Instituto Juan March
de Estudios e Investigaciones

134 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Leaf Development

Organized by

S. Hake and J. L. Micol

T. Altmann
T. Berleth
J. L. Bowman
N. G. Dengler
S. Hake
A. Hudson
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Introduction

J. L. Micol and S. Hake

Questions and answers about the development of plant leaves

The primary pathway for carbon and energy uptake by plants is the leaf, an organ of utmost importance in agriculture. However, little is known about the genetic controls underlying leaf development, in spite of the fact that its biotechnological manipulation offers great potential. Although Bateson realized the existence of inherited leaf shape variants as early as 1913, genetic investigations did not provide major insights into the dissection of leaf initiation and morphogenesis until the last decades of the XX century, when a large number of mutants with abnormally shaped leaves, most of them yet to be characterized, were isolated in model systems such as *Arabidopsis thaliana*, *Antirrhinum majus* and *Zea mays*.

Although most plant leaves are simple structures, many developmental processes are involved in leaf ontogeny. They include, among others, the positioning and initiation of leaf primordia at the flanks of the shoot meristem, the specification of leaf identity as opposed to that of other organs which are assumed to be modified leaves, the establishment of dorsal and ventral identities within the organ, the definition of domains such as ligule, sheath and blade in some monocotyledonous plants, as well as petiole and lamina in dicots, the control of cell division and expansion, the formation of patterns such as those of venation, trichomes or stomata, the mechanisms responsible for the diversity of compound and simple leaves and those that specify heteroblastic differences among different leaves within a plant. A large body of detailed information on what actually happens at a morphological level is available for most, if not all such processes. At the present time, an expanding number of studies on leaf variants including molecular and genetic analyses are being published for several plant species. Thanks to these efforts, answers are beginning to be available to the questions on the nature, action and interactions of the genes driving the sequence of developmental events that contribute to the making of a leaf.

We discussed at this workshop recent progress in the study of genetic mechanisms that control the elaboration of plant leaves. The topics covered were the specification of leaf identity, the definition of axes and polarities in the formation and growth of leaves and different aspects of cell differentiation, pattern formation, phase change and heteroblasty. As it can be seen in the following pages, answers to many of the above mentioned fundamental questions are coming from the study of specific genes, thanks to the enormous progress that has been made in recent years in the area of leaf development.

José Luis Micol and Sarah Hake

Session 1: Specification of leaf identity
Chair: John L. Bowman

The gibberellin pathway mediates KNOTTED1-type homeobox function in plants with different body plans

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In Arabidopsis, two *KNOX* genes, *STM* (*SHOOTMERISTEMLESS)* and *KNAT1(KNOTTED-LIKE* from *ARABIDOPSIS THALIANA1),* are expressed in partially overlapping domains of the SAM. Transcripts for both genes, however, are excluded from incipient leaf primordia, leading to the hypothesis that *KNOX* gene products are required for meristem function whereas their absence is required for leaf initiation (1) (2). Genetic evidence supports this hypothesis: loss of function mutations in *STM* result in a failure to initiate or maintain a SAM (2). Conversely, ectopic expression of *KNAT1* results in the formation of meristems on leaves and alteration of leaf shape (3). Recent data suggests that certain *KNOX* misexpression phenotypes may be mediated through growth regulator pathways (4) (5). Gibberellins are diterpenoid growth regulators that are important for many aspects of plant development including germination, stem elongation and flowering time (6). In tobacco the KNOX protein NTH15 represses transcription of *Ntc12*, a gene encoding a GA 20-oxidase required for GA biosynthesis (7). In contrast to species with simple leaves such as Arabidopsis and tobacco, the dissected leaves of tomato plants express *KNOX* genes. This expression pattern, combined with the increased leaf dissection obtained by overexpressing *KNOX* genes in tomato, has led to the suggestion that differential regulation of *KNOX* genes may be involved in the generation of dissected leaf morphology (8) (9). Here we discuss the role of the KNOX/GA regulatory module in controlling meristem activity in Arabidopsis and leaflet number in tomato.

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narrow sheath2 encodes a NITRILASE-like gene product required for recruitment of a lateral compartment in maize lateral organs

Michael J. Scanlon

Mutations in the *narrow sheath* (*ns*) genes cause the deletion of a lateral domain of the maize phytomer that includes the margins of the proximal blade, the sheath and the internode (Scanlon et al., 1996). Mutant plants have short stems and narrow leaves, although the leaf length is undisturbed. All vegetative and floral phytomers of the post-embryonic shoot are affected; no phenotype is detected in roots or non-foliar organs. The narrow sheath phenotype is a duplicate factor trait controlled by recessive mutations at two unlinked loci, *ns1* and *ns2* (Scanlon et al., 1996; Scanlon et al., 2000). A single non-mutant copy of either *ns* gene is sufficient for normal leaf development. KNOX immunolocalization studies and fate-mapping of mutant meristems (Scanlon and Freeling, 1997) reveal that a founder-cell domain, which normally contributes to the non-mutant leaf margins, is not recruited in ns mutant leaves. These data suggested a model whereby the founder cells of the maize leaf are comprised of two distinct lateral compartments. One compartment, the central domain, includes the midrib and upper leaf blade and does not require NS function for recruitment. The narrow sheath domain includes all lateral regions from the boundary of the central domain to the margin.

