting that the quiescent centre primarily arrests cell differentiation.

Together, the balance between the positional signals for proper differentiation coming from more mature cells and the short range signals from the quiescent centre required to keep cells in an undifferentiated state could be the basis for orchestrating pattern formation in the root meristem.

Although the nature of these positional signals in the root meristem is at present a matter of speculation, an analogy can be drawn towards the shoot meristem which also contains cells with reduced cell division rates. Here, the CLAVATA1 gene, encoding a putative leucine-rich-repeat containing receptor kinase, has been implicated to play an essential role in either regulating cell division or differentiation[6]. Evidence for the existence of root specific CLAVATA1 homologs and their possible function in the root meristem will be presented.

1. Meyerowitz, E.M. Cell 88, 299-308 (1997).
2. Dolan, L. et al. Development 119, 71-84 (1993).
3. Scheres, B. et al. Development 120, 2475-2487 (1994).
4. van den Berg, C. et al. Nature 378, 62-65 (1995).
5. van den Berg, C. et al. Nature (in press)
6. Clark, S.E. et al. Cell 89, 575-585 (1997).

POSTERS

Seed coat development in *Arabidopsis* is a three-stage process with precise timing of different cytological events.

Tom Beeckman[1], Riet De Rycke[2], Antje Rohde[2] and Ronald Viane[1]

[1]University of Gent, Department of Plant Morphology
[2]Flanders Interuniversitary Institute for Biotechnology, Department of Genetics, University of Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

A detailed analysis of *Arabidopsis* seed coat development using light and transmission electron microscopy disclosed the major morphological changes associated with the transition of the ovular wall into the mature seed coat. By the use of a metachromatic staining procedure, cytological events such as the production of phenolic compounds and acidic polysaccharides were followed.

Just before fertilization, the ovule wall is composed of 4 cell layers in the micropylar region, but contains 5 layers elsewhere. The outer integument consists of 2 cell layers (O.I.1 and O.I.2) throughout the entire ovule wall, whereas the inner integument is two-layered (I.I.1 and I.I.2) at the micropyle and three-layered elsewhere (I.I.1, I.I.1' and I.I.2). By any staining procedure the cells of the I.I.1 layer (or endothelium) show a darkly stained, dense cytoplasm without large vacuoles; they are mostly isodiametric indicating their probable meristematic nature.

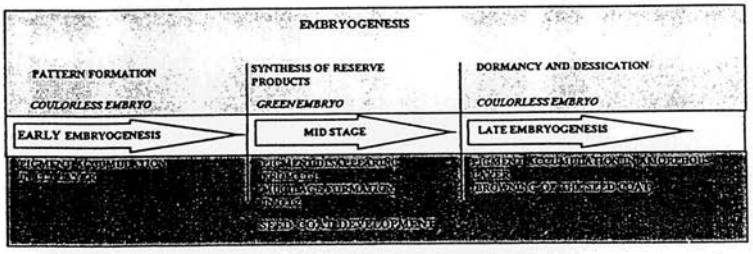
At the one-cell embryo stage, immediately after fertilization, the cells of the I.I.1 layer become vacuolated; from the two-cell stage onwards pigment accumulation is observed in the newly formed vacuoles. This pigment, easily demonstrated by toluidine blue, starts to disappear from the cytoplasm at the torpedo stage.

During the torpedo stage, the inner periclinal walls of the O.I.1 layer become thickened, while mucilage also starts to accumulate in the cells of the O.I.2 layer. Furthermore, starch grains accumulate against the inner tangential wall in the centre of each cell, resulting in the formation of small "columns" that persist until seed maturity.

At the maturation stage, a second pigment accumulation was observed in an amorphous layer derived from the remnants of the O.I.1, the I.I.1 and the I.I.2 layers, resulting in a brown-coloured pigment layer responsible for the brown seed colour.

Besides the integumentary cell layers, one endosperm layer contributes to the formation of the mature seed coat. It is characterized by the presence of numerous protein bodies and is considered as an aleurone layer also reported for other members of the Brassicaceae.

Our results indicate that seed coat formation is a three-stage process and we show in the following figure that the timing of cytological events (mainly pigment accumulation) coincides with three developmental phases of embryogenesis, suggesting a possible relationship between embryogenesis and seed coat development. To test this hypothesis we are now analysing seed coat formation in mutants that show aberrant timing of the mid and late embryogenetic stages.



FRÉDÉRIC BERGER
Reproduction et Développement des Plantes
UMR 9938 CNRS/INRA/ENS
Ecole Normales Supérieure de Lyon
46 allée d'Italie
69364 LYON (France)

Using laser ablation to investigate basic mechanisms in plant development.

Experimental embryogenesis based on ablation and grafts has provided a large body of information concerning the basics of animal embryo development. However in plants this kind of approach has been rather neglected. We have performed microsurgical manipulations of plant embryos and meristems to investigate the role of positional information and cell-cell communication in these processes. Laser beams allow to perform ablation of cells and subcellular structures. It is thus possible to remove a particular cell at a given time during development to investigate its role or to study the fate of the cells which will occupy the position of ablated cells. This has showed that both in the *Fucus* embryo and in the *Arabidopsis* root epidermis, cell fate is dictated by cell position rather than by lineage. Moreover it is possible to isolate protoplasts through localy ablated cell wall. In *Fucus* embryo this latter procedure showed that positional information relies at least partially on the presence of the cell wall.
Alternative experimental set-ups to realise cell ablation will be presented.

GENES ENCODING AN AP2 DOMAIN TRANSCRIPTION FACTOR AND A BURP DOMAIN PROTEIN ARE EXPRESSED DURING THE INDUCTION OF *BRASSICA NAPUS* MICROSPORE EMBRYOGENESIS

Kim Boutilier[1,2], Jiro Hattori[1], Thérèse Ouellet[1], Michiel van Lookeren Campagne[2], and Brian Miki[1]

[1]Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, Ontario Canada K1A-0C6, and [2]Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, 6700 AA Wageningen, The Netherlands.

The *Brassica napus* microspore embryo culture system is well suited for investigating the cellular and molecular changes involved in the initiation of embryogenesis *in vitro*, as only a short heat-stress treatment (32.5 °C, 8h), followed by transfer to normal growth temperatures, is required to simultaneously block further pollen development and induce embryogenesis. Using this system we have identified a number of genes whose expression is newly induced or up-regulated during the heat-stress induction of embryogenesis and which continues during the subsequent stages of embryo development. One of these genes, *BNM3*, belongs to the AP2 domain family of transcription factors, members of which include *APETALA2, AINTEGUMENTA, TINY, EREBP, GLOSSY15* and *CBF1*. RNA gel blot and mRNA *in situ* hybridization anlysis indicate that expression of *BNM3* is embryo-specific, spans the early to middle stages of seed development and is localized to all cell types of the developing embryo. The second gene identified, *BNM2*, encodes a polypeptide which contains a novel domain of unknown function. This domain is conserved in three other plant proteins: RD22, an *Arabidopsis thaliana* drought-induced protein; USP, an abundant *Vicia faba* non-storage seed protein and the ß subunit of polygalacturonase isozyme 1, which is expressed in ripening tomato fruit. We have named this region of similarity, the BURP domain for BNM2, USP, RD22, and PG1. The *BNM2* gene is expressed in the inner integument, endosperm and axis of the developing embryos during the early to middle stages of seed development. Both the temporal and spatial patterns of *BNM2* and *BNM3* gene expression are maintained in developing haploid microspore-derived embryos.

Venation pattern formation in *Arabidopsis thaliana* vegetative leaves

Héctor Candela, Antonio Martínez-Laborda and José Luis Micol
División de Genética. Universidad Miguel Hernández. Campus de San Juan. 03550. Alicante. Spain.

The problem of pattern formation is mainly concerned with the mechanisms by which genetic information is translated into spatial patterns of cellular differentiation. Plant vascular systems provide an excellent example of the formation of a complex pattern in a three-dimensionally growing body. Although several models have been proposed, the mechanism by which such complex structures are built is still unclear at the genetic level.

Here we report data on the venation pattern of *Arabidopsis thaliana* leaves, its variation with time and developmental stages and the differences it shows with Hemivenata, a monogenic trait found in one of the 266 ecotypes studied. Our work provides the basis for further genetic analyses, which will allow a more thorough study of the process of venation pattern formation.

Our data show that the area of the lamina increases exponentially in the early stages of leaf expansion. This period, in which the leaf is constituted of small cells showing meristematic activity, appears to be a critical phase in the elaboration of the vascular pattern, and so it is to be expected that the genes which play an important role in venation pattern formation will be active in the very early stages of leaf expansion. Subsequently, the rate of leaf expansion diminishes and new vascular elements are preferentially formed at the basal region of the leaf, whose cells differentiate later than those of the leaf apex. Finally, the lamina area of the mature leaf is reached by cell expansion processes and the increase in the airspace volume, with no further formation of vascular pattern elements being observed.

Two criteria have been established to quantitatively analyze the complexity of the venation pattern in vegetative leaves: the density of venation and the number of branching points per mm^2. Although the length of vascular bundles and the number of branching points increase during expansion, the ratios between them and the lamina area decline as leaf expansion progresses. For instance, the density of venation diminishes as leaf development progresses until it reaches a value of around 4 mm/mm^2 at maturity. A similar observation can be made with respect to the number of branching points per unit of area.

As another landmark for the study of the venation pattern, we have analyzed in *Arabidopsis* leaves the presence of hydathodes, which are guttation organs associated to the vasculature, observing that the more lateral teeth that are present in a leaf primordium, the more hydathodes there will be in the mature leaf. Considering mature leaves, it is a general rule that the later a leaf originates, the more hydathodes it contains.

A mutation in the *HEMIVENATA* (*HVE*) gene has been found which shows reduced values for the above mentioned parameters. This suggests a role for *HVE* in the construction of the venation pattern. Since the Hemivenata phenotype is characterized by incomplete venation both in leaves and cotyledons, we conclude that the *HVE* gene is required in both the embryonic and postembryonic stages of vascular development.

TORNADO GENES CONTROL DIRECTED CELL ELONGATION AND ORGAN DEVELOPMENT IN *ARABIDOPSIS*

Gerda Cnops[1,2], Xuan Wang[1], Liam Dolan[2], Tom Beeckman[3], Mark Van Montagu[1] and Mieke Van Lijsebettens[1]

Laboratorium Voor Genetica[1], Laboratorium Voor Plantenmorfologie[3], Universiteit Gent K.L. Ledeganckstraat 35 B-9000 Gent, Belgium; Cell Biology[3] Colney Lane, NR57UH Norwich, UK

Morphogenesis is an important aspect in the development of organ shape. Cell division and cell elongation processes define the final appearance of plants. Two Arabidopsis mutations perturbed in morphogenesis are described. Recessive mutations at the *TORNADO1 (TRN1)* and the *TORNADO2 (TRN2)* loci cause severe dwarfism combined with twisted growth. Embryonic pattern formation is not affected in these mutants. The first visible defects in both *TRN1* and *TRN2* become apparent in young seedlings with a reduction in elongation and twisting of the primary root. This twisting of cell files in *TRN* mutants is induced during organ elongation. More severe twisting in older roots coincides with the occurrence of grooves in the root epidermis and ectopic root hair formation. The mutant phenotype is highly pleiotropic since the shoot exhibits dramatic defects such as abnormal leaf shape, irregularities in the vascular organisation and reduced fertility.

Chromosome walking towards the *TRN1* locus is in progress. *TRN1* is mapped to the bottom half of chromosome 5, relative to genetic and RFLP markers. Recombinants, within the approximately 3 cM region around *TRN1*, were used to position 17 molecular markers, generated by the AFLP technique. This approach allowed us to land at *TRN1*. Linked and co-segregating markers hybridised to two CIC YAC clones (Mol. Gen. Genet. *253*, 32-41). Three AFLP markers have been cloned and used to screen BAC and cosmid libraries. Positive clones are being analysed and used for complementation analysis of the *trn1* mutant. Two putative T-DNA tagged *TRN2* alleles were obtained and linkage analysis is currently undertaken. To examine the interaction between *TRN1* and together with the cloning and molecular analysis of *TRN1* and *TRN2* will enable us to study the function of these genes in directed cell elongation and onset of twisting in plants.

Discovering the developmental role of MADS-box factors.

Brendan Davies*, Patrick Motte*, Zsuzsanna Schwarz-Sommer* and Hans Sommer*.
*Centre for Plant Biochemistry and Biotechnology,
University of Leeds, Leeds LS2 9JT, UK.
*Department of Molecular Plant Genetics,
Max-Planck-Institut für Züchtungsforschung, D-50829 Köln, Germany.

Plant MADS-box factors were first identified using floral homeotic mutants of *Antirrhinum majus*. These factors show striking similarities to well characterised yeast and animal transcription factors, in a region known to be important for DNA binding. Although a few MADS-box genes have been discovered in most eukaryotes it is in plants that they are most numerous and search of the databases currently reveals >130 MADS-box genes from 22 different plant species. Plant MADS-box genes are subject to spatial and temporal constraints on their expression, supporting the assumption that they are involved in different developmental processes.

Despite this proliferation of cDNA sequence information and an extensive molecular analysis of certain members of this family, the developmental function of most of the genes remains a matter of informed guesswork. Since the few MADS-box genes for which developmental roles have been identified control functions such as floral organ identity and meristem identity, it is likely that other members of this family are also important for plant development.

We have begun a systematic programme to inactivate the MADS-box genes of *Antirrhinum majus* by selecting for transposon insertions using PCR. To test the feasibility of this approach we started with a gene, *DEFH1*, which by virtue of its predicted amino acid sequence, spatial and temporal expression pattern, ectopic expression in transgenic plants, DNA binding properties and protein-protein interactions as determined in a yeast two-hybrid screen, was predicted to be a second C-function homeotic gene.