In support of this model, clonal analyses demonstrate that NS1 function is localized to two discrete focal points (one for each leaf margin) in the L2 tissue layer of the maize apex (Scanlon 2000). NS1 is not required for initiation of the central domain, and loss of wild type NS1 function in founder cell regions marginal to the narrow sheath foci has no effect on leaf development. These data indicate that NARROW SHEATH1 function is non cell-autonomous within the narrow sheath domain, and is required for initiation, but not propagation, of the founder-cell recruitment signal. In order to identify the nature of the NS recruitment signal, *ns2* was cloned by transposon-tagging. NS2 encodes a NITRILASE-like product; nitrilases are implicated in the production of IAA in plant shoots. Moreover, the ns mutant phenotype of maize bears a striking similarity to tobacco and petunia plants that overexpress IAA-biosynthetic genes (Klee et al., 1987; Sitborn et al., 1992). These data contribute to existing models in which induction and lateral expansion of plant lateral organs is regulated by the production and/or transport of IAA in plant shoots (Reinhardt et al., 2000; Berleth and Sachs, 2001). Surprisingly, *ns2* and the duplicate factor gene *ns1* are non-homologous sequences. Evidence is presented to test the model that the *ns* duplicate genes encode redundant, parallel functions in the control of hormone levels in the maize shoot apex.

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The phytomer concept revisited - the relationships between axillary meristems, leaves and internodes

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In higher plants, morphogenetic events occur at the shoot apical meristem (SAM), a self-organizing group of stem cells. Activities of the SAM lead to leaves, flowers and stem tissues. The relationship between these different plant parts has been studied by morphologists and now by molecular biologists. The concept of the phytomer has been used to describe the repeating pattern of leaf, stem and internode and to explain different parts of grass flowers (Galinat, 1959). Clonal analyses in maize suggest that the different plant parts originate from the same population of SAM cells. We have addressed the question of relationship between axillary meristem, leaf and internode by examining mutants in Arabidopsis and maize.

KNOTTED1-like homeobox (*KNOX*) genes are expressed in specific patterns in the SAM and may contribute to establishing the different fates of meristem cells (Jackson et al., 1994). We isolated and analyzed a recessive loss-of-function allele at one of the *KNOX* genes in *Arabidopsis thaliana*, *KNAT1*. The mutation is allelic to *brevipedicellus-1* (*bp-1*). *bp* plants are short, siliques point downward and the shape of the inflorescence is altered. Scanning electron microscopy was employed to examine the patterning of floral primordia. In wild-type plants, inflorescence meristems produce flowers and internodes in regular patterns such that adjacent floral primordia are positioned at regular angles separated by well defined internodes. In *bp*, both the angle and distance between flowers is inconsistent. The patterning of floral and internode primordia are both affected in *bp*, suggesting that *BP* is involved in the process of allocating cells to floral or internode primordia, possibly through the establishment of boundaries between floral and internode initials. The *in situ* mRNA accumulation pattern of *BP* in the inflorescence meristem supports this hypothesis. It appears as a boundary around the incipient primordia.

A consequence of the boundary of *BP* expression has also been described for ectopic expression in the leaf (Ori et al., 2000). Overexpression throughout the leaf does not have as dramatic an effect on the phenotype, as does the juxtaposition of *BP*-expressing and non-expressing cells, which leads to dramatic leaf lobing.

Another mutant that reveals an intimate relationship between internode and leaf is *terminal ear* in maize. In *te1* plants, leaves are produced faster and the phyllotactic pattern is affected (Veit et al., 1998). Like *bp*, very short internodes are found on *te1* plants and they are often interspersed with long internodes. The internodes also curve from side to side. The shorter side of the curved internode is directly below the midpoint of the clonally-related leaf. *te1* is also expressed in a subdomain of the shoot apical meristem in a pattern not too different from the maize orthologs of *BP*, the duplicate loci *gnarley* and *rough sheath1*.