Two independent integrations were identified and loss of gene function was verified by Northern analysis. The homozygous mutant plants showed a completely unexpected phenotype which could never have been predicted from our extensive studies. Double mutant analysis with other homeotic mutants also revealed unexpected results. These experiments demonstrate the importance of obtaining mutants in order to identify the function of genes isolated by homology. They also reveal another level of complexity underneath the basic ABC model of floral development. It is hoped that extending this type of analysis to other MADS-box genes will reveal further examples of their different roles in plant morphogenesis.

A pectate lyase is induced during tracheary element differentiation in *Zinnia*

Domingo, C.; Roberts, K., Stacey, N.J. and McCann

Department of Cell Biology, John Innes Centre, Colney, Norwich NR4 7UH, UK

Mesophyll cells from the leaves of *Zinnia elegans* trans-differentiate into tracheary elements (TEs) when cultured during 96h in a medium containing a specific ratio of auxin and cytokinin, while in the presence of auxin alone the cells simply elongate. In a search for genes involved in modifications to cell-wall architecture before any overt signs of cell differentiation, a differential hybridisation of a 72 h cDNA library with probes from mRNA at time-points of 24 h and 72 h was done revealing a number of transcripts up-regulated between these times. One of these cDNAs shows homology to pectate lyase, an unusual pectin-degrading enzyme. The complete cDNA sequence (ZePel) corresponds to a translated protein of 44 kDa with an N-terminal signal peptide of about 2 kDa, and one potential N-glycosylation site. Northern analysis confirms that the strong expression of this gene during TE induction occurs at a very early stage of the process and is due solely to the presence of auxin in the induction medium. In situ hybridisation studies in young *Zinnia* stems show that ZePel expression is associated with vascular bundles and shoot primordia. Recombinant protein made in *E. coli* possesses calcium-dependent pectate lyase activity. Pectate lyase activity is detected in elongating and differentiating in vitro cell populations. The role of this enzyme in remodelling the cell wall during cell elongation and differentiation will be discussed.

The attainment of embryonic symmetry in monocots: influence of auxin polar transport

Fischer Ch, Speth V, Eberenz S, Neuhaus G.

University of Freiburg, Institute of Biology II, Cell Biology, Schänzlestr. 1, D- 79104 Freiburg

To study the influence of auxin on the attainment of bilateral symmetry from radial symmetry during monocot embryogenesis, the fate of young wheat (*Triticum aestivum* L.) zygotic embryos has been manipulated *in vitro* by adding selected auxin transport inhibitors to the culture medium. Although NPA (N-1-Naphthylphthalamic acid) and quercetin (3,3',4',5,7-Pentahydroxyflavone) belong to two different classes of auxin transport inhibitors, the phytotropins and the flavonoids, they induced the same specific abnormal phenotypes during embryo development. These abnormal embryos differentiated multiple shoot and/or root meristems, and multiple organs *e.g* multiple coleoptiles and scutella. Different classes of increasingly affected phenotypes were observed. Strongly affected embryos showed a complete duplication of the embryonic axis and the scutellum. The occurence of multiple shoot meristem phenotypes depended on the concentration of the inhibitor and on the developmental stage of the isolated embryo. Embryos treated with NPA or quercetin differentiated multiple roots less frequently than multiple shoots. Our data support the hypothesis that polar transport of auxin plays an important role in the establishment of the embryonic symmetry in *Poaceae*, an important monocot family. The different abnormal phenotypes obtained were used to develop a hypothetical model of auxin synthesis and transport in the globular and early transition embryos.

TOSHI M. FOSTER

University of California Berkeley
Department of Plant Biology
Plant Gene Expression Center
BERKELEY, CA. 94720 (USA)

The *Gnarley1* mutation of *Zea mays* alters proximal-distal patterning of the leaf and specifically disturbs sheath histology.

The *Gn1* locus of is defined by two dominant mutations that alter basic pattern elements of the maize leaf. The *Gn1* mutations perturb the ligule and auricle, specialized tissues that mark the boundary between blade and sheath regions of the leaf. In *Gn1* mutants, the ligule is displaced proximally into the blade, and auricle initiates at novel positions on the leaf. A second aspect of the *Gn1* mutant phenotype involves changes in cell dimensions within sheath tissue. Mutant sheath cells do not fully elongate in the proximal-distal dimension, but over-expand in both the lateral (midrib -margin) and dorsal-ventral (abaxial-adaxial) dimensions. Analysis of leaves genetically mosaic for *Gn1*-O reveals that the two basic aspects of the *Gn1*-O mutant phenotype, the leaf pattern defect and cell dimension defect, are independent of one another.

Molecular data indicate that *Gn1* corresponds to *knox 4*, a member of the *kn1*-like family of homeobox genes. *knox 4* is expressed in wild type vegetative meristems, ear and tassel primordia, and roots. In situ localization of *knox 4* mRNA in the vegetative meristem indicates that *knox 4* is expressed in horizontal rings below the incipient leaf primordia P(0) and P(-1). A thicker ring of *knox 4* expression is located at the leaf insertion point of older leaves. These observations suggest that *knox 4* may be involved in designating the basal components of the vegetative phytomer; node, internode, and axillary bud. A repetitive, ring-like pattern of *knox 4* expression is also seen in young ear primordia and within lateral meristems on the ear.

knox 4 is not expressed in normal leaves, but is ectopically expressed in young *Gn1* mutant leaves. In situ analysis indicates that *knox 4* is expressed in mesophyll cells towards the base of young *Gn1* leaves, from P(1-5). The ectopic expression of *knox 4* is spacially and temporally correlated with the elaboration of the sheath, implicating that the misexpression of *knox 4* results in a major disruption to early patterning events within *Gn1* leaves.

Cytological and molecular changes induced in plant roots by sedentary nematodes

Godelieve Gheysen

Laboratorium voor Genetica, Departement Genetica, Vlaams Interuniversitair Instituut voor Biotechnologie (VIB), Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium and Vakgroep Plantaardige Productie, Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

Sedentary plant-parasitic nematodes induce specialized feeding sites in the roots of their host plants. The elaborate structural and metabolic changes occurring during nematode infection are probably the result of interactions between nematode secretions and the regulation of plant gene expression. The initial stimulus from the nematode and the transduction pathway of signals linking this with the final plant cell response has yet to be clarified. Although many fragmentary data point toward the involvement of plant hormones in plant—nematode interactions, a clear and coherent picture has not yet emerged. The formation of a nematode feeding site is a developmental process. Several plant genes that are turned on/off during this differentiation have been identified. For example, cell cycle genes such as *cdc2aAt* or *cyc1At* are induced during initiation of feeding sites, syncytia as well as giant cells. This was unexpected since it is generally accepted that syncytia develop through dissolution of cell walls without the involvement of mitosis. In contrast, giant cells are formed by repeated mitosis without cytokinesis. Both types of feeding cells have multiple nuclei that are enlarged due to endoreduplication. The cytological and molecular study of the root cell changes induced by the nematodes are not only bringing insight into the pathogenesis but also provide data on normal plant cell differentiation and on the role of specific proteins in the plant cell cycle.

The *Arabidopsis* gene *MONOPTEROS* encodes a transcriptional regulator mediating embryo axis formation and vascular development.

Christian S. Hardtke and Thomas Berleth, Insitut für Genetik und Mikrobiologie, Maria-Ward-Str. 1A, 80638 München, Germany

Mutations in the *MONOPTEROS* gene (*MP*) of *Arabidopsis thaliana* interfere with the initiation of the body axis in the early embryo and the formation of vascular strands at all stages. We have isolated the *MP* gene by positional cloning. The gene was located by RFLP mapping. Starting from nearby RFLP markers, some of which were converted into CAPS markers, a chromosome walk using yeast artificial chromosomes (YACs) was assembled, spanning ca. 2200kb and 5.5cM. A YAC bridging the gene was used as a probe to isolate homologous clones from a cosmid library. Mapping of several of these cosmids revealed one clone, which detected a polymorphism that cosegregated with the *mp* locus on a population of 1796 meiotic events. Around this clone, a local contig of ca. 60kb was generated using bacterial artificial chromosomes and cosmids. In this contig, eight different cDNA clones were isolated. One of them detected an allele-specific polymorphism in an X-ray induced *mp* allele. The identity of this cDNA clone with the *MP* transcription unit was confirmed by revealing point mutations in the corresponding genomic sequences of five more independent mutant alleles. The complete sequence of the *MP* transcription unit was determined by RACE-PCR, and the intron-exon structure by sequencing a genomic fragment. The presumptive MP protein encodes a putative transcription factor. Functionality of putative nuclear localization signals within the protein has been proven by assaying subcellular localization of MP-GUS fusion proteins in transiently transformed onion cells. The expression pattern of the gene has been determined by investigating the distribution of *MP* transcript over the whole life cycle of the plant in *in situ* hybridization experiments. In addition, transgenic plants carrying promoter-GUS fusions have been generated. The correlation of mutant defects and the *MP* expression pattern as well as the identity of the MP protein suggest a regulatory role of *MP* in mediating embryo axis formation and vascular development.

Pursuite of *In vitro* zygote development in wheat

Jochen Kumlehn, Horst Lörz and Erhard Kranz
Institut für Allgemeine Botanik, AMP II, Ohnhorststraße 18, 22609 Hamburg, Germany

We have established a method to cultivate isolated wheat (*Triticum aestivum* L.) zygotes which undergo direct embryogenesis and plant regeneration. The zygotes are embedded in an agarose matrix on a microscopic glass slide, which allows observation of individual cells at high magnification. A developmental series of an individually cultivated zygote will be presented.

The zygotes were isolated about 3 hours after hand-pollination (hap) of emasculated spikes. Cell isolation was carried out using fine-tipped glass needles in an 0.5 M mannitol solution. The isolated zygotes were taken up with a glass capillary interfaced to a computer-controlled micropump through an oil-filled teflon tube. The zygotes were released into an agarose droplet which had been placed centrally into a 10-mm glass ring fixed with silicon adhesive onto a glass slide. Liquid culture medium, supplemented with a suspension of barley microspores which had been induced to sporophytic development, was poured into the glass ring after solidification of the agarose. Co-culture with barley microspore-derived aggregates was found to be essential for further development of isolated wheat zygotes. Under optimized conditions, almost every zygote developed into an embryo.

The unpollinated wheat egg cell is characterized by one large nucleolus. In the cultivated zygotes, up to 3 smaller additional nucleoli appeared between 5 and 11 hap. Around 18 hap, all nucleoli of a zygote disappeared within a few minutes, indicating mitotic prophase. The first cell division was completed in all zygotes within 23 hap. The early cellular events are likely to be analogous to those *in planta* since this result confirms earlier studies based on fixed material and since the zygotes subsequently mimic normal embryonic development. For this reason, this method could be used as a system for detailed investigations, e.g. on DNA replication or early pattern formation during zygotic development. To extend the analysis to earlier stages of zygotic development, it could be coupled with *in vitro* fertilization techniques, which have previously been developed in our laboratory.

Although the zygotes develop quite synchronously in culture, there is considerable variation between individuals. Therefore, for detailed analyses at the molecular level, it would be advantageous to have optical control during cell selection. Through this novel approach, we have monitored cellular events which can easily be observed in every cultivated wheat zygote using light microscopy. Using this method, it is possible to sample cells of a strictly defined developmental stage for molecular approaches, to apply detailed microscopic examination after differential staining, or *in situ* hybridization directly on the same slide.

MYB TRANSCRIPTION FACTORS ARE ENCODED BY A UNUSUALLY LARGE GENE FAMILY OF ABOUT ONE HUNDRED MEMBERS.

Ignacio Romero, Antonio Fuertes, Antonio Leyva, and Javier Paz-Ares
Centro Nacional de Biotecnología-CSIC. Campus de Cantoblanco, 28049 Madrid, Spain

Transcription factors belonging to the R2R3-MYB family contain the related helix-turn-helix repeats R2 and R3. We isolated partial cDNA and/or genomic clones of 78 *R2R3-MYB* genes and estimated that about one hundred of these genes are present in the *Arabidopsis thaliana* genome, representing the largest regulatory gene family currently known in plants. In contrast, no more than three *R2R3-MYB* genes have been reported in any organism from other phyla. DNA-binding studies showed that there are differences but also frequent overlaps in binding specificity among plant R2R3-MYB proteins, in line with the distinct but often related functions that are beginning to be recognized for these proteins. The available *myb* clones have been used as probes in a PCR-based strategy to search for *myb* mutants in T-DNA tagged lines and their phenotypic analysis should shed further light on the functional diversity/relationships between the members of the MYB family.

HOMEODOMAIN PROTEINS IN REGULATION OF RICE DEVELOPMENT

Annemarie H. Meijer and J. Harry C. Hoge

Institute of Molecular Plant Sciences, Leiden University, Clusius Laboratory, P.O. Box 9505, 2300 RA Leiden, The Netherlands