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Genetic architecture of leaf morphogenesis in *Arabidopsis thaliana*

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In an attempt to identify genes involved in leaf morphogenesis in *Arabidopsis thaliana*, we screened for new mutants showing abnormal leaves and conducted genetic analyses of already obtained mutants. Our large-scale mutant search, which got close to but did not reach saturation of the genome, showed that the lines obtained fell into 94 complementation groups. Many of these genes were mapped using a high-throughput linkage analysis method, based on the simultaneous PCR coamplification of 21 polymorphic microsatellites and the fluorescent semiautomated detection of their products. In addition, in an attempt to ascertain wether intraspecific variability might be a source of information on the genetic controls underlying plant leaf morphogenesis, we analyzed variations in the architecture of vegetative leaves in a large sample of *Arabidopsis thaliana* natural races, concluding that such morphological traits are unlikely to develop under monogenic controls. Hence, a mapping population of the recombinant inbred lines of Lister and Dean was analyzed to identify quantitative trait loci (QTL) harboring naturally occurring alleles that contribute to natural variations in leaf architecture and to eventually correlate their intervals with the map positions of genes identified by mutation.

Session 2: Axes and polarities in leaf formation
Chair: Jane A. Langdale

Establishment of polarity in lateral organs of seed plants

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Plant architecture forms by a well-defined repeatable pattern for each and every plant species. However, enormous variation exists in shape, size and spacing of different plants lateral organs such as leaves and floral organ. This variation reflects the unique adaptations into various growth habitats and environments. The ability to determine the plane and magnitude of blade growth provides a powerful mechanism to regulate shape. The flexibility of ab/ad relations, and the quantitative rather than qualitative relationships between them could account for the enormous variation in lateral organ shape and size that characterizes the plant kingdom.

Recent studies in model species as *Antirrhinum* and *Arabidopsis* have demonstrated that specification of lateral organ polarity along the abaxial/adaxial organ axis has major consequences for the overall organ shape. Polar morphology results in specific adaptations of the leaf with an adaxial (top) surface specialized for light capture and an abaxial (bottom) surface specialized for gas exchange. Furthermore, the establishment of polarity is required for proper lamina development. A model of leaf blade development by Waites and Hudson (1995) proposed that juxtaposition of abaxial and adaxial domains is required for lamina outgrowth. As lateral organs are derived from the flanks of apical meristems, there exists an inherent positional relationship between them – the adaxial side of the lateral organ primordia is adjacent to the meristem and the abaxial side is at a distance from it. The fundamental positional relationship of leaves relative to the shoot apical meristem (SAM) was suggested to form the physiological basis for their asymmetric development (Wardlaw, 1949). This view was further supported by experiments in which the lateral organ primordia were separated from the apical meristem by incision (Sussex, 1955; Snow and Snow, 1959). When young potato leaves were separated from the shoot apical meristem (SAM), a small radial leaf was formed, suggesting that the meristem may act as a source for a signal required for proper polarity establishment in lateral organs (Sussex, 1955). Furthermore, the establishment of polarity is required for proper lamina development with the juxtaposition of abaxial and adaxial domains responsible for induction of lamina outgrowth (Waites and Hudson, 1995). The end result in most plants is a laminar leaf with an adaxial (top) surface specialized for light capture and an abaxial (bottom) surface specialized for gas exchange.

An emerging picture from classical and molecular genetic analyses is that as incipient lateral organ primordia develop from the flanks of the shoot apical meristem, factors both intrinsic and extrinsic to the organ primordia contribute to the specification of cells as adaxial or abaxial. The apical meristem itself likely provides a signal(s) that promotes adaxial cell fate (Sussex, 1955), whose perception may be mediated through *PHB/PHV/REV* (McConnell and Barton, 1998; McConnell et al., 2001). The ultimate source and biochemical nature of the ligand is unknown. *PHB*, *FIL* and *KAN* are all expressed in the leaf anlagen, but their expression becomes confined to mutually exclusive domains as the primordia form (Siegfried et al., 1999; Sawa et al., 1999; McConnell et al., 2001; Kerstetter et al., 2001).

Abaxial cell fate, which is promoted by both YABBY and KANADI genes (Siegfried et al., 1999; Sawa et al., 1999; Eshed et al., 1999; Eshed et al., 2001; Kerstetter et al., 2001) may be a 'default' in the absence of signal, for instance, if the lateral organ primordia are separated from the apical meristem. This 'default' state could be the result of the failure to repress genes promoting abaxial identity (e.g. *YABBY* and *KANADI* genes) which are initially activated throughout the anlagen (Siegfried et al., 1999; Sawa et al., 1999; Kerstetter et al., 2001). Surgical experiments indicate that while polarity is labile in P1 it is irreversibly established by P2 (Sussex, 1955). Subsequent interactions between the juxtaposed adaxial and abaxial domains, perhaps mediated by relative levels of KANADI and YABBY activity, are required for lamina outgrowth (Waites and Hudson, 1995).

We will present our recent results examining the interactions between the YABBY, PHB-like, and KANADI genes.

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Comparative genetics of leaf development in *Antirrhinum*

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To investigate the genetic control of lateral organ development in the snapdragon, *Antirrhinum majus*, we isolated the *PHANTASTICA* (*PHAN*) gene that is required redundantly for organ initiation and dorsal (adaxial) cell identity in leaves, bracts and petals [1]. It encodes a MYB transcription factor that is expressed throughout lateral organ initials and primordia. In *Arabidopsis*, dorsal organ identity is specified by *PHABULOSA* (*PHB*) and related genes which restrict expression of *YABBY* (*YAB*) genes to ventral organ domains [2,3]. In *Antirrhinum*, PHAN activity is needed for dorsal expression of *PHB*-like genes and for repression of *YAB*-like genes, suggesting that *PHAN* may affect organ asymmetry by promoting PHB-like activity. *GRAMINIFOLIA* (*GRAM*) is a member of the *Antirrhinum YAB* gene family that is expressed ventrally in developing organs and needed non-cell autonomously for lateral growth of leaves and petals and identity of cells at the ventral leaf margins. Although *GRAM* is expressed ectopically in *phan* mutant leaves it is not required for ectopic ventral cell identity.

A further target of *PHAN* regulation was identified as the *knotted1*-like homeobox (*knox*) gene, *HIRZINA* (*HIRZ*) - one of two *STM* orthologues in *Antirrhinum*. Expression of *HIRZ*, and the closely related *INVAGINATA* (*INA*), is normally confined to the shoot apical meristem (SAM) and internode initials [4]. Although ectopic expression of *HIRZ*, but not *INA*, occurs in *phan* mutant organs, genetic evidence suggests that *PHAN*'s role in promoting organ formation and asymmetry is independent of *HIRZ* repression. Spontaneous gain-of-function mutations have been identified in *HIRZ* and *INA*. Both lead to ectopic expression in developing petals and formation of an ectopic petal tube. This tube resembles the nectar spur found in close relatives of *Antirrhinum*, suggesting that spurs might have evolved by redeployment of the mechanisms controlling petal tube formation and that changes in *knox* gene expression can cause such redeployment.

The role of *PHAN* in repression of Class I *knox* genes is conserved with the orthologous *Arabidopsis* gene, *ASYMMETRIC LEAVES1* (*AS1*) [5]. However, the targets of repression have diverged – *AS1* is needed to repress *KNAT1*, *2* and *6*, but not *STM* in organs. Loss of *KNAT1* or *KNAT2* activity does not affect the phenotypes of *as1* mutant organs, suggesting that *AS1* has other targets. We have identified one potential target, *SYMMETRICA* (*SYM*), as a suppressor of the as1 mutant phenotype which epistasis experiments suggest might function between *AS1* and *KNOX* genes. In the SAM, *STM* is required to repress *AS1* expression. One explanation for the loss of SAM activity in *stm* mutants is that ectopic *AS1* activity represses expression of other *knox* genes which would normally act redundantly with *STM*. This view is consistent with observed changes in gene expression, *KNAT*-dependent suppression of the stm phenotype by *as1* mutations, and the ability of KNAT proteins to substitute for STM.

We have also begun to exploit *Antirrhinum* to examine the genetic basis for evolutionary differences in organ shape and size. The genus *Antirrhinum* consists of ~20 species that differ markedly in morphology but form inter-fertile hybrids. Analysis of hybrids between the large

leafed *A. majus*, and the smaller leafed alpine, *A. molle* suggest that several genes control overall leaf size or length and width independently. Having obtained a molecular map for *Antirrhinum*, we have started to map these genes as quantitative trait loci and to examine when and how they exert their effects.

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Dorsoventral patterning of the maize leaf

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The first steps in lateral organ formation include the recruitment of founder cells from the meristem and the establishment of new developmental axes relative to the main body axis. Characterization of the maize *leafbladeless1*(*lbl1*) mutant phenotype suggested that *lbl1* is required to establish adaxial cell identity in leaves and leaf-like lateral organs. In the absence of LBL1 activity, cells maintain an abaxial identity that results in the formation of radially symmetric, abaxialized leaves. The number of founder cells incorporated into such leaves is strongly reduced, suggesting that *lbl1* is directly or indirectly required for the down-regulation of *knox* genes during leaf initiation. Less severe leaf phenotypes include the formation of ectopic laminar outgrowths at the boundaries of abaxialized sectors on the adaxial leaf surface, and the bifurcation of leaves.

The semi-dominant *Rolled1-O* (*Rld1-O*) mutant of maize also affects dorsoventral patterning in that the polarity of the leaf is inverted. However, this mutation has no apparent effect on founder cell recruitment. Double mutants between *Rld1-O* and *lbl1* result in a mutual suppression of both phenotypes suggesting that *lbl1* and *rld1* act in an opposing fashion on the same pathway, or that *lbl1* and *rld1* negatively regulate each other.