Homeodomain proteins are well known for their key function in cell fate determination in animals and there is accumulating evidence that these transcription factors are also of major importance during plant development. In order to identify homeobox genes with possible functions in regulation of morphogenesis and embryogenesis in the model cereal rice, we have constructed cDNA libraries of seedlings, and of embryos at well-defined developmental stages before, during and after organ differentiation. Extensive screenings of these libraries resulted in the isolation of a number of different cDNA clones encoding homeodomain proteins either with (HD class) or without (HD-Zip class) a leucine zipper dimerization motif adjacent to the homeodomain. The cDNA clone *Oshox1*, encoding a protein of the HD-Zip class was selected for functional analysis. Expression studies indicated that *Oshox1* may operate in various tissues and at different developmental stages, from at least as early as the organ differentiation phase in embryogenesis up to the mature flowering plant. Transgenic *Arabidopsis* plants expressing *Oshox1* showed retarded growth, tiny lancet-shaped leaves and reduced seed set, suggesting that the Oshox1 protein functions as a developmental regulator. Transgenic expression studies in rice are in progress. Yeast two-hybrid assays revealed that Oshox1 protein can homodimerize, but can also form heterodimers with an *Arabidopsis* HD-Zip protein. This suggests that protein-protein interactions may also occur between different HD-Zip proteins in rice, which would provide enormous versatility for generating specific gene control mechanisms. To further investigate this possibility efforts are underway to clone additional members of the rice HD-Zip family. In addition, attempts to identify other putative Oshox1 interaction partners are in progress. A two-hybrid screening with Oshox1 as a bait resulted in several positive clones, among which a transcriptional coactivator and a zinc finger transcription factor. Currently we are testing whether these represent true interaction partners of Oshox1. To confirm that Oshox1 represents a transcription factor DNA-binding studies were performed. These revealed that Oshox1 interacts with the pseudopalindromic sequence CAAT(C/G)ATTG both *in vitro* and *in vivo*. Oshox1 was found to repress reporter gene activity in rice suspension cells, most likely by a mechanism of active transcriptional repression. Repression was strictly dependent on the presence of upstream Oshox1 binding sites in the reporter gene constructs and a function of the N-terminal region of Oshox1, preceding the homeodomain. By means of genetic selection in yeast we are currently trying to identify natural target genes of Oshox1.

Pattern formation in Arabidopsis flowers

François Parcy, Ove Nilsson, and Detlef Weigel

Plant Biology Laboratory, The Salk Institute for Biological Studies, 10010 N. Torrey Pines Rd., La Jolla, CA 92037, USA; fparcy@aim.salk.edu

In *Arabidopsis*, flower development proceeds in two discrete steps, the specification of a flower meristem followed by the specification of floral organs (Weigel and Meyerowitz, 1994). The first step is under control of meristem-identity genes such as *LEAFY (LFY)* (Weigel *et al.*, 1992) Both the study of *lfy* mutants and plants ectopically expressing *LFY* have established that this gene plays an important role in triggering the first step, the formation of a flower meristem. The second step is under the control of the ABC organ-identity genes, whose combined activities control to the differentiation of the four types of floral organs(Weigel and Meyerowitz, 1994). However, the link between meristem-identity and organ-identity genes is not yet clearly established. In particular, the mechanism by which the region-specific expression of organ-identity genes is established is unknown.

We are studying the role played by *LFY* in this process. *lfy* loss-of-function mutants have not been very helpful in understanding this aspect of LFY function, since the mutant is already impaired in the first step and never forms a normal flower meristem. In addition, it has been shown that ABC genes can be eventually activated in a *lfy* mutant showing that *LFY* is not absolutely required for their induction (Weigel and Meyerowitz, 1993).

To better understand the role played by *LFY* in activating organ-identity genes, we have initiated experiments in which the two processes of flower-meristem specification and activation of organ-identity genes are uncoupled. We have achieved this either by changing *LFY* expression or LFY activity. Our first type of experiment involved *35S::LFY* plants, in which *LFY* is ectopically expressed. By crossing such transgenics to *GUS* reporter plants, we have shown that *LFY* can induce the promoter of *APETALA1 (AP1)*, but not of *APETALA3 (AP3)* or *AGAMOUS (AG)*, in leaf primordia in the absence of flower formation.

A variant of this approach involved the gene *UNUSUAL FLORAL ORGANS (UFO)*, which plays a role in activating *AP3* expression. Using *35S::LFY 35S::UFO* plants, we have shown that *LFY* and *UFO* together are able to activate *AP3* in all seedling organs in the absence of floral meristem formation. *AG* expression is, however, not induced in these transgenics.

The rationale of the second type of experience was to create an activated form of LFY by inserting sequences encoding the viral VP16 activation domain into the *LFY* coding sequence. This fusion was introduced in plants under the control of *LFY* regulatory elements (*"LFY:VP16"*). *LFY:VP16* flowers display a range of organ modifications, suggesting *AG* expression in all four whorls. We have shown by in situ hybridization and with reporter gene fusions that *AG* is indeed activated earlier and at a higher level than in wild type and throughout all four whorls of the flower. AG is also induced in the subapical region of the shoot meristem. We have also shown that *AG* is responsible for the LFY:VP16 phenotype, as the phenotype is suppressed by the *ag-1* mutation. These findings suggest that *LFY* is directly involved in controlling *AG* expression during normal flower development and that modulation of *LFY* activity (for example, by a localized cofactor) is responsible for the wild type *AG* expression pattern. This hypothesis will be discussed in relation to previous models involving repression by factors such as AP2.

This set of experiments has allowed us to show that *LFY* directly induces ABC genes, and that this does not require formation of a functional flower meristem. In addition, we have identified differences in the mechanisms of regulation of ABC genes, helping us to understand how a single uniformly expressed gene can activate targets with specific expression pattern.

D. Weigel, J. Alvarez, D. R. Smyth, M. F. Yanofsky and E. M. Meyerowitz (1992) *Cell*, 69, 843-59.
D. Weigel and E. M. Meyerowitz (1993) *Science*, 261, 1723-1726.
D. Weigel and E. M. Meyerowitz (1994) *Cell*, 78, 203-209.

Isolation of genes whose products interact with APETALA1 and CAULIFLOWER using the yeast 2-hybrid system.

Soraya Pelaz, Cindy Gustafson-Brown, Susanne Kohalmi*, Bill Crosby* and Martin Yanofsky

Department of Biology, University of California at San Diego, La Jolla, CA 92093-0116
* Plant Biotech Institute, 110 Gymnasium Place, Saskatoon, SK S7N-0W9 NRC Canada

APETALA1 (AP1) and *CAULIFLOWER* (CAL) encode closely related and partially functionally redundant MADS-box genes that specify flower meristem identity in *Arabidopsis*. Although these genes likely function as transcriptional regulators, little is known about the upstream factors that regulate their expression, their downstream targets, or potential factors with which they directly interact. As a start toward identifying genes whose products interact with AP1 and CAL, we screened expression libraries using the yeast two-hybrid system. The screen using CAL as bait yielded four different MADS-box genes, including *AGL9*, *AGL20*, *AGL22*, and *AGL24*. Subsequent studies showed that these four MADS-box gene products also interact with AP1 in the yeast two-hybrid assay. The screen using AP1 as bait also yielded several distinct MADS-box genes, as well as two independent clones encoding a putative RNA binding protein. Further characterization of these interactions will be presented.

Genetic dissection of the phytochrome A signal transduction pathway in *Arabidopsis*

Pilar Puente, Thierry Desnos, Garry C. Whitelam[1] and Nicholas P. Harberd.
John Innes Centre. Colney, Norwich NR4 7UH, U.K. [1]Botany Department, University of Leicester, Leicester LE1 7RH, U.K.

Light is arguably the most important environmental factor regulating plant morphogenesis. In *Arabidopsis* from seed germination to flowering time, a number of developmental features depend upon light quality, quantity, duration and direction and the ability of the plant to monitor and respond to them all. In *Arabidopsis* several photoreceptors mediate light perception: UV-light photoreceptor(s), red/far-red (R/FR) photoreversible photoreceptors or phytochromes and blue/UV-A-light photoreceptor(s) or cryptochrome(s). The isolation of mutants in the phyA locus and the characterization of their phenotypes under different light conditions has unravelled the involvement of this photoreceptor in the high-irradiance far-red light signalling pathway: *phyA* mutants grown under cFR-light resemble wild type seedlings grown in darkness, which strongly suggests that the phyA photoreceptor is the only one mediating this response. Thus this system is a powerful model to genetically dissect the phyA signal transduction pathway and mutants in two loci (*fhy1* and *fhy3*) defining steps in this pathway have already been identified (Whitelam et al, 1993. Plant Cell 5, 757).

Here we report the isolation of new alleles of *phyA*, *fhy1* and *fhy3* together with mutant alleles at new loci. The material screened came from various sources (fast-neutrons, γ-ray or EMS mutagenized seeds and T-DNA collections). We have defined complementation groups and we are currently characterizing the mutants at different levels.

We will be presenting our results on specific developmental phenotypes such as etiolation under cFR-light, white light-induced greening after cFR-light treatments and flowering time.

Together with other genetic and molecular data, these results will allow us to contribute to the understanding of light signalling as developmental factor in plants.

Fast neutron induced *Arabidopsis thaliana* mutants with abnormal leaves

Pedro Robles and José Luis Micol
División de Genética. Universidad Miguel Hernández. Campus de San Juan. 03550. Alicante. Spain.

As a part of a search for *Arabidopsis thaliana* mutants showing morphological abnormalities in vegetative leaves, we screened 23,710 M_2 seeds, the progeny of 2,964 M_1 *Ler* individuals mutagenized by fast neutron bombardment. Most of the 904 M_2 putative mutants isolated showed an unstable phenotype, which was lost after one or two generations of selfing. Some mutants, whose phenotypes were transmitted with complete penetrance and almost constant expressivity, were subjected to genetic analysis.

The fast neutron induced mutants under study were included in the following complementation groups. *DENTICULATA18* and *DENTICULATA19*: mutants with pointed leaves, several marked teeth in the leaf margin and conspicuous venation pattern; the spacial arrangement of leaf cells is disrupted, both the spongy mesophyll and palisade mesophyll showing fewer cells than those of wild type individuals. *TRANSCURVATA4* and *TRANSCURVATA5*: mutants with leaves that are transversally curled towards the abaxial surface. *ULTRACURVATA2*: the lamina of these mutants rolls down along the midvein, resulting in a very compact rosette. *INCURVATA8* and *INCURVATA10*: the Incurvata phenotype is characterized by leaves with involute margins.

The *ultracurvata*, *transcurvata* and *incurvata* mutants described here are the most likely candidates to be affected in genes that control coordination between the growth of the dorsal and ventral surfaces of the leaf. Characterization of some of these genes might provide information about the mechanisms coordinating groups of cells in proliferation during development.

Abstract: Judith L. Roe-

Patterning of tissues in floral organs of Arabidopsis

Each mature floral organ type is composed of an arrangement of unique tissues. The regional specification of tissues in an organ primordium proceeds by controlled growth and differentiation of cells in response to positional information. Mutational analysis has uncovered genes which play a role in these events.

Mutations at the *TOUSLED* locus in *Arabidopsis* cause a reduction in organ number in the flower, and also a loss of apical tissues in the gynoecium. The *TSL* gene encodes a nuclear protein kinase which has been highly conserved during evolution and members of the TSL-like kinase family have now been found in animals as well as other plant species. Double mutants of *tsl* and other mutations which affect gynoecium morphology have uncovered steps in carpel development which can be separated genetically, indicating the presence of specific pathways involved during morphogenesis of the different regions of the gynoecium which include the style, stigma, transmitting tract, valve and placentae. Also,*tsl* enhances a weak allele of *ettin*, a mutation which causes basalization of apical cell types in the gynoecium, suggesting that the two loci interact during carpel development.

A mutation at the *FENNEL* (*FNL*)locus affects only certain cell types within the floral organs, and does not alter organ numbers. *fnl* petals show a great reduction in blade tissue and an increase in claw tissue, suggesting that *FNL* may participate in apical/basal patterning in the petal primordia. *fnl* flowers also show an increase in apical tissues (style) in the gynoecium, an abnormal pattern of cell sizes in the sepals, and slightly altered morphology of the stamens. *FNL* may thus play a role in patterning of tissues in each type of floral organ primordium.

Judy Roe
Dept. Plant and Microbial Biology
University of California, Berkeley

USE OF DIFFERENTIAL DISPLAY TO IDENTIFY THE PLANT GENES INVOLVED IN THE INTERACTION BETWEEN *N. TABACUM* AND *RHODOCOCCUS FASCIANS*

Carmen Simon-Mateo, Carmem-Lara O. Manes, Denise Thorin, Marc van Montagu and Koen Goethals. Flanders Interuniversity Institute for Biotechnology, University of Gent, Belgium.

Rhodococcus fascians is a plant pathogenic bacterium that causes the formation of leafy galls (fasciation), a symptom accompanied by a loss of apical dominance and the production of multiple shoot primordia in the infected plant. Fasciation is a process involving signals by the pathogen and concomitant changes in the developmental programming of the infected plant host. Thus, an interesting approach to understand the hormone action and shoot development in plants is to investigate the plant genes induced or repressed after infection with *R. fascians*. To achieve this goal we used a powerful technique called 'differential display'. By using this technique we compared the mRNA content of *N. tabacum* plants infected with a pathogenic strain from *R. fascians* (D188) and of plants infected with a non pathogenic strain (D188-5) at different times after infection (1, 3 and 7 days). Four PCR bands were detected which were uniquely present in the PCR patterns corresponding to RNA of D188 infected plants and one PCR band was detected only in the samples from D188-5-infected plants.

DD1, a Light-Regulated Arabidopsis Gene

Guy Vancanneyt and José J. Sánchez-Serrano
Centro Nacional de Biotecnologia (CSIC), UAM Campus Cantoblanco, E-28049 Madrid.

We have been studying a novel light-responsive gene in *Arabidopsis thaliana*. The clone, DD1 (Down in the Dark), was isolated from a leaf cDNA library. It is encoded by a single copy gene, located on chromosome 4. Its 2 Kb transcript reveals an open reading frame of 443 amino acids, which cannot be correlated with any protein with known function in the databases. Interestingly, the protein is conserved in a range of plant species, including pine and maize. The primary structure of this polypeptide does not reveal any obvious targeting signal. Immunological studies indicate that the protein is probably not located in chloroplasts.

The gene corresponding to the DD1 transcript was isolated from a genomic Arabidopsis library. The DD1 gene is composed of an intronless untranslated RNA-leader of about 500bp, and a protein-codifying part which is interrupted by several introns.