In order to further characterize the *lbl1*, *Rld1* and *lbl1;Rld1* double mutant phenotypes, we have isolated several maize homologs of the *Arabidopsis Yabby* genes which are expressed in the incipient primordium and in the abaxial domain of developing leaves and floral organs. As in *Arabidopsis*, the maize *yabby* genes are expressed throughout the incipient primordium, but interestingly, expression later in development becomes restricted to the adaxial side of the leaf. Preliminary data suggest that the *yabby* expression pattern is altered in the *lbl1* and *Rld1* mutants. Together these data suggest that *lbl1*, *rld1* and the *yabby* genes play a role in adaxial/abaxial patterning in maize and that the polarity of the maize leaf may be inverted relative to the dicot leaf.

Polarities in leaf shape: polar cell elongation, central axis, and meristematic activities in leaf blades

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Focusing on mechanisms that govern the polarized growth of leaf blades in Arabidopsis, we found that two genes act independently in the polar elongation of leaf cells. The ANGUSTIFOLIA (AN) gene regulates the width of leaves and the ROTUNDIFOLIA3 (ROT3) gene regulates the length of leaves (Tsukaya et al. 1994, Tsuge et al. 1996, Kim et al. 1998, 1999).The an mutant shows abnormal arrangement of cortical MTs in leaf cells, suggesting that the AN gene regulates the polarity of cell growth by changing the arrangement of cortical microtubules. The AN gene was the first known plant member of CtBP, which acts as a transcriptional repressor in the animal kingdom (Kim et al., submitted). To understand the function of the AN gene as a transcriptional regulator of other genes, microarray analysis was carried out using the Monsanto Arabidopsis Microarray Program.

As a result, it was found that the an mutant over-expresses some genes whose expression is reversed by overexpression of the AN gene, suggesting that the AN gene represses some genes. A yeast two-hybrid system revealed that AN protein self-associates. Screening of AN-associating proteins is underway. The ROT3 gene is a member of the CYP90 cytochrome P450 family, and might be involved in the biosynthesis of a specific steroid. While mutational defects in the biosynthesis of the steroid hormone brassinosteroid decreases both the number and size of leaf cells (Nakaya et al., in press), the rot3-1 null mutation causes a defect only in the size of leaf cells (Tsuge et al., 1996). The stunted leaf shape with normal width seen in the rot3-1 mutant reflects the polar-dependent defect in leaf-cell expansion (Tsuge et al. 1996), which is reversed by overexpression of the wild-type ROT3 gene (Kim et al. 1999). An antisense construct of a homolog of the ROT3 gene in Arabidopsis causes severe dwarfism under the background of the rot3-1 null mutation; however, it has no effect under the wild-type background. Biochemical analyses of these strains are underway.Besides the above-mentioned two polarities, we found that ASYMMETRIC LEAVES2 (AS2) and BLADE-LIKE PETIOLE (BLP) are involved in positioning of the central axis and proximo-distal axis of Arabidopsis leaf blades, respectively. The as2 mutation produces abnormal patterning of vascular bundles in the leaf blades and develops leaflet-like structures that might be due to loss of the midvein (Semiarti et al., 2001). The blp mutant develops leaf-blade-like structures on the petioles and severely enhances the morphological abnormalities seen in as2. Combination of the blp mutation with overexpression of a class-I KNOX gene causes a "super-compound leaf" phenotype, whereas the blp phenotype is not suppressed by the bp (= knat1) mutation. Both as2 (Semiarti et al., 2001) and blp show ectopic expression of class-I KNOX genes in their leaf blades, suggesting a tight relationship between the meristematic status of leaf blades and the establishment of the central and proximo-distal axes in leaf blades.

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Three *Brick* genes promote the polarization of maize leaf epidermal cells

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Leaf cells have a variety of shapes related to their diverse functions. Plant cell shapes are defined by the walls surrounding them, but the cytoskeleton plays a critical role in cell shape determination by influencing the patterns in which wall materials are deposited in expanding cells. Most cells expand by means of polarized diffuse growth: expansion distributed across the cell surface that is oriented preferentially in one or more directions. Microtubules have been proposed to determine the orientation of diffuse growth by guiding the deposition pattern of cellulose microfibrils, which constrain cell expansion [1]. Some highly elongated cell such as pollen tubes and root hairs expand by means of tip growth, which is focused at a single site on the cell surface. Unlike diffuse growth, tip growth depends mainly on the actin cytoskeleton [2]. A well established role for actin in tip growth is to deliver vesicles containing cell wall materials to the growth site; additional roles for actin have been suggested, but are not well understood [2]. Most cells in both epidermal and internal tissue layers of the leaf have lobed shapes. These shapes are acquired by means of a polarized growth pattern that has been thought to be determined by the arrangement of cortical microtubules [3-6].

We have isolated mutations in three *Brick* (*Brk*) genes required for lobe formation along the margins of epidermal pavement cells [7,8]. Mutant epidermal cells expand to the same extent as wild type cells, but fail to establish polar growth sites along the cell margins from which lobes arise. *Brk* genes are not required for the lobing of mesophyll cells, but do play roles in the morphogenesis of other epidermal cell types: stomatal subsidiary cells are often formed abnormally, and epidermal hairs are shorter and blunter than in wild type cells. Analysis of the cytoskeleton in *brk* mutants showed that microtubule organization in expanding *brk* mutant pavement cells differs little from that in wild type cells. However, localized patches of cortical F-actin found in wild type cells at sites where lobes are initiating and at the tips of elongating lobes are never observed in mutant cells [7,8]. These observations suggest that pavement cell lobes in maize arise through an actin-dependent, tip growth-like polarized growth process, which fails to occur in *brk* mutants. Moreover, stomatal abnormalities in *brk1* leaves could be attributed to a polarity defect in subsidiary mother cells that is also associated with failure to form a localized patch of cortical F-actin [9]. These observations suggest roles for *Brk* genes in multiple, actin-dependent cell polarization in the developing maize leaf epidermis.

Mosaic analyses revealed that *Brk2* act cell autonomously, *Brk1* acts non cell autonomously, while *Brk3* acts cell autonomously to promote epidermal lobe formation and non autonomously to promote normal subsidiary mother cell divisions [8]. In addition, we found that *brk1;brk2*, *brk1;brk3* and *brk2;brk3* double mutants all have the same phenotype as *brk* single mutants [8]. Together, these observations lead to the conclusion that all three *Brk* genes act in a common pathway or process in which each gene has a distinct function. The non cell autonomous action of *Brk1* is consistent with the finding that this gene encodes a very small protein (8kD), which is likely to move between adjacent cells through

plasmodesmata. The BRK1 protein is unrelated to proteins or motifs of known function, but is highly conserved thoughout the plant kingdom. Moreover, a corresponding family of proteins is also highly conserved throughout the animal kingdom [7]. These findings suggests that BRK1-like proteins may function in actin-dependent aspects of cell polarization in a wide spectrum of eukaryotic organisms, and that the entire pathway or process in which all three *Brk* genes function is also conserved among eukaryotes. We speculate that the BRK1 protein may be directly involved in the regulation of localized actin polymerization.

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Session 3: Cell differentiation
Chair: R. Scott Poethig

GLK genes function in diverse species to regulate cellular differentiation in the leaf

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Chloroplast biogenesis is a complex process that requires close co-ordination between two genomes. Many of the proteins that accumulate in the chloroplast are encoded by the nuclear genome and the developmental transition from proplastid to chloroplast is regulated by nuclear genes. We have shown that a pair of *Golden 2*-like (*GLK*) genes regulate chloroplast development in maize and *Arabidopsis*. The GLK proteins are members of the GARP superfamily of transcription factors and phylogenetic analysis demonstrates that the maize, rice and *Arabidopsis GLK* gene pairs comprise a distinct group within the GARP superfamily. Further phylogenetic analysis suggests that the gene pairs arose through separate duplication events in the monocot and dicot lineages. In the C_4 plant maize, each *GLK* gene is expressed in a specific C_4 photosynthetic cell-type and loss of function of one gene perturbs the differentiation of chloroplasts in only one cell-type. In C_3 rice and *Arabidopsis*, however, *GLK* genes are expressed in partially overlapping domains in all photosynthetic tissue.

Insertion mutants demonstrate that this expression pattern reflects a degree of functional redundancy as single mutants display normal phenotypes in most photosynthetic tissues. However, double mutants are pale green in all photosynthetic tissues and chloroplasts exhibit a reduction in granal thylakoids. Products of several genes involved in light harvesting also accumulate at reduced levels in double mutant chloroplasts. *GLK* genes therefore regulate chloroplast development in diverse plant species.

The control of leaf palisade development in *Antirrhinum majus*

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We are interested in the genetic control of cellular specialisation, and the mechanisms of cellular differentiation. Although specialised form and function is adopted by the palisade layer of leaves in many species, the processes controlling this relatively late differentiation stage in leaves are little understood and there are few molecular markers that can be used to distinguish palisade from spongy mesophyll cells. There is a strong environmental influence on palisade development as well as inherent morphogenetic control that both influence the number of cell layers that make up the palisade.

The influence of two mutations on leaf palisade development in *Antirrhinum majus* will be described. One, the dag mutant, does not influence the commitment of cells to form the palisade, but does influence their ability to divide and expand to adopt their normal form. The primary effect of the *dag* mutation is on chloroplast development. Its influence on palisade cell expansion connects chloroplast development to cellular development, and establishes that a signal, that works principally on an intracellular level, links the two aspects of cell specialisation. The *DAG* gene probably influences chloroplast development through the activity of the nuclear encoded polymerase (NEP), and provides a new role for a family of plant-specific genes in regulating aspects of regulation of the transcription of plastids and specialised chloroplasts.