DD1 is highly expressed in green Arabidopsis tissues. However steady state mRNA levels are very rapidly reduced upon transfer of plants from light to dark, and rapidly accumulate upon transfer from dark to light. The complete DD1 gene was introduced in a heterologous plant, tobacco, where the transgene is similarly regulated with respect to its down-regulation in the dark. Moreover promoter-GUS fusion were prepared and introduced in tobacco and Arabidopsis. These transgenic plants will be used to address the transcriptional and/or post-transcriptional regulation of the DD1 gene.

List of Invited Speakers

Workshop on

PLANT MORPHOGENESIS

List of Invited Speakers

Thomas Altmann — Max-Planck-Institut für Molekulare Pflanzenphysiologie, Karl-Liebknecht-Strasse 25, Haus 20, 14476 Golm (Germany).
Tel.: 49 331 977 23 00
Fax : 49 331 977 23 01
E-mail: altmann@mpimp-golm.mpg.de

Thomas Berleth — Universität München, Genetics Institute, Maria-Ward-Strasse 1A, D-80638 München (Germany).
Tel.: 49 89 179 198 41
Fax : 49 89 179 198 20
E-mail: uj44205@sunmail.lrz-muenchen.de

Ton Bisseling — Department of Molecular Biology, Agricultural University of Wageningen, Dreijenlaan 3, 6703 Wageningen (The Netherlands).
Tel.: 31 317 48 32 65
Fax : 31 317 48 35 84
E-mail: Ton.Bisseling@MAC.MB.WAU.NL

Liam Dolan — Department of Cell Biology, John Innes Centre, Norwich NR4 7UH (U.K.).
Tel.: 44 1603 45 25 71
Fax : 44 1603 50 17 71
E-mail: LIAM DOLAN@bbsrc.ac.uk

Hiroo Fukuda — Botanical Gardens, Faculty of Science, University of Tokyo, 3-7-1 Hakusan, Bunkyo-ku, Tokyo 112 (Japan).
Tel.: 81 3 5684 85 97
Fax : 81 3 3814 01 39
E-mail: sfukuda@hongo.ecc.u-tokyo.ac.jp

Sarah Hake — Plant Gene Expression Center, 800 Buchanan St., Albany, CA. 94710 (USA).
Tel.: 1 510 559 59 07
Fax : 1 510 559 56 78
E-mail: maizesh@nature.Berkeley.EDU

Dirk Inzé

Laboratorium voor Genetica, Departement Genetica, Vlaams Interuniversitair Instituut voor Biotechnologie,Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent (Belgium)
Tel.: 32 9 264 51 92
Fax : 32 9 264 53 49
E-mail: diinz@gengenp.rug.ac.be

Gerd Jürgens

Lehrstuhl für Entwicklungsgenetik, Universität Tübingen, Spemannstr. 37-39, 72076 Tübingen (Germany).
Tel.: 49 7071 601 861
Fax : 49 7071 601 862
E-mail: geju@mpib-tuebingen.mpg.de

Jane A. Langdale

Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB (U.K.).
Tel.: 44 1865 27 50 99
Fax : 44 1865 27 51 47
E-mail: jane.langdale@plants.ox.ac.uk

M. David Marks

Department of Genetics and Cell Biology, University of Minnesota, St. Paul, MN. 55108(USA).
Tel.: 1 612 625 67 37
Fax : 1 612 625 57 54
E-mail: dmarks@biosci.cbs.umn.edu

David W. Meinke

Department of Botany, Oklahoma State University, Stillwater, OK. 74078 (USA).
Tel.: 1 405 744 65 49
Fax : 1 405 744 70 74
E-mail:
meinke@osuunx.ucc.okstate.edu

José Luis Micol

División de Genética, Universidad Miguel Hernández,, Campus de San Juan, Ctra. de Valencia Km. 87, 03550 Alicante (Spain).
Tel.: 34 6 591 94 55
Fax : 34 6 591 94 34
E-mail: jlmicol@umh.es

Scott Poethig

Department of Biology, University of Pennsylvania, Philadelphia, PA. 19104-6018 (USA).
Tel.: 1 215 898 89 15
Fax : 1 215 898 87 80
E-mail: spoethig@sas.upenn.edu

Kevin A. Pyke	School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey TW20 0EX (U.K.) Tel.: 44 1784 44 34 07 Fax : 44 1784 47 07 56 E-mail: K.Pyke@rhbnc.ac.uk
Tsvi Sachs	Department of Plant Sciences, The Hebrew University, Jerusalem 91904 (Israel). Fax : 972 2 658 44 25 E-mail: tsachs@vms.huji.ac.il
John Schiefelbein	Department of Biology, University of Michigan, 830 North University Avenue, Ann Arbor, MI. 48109 (USA). Tel.: 1 313 764 35 80 Fax : 1 313 647 08 84 E-mail: schiefel@biology.Isa.umich.edu
Jan Traas	INRA, Laboratoire de Biologie Cellulaire, Route de Saint Cyr, 78026 Versailles cedex (France) Tel.: 33 1 30 83 30 58 Fax : 33 1 30 83 30 99 E-mail: traas@versailles.inra.fr
Chris van der Schoot	ATO-DLO, P.O. Box 17, NL-6700 AA Wageningen (The Netherlands). Tel.: 31 317 47 51 02 Fax : 31 317 47 53 47 E-mail:C.VANDERSCHOOT@ato.dlo.nl
Marc Van Montagu	Lab of Genetics, Flanders Inter University Institute for Biotechnology, K.L. Ledeganck-straat 35, 9000 Gent (Belgium). Tel.: 32 9 264 51 70 Fax : 32 9 264 53 49 E-mail: MAMON@GENGENP.RUG.AC.BE
Robert W. Williams	California Institute of Technology, Division of Biology 156-29, Pasadena, CA. 91125 (USA). Tel.: 1 818 395 49 36 Fax : 1 818 449 07 56 E-mail: bobbyw@cco.caltech.edu

List of Participants

Workshop on

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List of Participants

Tom Beeckman	University of Gent, Department of Plant Morphology, K.L. Ledeganckstraat 35, B-9000 Gent (Belgium). Tel.: 32 9 264 50 63 Fax : 32 9 264 53 34 E-mail: tom.beeckman@rug.ac.be
José Pío Beltrán	Instituto de Biología Molecular y Celular de Plantas, UPVA-CSIC, Departamento de Biología del Desarrollo, Campus de la Universidad Politécnica de Valencia, Camino de Vera s/n., 46022 Valencia (Spain). Tel.: 34 6 387 78 50 Fax : 34 6 387 78 59 E-mail: jbeltran@ibmcp.upv.es
Frédéric Berger	Reproduction et Développement des Plantes, UMR 9938 CNRS/INRA/ENS, Ecole Normale Supérieure de Lyon, 46 allée d'Italie, 69364 LYON (France). Tel.: 33 4 72 72 86 14 Fax : 33 4 72 72 86 00 E-mail: frederic.berger@ens-lyon.fr
Kim Boutilier	Centre for Plant Breeding and Reproduction Research CPRO-DLO, P.O. Box 16, 6700 AA Wageningen (The Netherlands). Tel.: 31 3174 77 167 Fax : 31 3174 18 094 E-mail: a.boutilier@cpro.dlo.nl
Héctor Candela	División de Genética, Universidad Miguel Hernández, Campus de San Juan, 03550 Alicante (Spain). Tel.: 34 6 591 93 46 Fax : 34 6 591 94 34 E-mail: HCANDELA@UMH.ES
Gerda Cnops	John Innes Centre, Department of Cell Biology, Colney Road, Colney Lane, Norwich NR5 7UH (U.K.) Tel.: 44 1603 45 25 71 Fax : 44 1603 50 17 71 E-mail: Cnops@bbsrc.ac.uk

Brendan Davies	University of Leeds, Centre for Plant Biochemistry & Biotechnology, Leeds LS2 9JT (U.K.). Tel.: 44 113 233 31 23 Fax : 44 113 233 31 44 E-mail: bdavies@bmb.leeds.ac.uk
Concha Domingo	Department of Cell Biology, John Innes Centre, Colney, Norwich NR4 7UH (U.K.). Tel.: 44 1603 45 25 71 Fax : 44 1603 50 17 71
Carmen Fenoll	Departamento de Biología, Facultad de Ciencias, Universidad Autónoma, Ciudad Universitaria de Cantoblanco, 28049 Madrid (Spain). Tel.: 34 1 397 81 98 Fax : 34 1 397 83 44 E-mail: Carmen.Fenoll@uam.es
Christiane Fischer	University of Freiburg, Institute of Biology II, Cell Biology, Schänzlestr. 1, D-79104 Freiburg (Germany) Tel.: 49 761 203 26 77 Fax : 49 761 203 26 75 E-mail: Fischer@uhura.biologie.uni-freiburg.de
Toshi M. Foster	University of California Berkeley, Department of Plant Biology, Plant Gene Expression Center, Berkeley, CA. 94720 (USA). Tel.: 1 510 559 59 00 Fax : 1 510 559 56 78 E-mail: toshi@nature.Berkeley.edu
Antonio Fuertes	Centro Nacional de Biotecnología, CSIC, Campus de Cantoblanco, 28049 Madrid (Spain). Tel.: 34 1 585 45 14 Fax : 34 1 585 45 06 E-mail: Fuertes@samba.cnb.uam.es
José Gadea	Instituto de Biología Molecular y Celular de Plantas, Universidad Politécnica de Valencia, Camino de Vera s/n, 46021 Valencia (Spain). Tel.: 34 6 387 78 73 Fax : 34 6 387 78 59 E-mail: jgadea@ibmcp.upv.es

Godelieve Gheysen	Laboratorium voor Genetica, Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent (Belgium) Tel.: 32 9 264 51 82 Fax : 32 9 264 53 49
Christian S. Hardtke	Institut für Genetik und Mikrobiologie, Maria-Ward-Str. 1A, 80638 München (Germany) Tel.: 49 89 1791 98 41 Fax : 49 89 1791 98 20 E-mail: uj44246@sunmail.lrz-muenchen.de
Renze Heidstra	Department of Molecular Cell Biology, Utrecht University, Padualaan 8, 3584 CH-Utrecht (The Netherlands). Tel.: 31 30 253 34 36 Fax . 31 30 251 36 55 E-mail: n.heidstra@biol.ruu.nl
Jochen Kumlehn	Institut für Allgemeine Botanik, AMP II, Ohnhorststraße 18, 22609 Hamburg (Germany) Tel.: 49 40 822 82 322 Fax : 49 40 822 82 229
Antonio Leyva	Centro Nacional de Biotecnología, CSIC, Campus de Cantoblanco, 28049 Madrid (Spain). Tel.: 34 1 585 45 14 Fax : 34 1 585 45 06 E-mail: aleyva@samba.cnb.uam.es
Annemarie H. Meijer	Institute of Molecular Plant Sciences, Leiden University, Clusius Laboratory, P.O. Box 9505, 2300 RA Leiden (The Netherlands). Tel.: 31 71 527 50 52 Fax : 31 71 527 49 99 E-mail: meijer@rulsfb.leidenuniv.nl
Jim A.H. Murray	Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QT (U.K.) Tel.: 44 1223 33 41 66 Fax : 44 1223 33 41 62 E-mail: j.murray@biotech.cam.ac.uk

François Parcy	Plant Biology Laboratory, The Salk Institute for Biological Studies, 10010 N. Torrey Pines Rd., La Jolla, CA. 92037 (USA). Tel.: 1 619 453 41 00 Fax . 1 619 558 63 79 E-mail: fparcy@aim.salk.edu
Soraya Pelaz	Department of Biology, University of California at San Diego, La Jolla, CA. 92093-0116 (USA). Tel.: 1 619 534 72 98 Fax . 1 619 534 71 08
Pilar Puente	John Innes Centre, Colney, Norwich NR4 7UH (U.K.) Tel.: 44 1603 45 25 71 Fax : 44 1603 45 68 44 E-mail: puente@bbsrc.ac.uk
Pere Puigdomènech	Departamento de Genética Molecular, CID-CSIC, Jordi Girona 18, 08034 Barcelona (Spain). Tel.: 34 3 400 61 29 Fax : 34 3 204 59 04 E-mail: pprgmp@cid.csic.es
Pedro Robles	División de Genética, Universidad Miguel Hernández, Campus de San Juan, 03550 Alicante (Spain). Tel.: 34 6 591 93 46 Fax : 34 6 591 94 34 E-mail: PROBLES@UMH.ES
Judith L. Roe	Department of Plant and Microbial Biology, University of California, 111 Koshland Hall, Berkeley, CA. 94720 (USA). Tel.: 1 510 643 92 04 Fax : 1 510 642 49 95 E-mail: jlr@nature.berkeley.edu
Carmen Simon-Mateo	Flanders Interuniversity Institute for Biotechnology, University of Gent (Belgium). Tel.: 32 9 264 51 83 Fax : 32 9 264 53 49 E-mail: casim@gengenp.rug.ac.be
Virginia Stiefel	Centre d'Investigació i Desenvolupament, Jordi Girona 18-26, 08034 Barcelona (Spain). Tel.: 34 3 400 61 00 Fax : 34 3 204 59 04

Guy Vancanneyt

Centro Nacional de Biotecnología,
CSIC, UAM Campus Cantoblanco, 28049
Madrid (Spain).
Tel.: 34 1 585 45 65
Fax : 34 1 585 45 06
E-mail: vancanneyt@cnb.uam.es

Mieke Van Lijsebettens

Department of Genetics, affliated
to Flanders Interuniversity
Institute for Biotechnology,
University Gent, K.L.
Ledeganckstraat 35, 9000 Gent
(Belgium).
Tel.: 32 9 264 51 70
Fax : 32 9 264 53 49
E-mail: milij@gengenp.rug.ac.be

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