The second mutation, currently named 'mutant x', has narrow leaves and narrow split petals. It develops an abaxial layer of palisade in addition to the normal adaxial layer(s). The other cell layers of the leaves show normal dorsi-ventral polarity, suggesting that gene *X* is not involved in establishing the dorsi-ventral axis. The mutation is recessive suggesting that commitment to palisade development is normally under negative control. The environment, especially light quality influences the degree of extra palisade production in the mutant. Double (abaxial and adaxial) palisade layers are found in some plants and may be adaptations to dry conditions. Progress towards molecular identification of locus 'X' will be described.

The role of cell cycle regulation during leaf development

Gerrit Beemster

After initiation a leaf goes through three distinct phases of development: fully meristematic, transition phase when division stops in a tip to base gradient and an expansion phase when cells expandind but no longer divide. The final size of the leaf depends on duration of the meristematic phase, the rate of cell division and the size that cells reach at maturity. The first two of these parameters are cell cycle regulated. Mature cell size has been postulated to be dependent on the ploidy levels of cells which in *A. thaliana* is determined by the number of endoreduplication cycles, and thus also possibly cell cycle regulated. We use a kinematic analysis of the abaxial epidermis of the first leaf pair to address the relationship between the molecular regulation of the cell cycle and these growth parameters. The results show that in wild type plants average cell division rates over the whole of the leaf are approximately constant till day 9, whereafter they decline over a 4 day period. At the same time that cell division is reducing we observe a gradual increase in the ploidy levels of cells indicating the onset of endoreduplication. Promotor-Gus expression of a number of cell cycle genes showed a striking correlation with the calculated cell division activity, with the exception of CDKA, which expressed at lower levels over a longer period, suggesting involvement with endoreduplication. Overexpression of various cell cycle genes differentially affects individual growth parameters, suggesting that they are under control of independent regulatory pathways.

Uncoupling the cell cycle from cell differentiation in developing *Arabidopsis* and tobacco leaf epidermis

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During plant organogenesis cell growth and terminal cell differentiation are coupled with the cell cycle switch*. Important cell cycle switches include withdrawal from the mitotic cell cycle and entry into the endoreduplication cycle, where the genome is duplicated in the absence of cell division**. The coupling of cell differentiation with the cell cycle switch is easily observed in the developing leaf epidermis, where protodermal cells differentiate into functionally and structurally distinct cells, such as hair cells (or trichome) and guard cells***. However, the significance of this coupling in proper cell differentiation or epidermal pattern formation has not been examined. I have generated transgenic Arabidopsis and tobacco plants in which the activities of cell cycle regulators involved in the cell cycle switches were altered in order to determine the effects of the misrepresented cell cycle switches on cell differentiation and pattern formation of the transgenic leaf epidermis. Continued mitosis and prevention of the entry into endoreduplication were achieved by the altered expression of D-type cyclins, which trigger the G1-S transition and are essential for the re-accumulation of mitotic cyclins. Altered expression of particular D-type cyclins caused all epidermal cells in transgenic plants became more compartmentalized than normal, resulting in the leaves composed with an enormous number of small cells. However, cell differentiation, cell morphogenesis, and primary epidermal patterning proceeded normally.

For example, the Arabidopsis unicellular trichome, which normally grows by endoreduplication, became a multicellular structure divided into as many as 16 cells, yet its basic tri-branched morphology was maintained. This phenotype indicates that cell growth and morphogenesis accompanied by progression of endoreduplication can be substituted by continued cell proliferation, and thus, the entry into endoreduplication is not essential for cell morphogenesis of the Arabidopsis trichomes.

To attain precocious withdrawal from cell divisions and induce the onset of endoreduplication in developing leaf epidermis, I created transgenic plants in which overall mitotic cyclin activity is down regulated. In these transgenic plants, despite significant decreases in cell numbers and increase in cellular volume of the mature leaf epidermis, cell differentiation occurred nearly normally. For instance, normal multicellular tobacco hairs were transformed into high polyploid, unicellular hairs without affecting their occasional branching patterns. Furthermore, fully differentiated guard cells with high ploidy nuclei, instead of normal diploid nuclei, were observed. These cellular patterns clearly indicate that cell growth and morphogenesis by mitotic cell cycle can be substituted by endocycles without affecting cell differentiation.

The above observations together demonstrate that patterned cell cycle transition can be uncoupled from determination and progression of cell differentiation in leaf epidermis. I would further discuss the effects of altered cell cycle switches on leaf epidermal cell patterning.

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Cell cycling and cell enlargement in leaf histogenesis

Nancy G. Dengler

Almost every aspect of development in plants, including organ morphogenesis and tissue histogenesis, involves cell division. Despite this consistent coupling of cell cycling and development, the precise role of cell cycling in these processes is not well-understood (Smith, 1996; Doonan, 2000). Results of both experimental manipulations and analyses of mutant phenotypes support the idea that morphogensis can accommodate considerable perturbation of cell cycling. Typically, expansion compensates for reduced rates of cell cycling and/or defective planes of cytokinesis, often resulting in smaller organs, but ones with normal shape and proper tissue organization (Doonan, 2000).

Histogenesis, the development of cells and tissues from undifferentiated precursors, is predicted to be less tolerant of perturbation (Smith, 1996). The balance between whole tissue expansion and division into individual cells that produces cell units of correct size for mature function is likely to be under more stringent constraints. Currently little is known about such constraints or even the specific roles of cell cycling in histogenesis. Characterization of spatial and temporal patterns of cell cycling during histogenesis should provide a more robust basis for interpretation of experimental results and mutant phenotypes (Huntley and Murray, 1999).

During normal development, cell cycling contributes to histogenesis in at least three ways. First, precursors of the three primary meristematic tissues are delimited by distinctive planes of cell division. Second, histogenesis involves differential patterns of cell proliferation in each of the primary meristematic tissues. Third, cell differentiation often requires precise regulation of the time, place and orientation of cell divisions. Our approach has been to use a B cyclin-GUS reporter construct to characterize spatial and temporal patterns of cell cycling during leaf development in Arabidopsis (Donnelly et al., 1999). Superimposed on a general longitudinal gradient of cell cycling frequency, tissue layers differ in the pattern of proliferative divisions. For example, cell cycling of palisade mesophyll precursors is prolonged in comparison to that of pavement cells of the adjacent epidermal layers, and cells exit the cycle at different characteristic sizes. Cell divisions directly related to formation of stomates and vascular tissue from their respective precursors occur throughout the period of leaf extension and reflect superposition of cycling related to cell differentiation on more general tissue proliferation.

Development of leaf venation is still one of the least understood histogenetic processes. While protoderm and ground meristem of leaf primordia are derived from surface and subsurface layers of the shoot apical meristem respectively, procambium is formed *de novo* from ground meristem precursors within the leaf primordium (Nelson and Dengler, 1997; Dengler and Kang, 2001). Experimental manipulations using auxin application and auxin transport inhibitors, as well as characterization of mutant phenotypes, all indicate that leaf vein pattern is formed in response to a canalized auxin signal (reviewed in Berleth et al., 2000). How such a signal is translated into the distinctive cytological properties of procambial cells is currently unknown. In a recent study we have followed early events in procambial development using the same cyclin reporter construct (Kang and Dengler, unpublished). We have shown quantitative differences in cell cycling frequency among the hierarchical elements of the leaf venation system (primary, secondary, and tertiary veins) and striking differences in

the duration of cell divisions among these vein classes during leaf expansion. We used a GUS reporter construct for the HD-ZIPIII homeobox gene *ATHB-8* as a molecular marker of early procambial development (Baima et al., 2001) and found an almost exact correlation in spatial and temporal pattern of cell cycling and *ATHB-8* expression in veins of developing Arabidopsis leaves. The striking co-occurrence of expression patterns suggest that one role played by *ATHB-8* might be to maintain procambium-specific patterns of cell cycling during leaf histogenesis.

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Cell differentiation and patterning: Trichomes in *Arabidopsis* as a model system

Martin Hülskamp

The unicellular Arabidopsis trichomes are used as a model system to study how single epidermal cells are selected to adopt a different cell fate (patterning), how in the course of their differentiation the cell cycle is modified to endoreduplications cycles and how the cells establish their highly regular three-dimensional form (Hülskamp et al., 1994). The recent cloning of the TRIPTYCHON gene, which is involved as a negative regulator in trichome patterning, revealed that it encodes a MYB-related transcription factor. We further show that TRIPTYCHON acts redundantly with its homolog CAPRICE in root hair patterning. This suggests that the establishment of root hair and non-root hair files involves cellular interactions between epidermal cells rather than becoming simply determined by the underlying cortex cells. In order to study the regulation of the switch from mitosis to endoreduplication we have generated transgenic lines expressing known cell cycle genes in a trichome-specific manner. We show that the expression of specific B-type and D-type cyclins suppresses endoreduplication(Schnittger et al., in press). As a consequence trichomes are multicellular. Cell morphogenesis is studied using a class of mutants with fewer or more branches. The cloning of several branching genes is reported and discussed.

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Session 4: Pattern formation
Chair: José Luis Micol

Genetic regulation of vascular tissue patterning in leaves

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The formation of the vascular pattern in leaves has developmental consequences beyond the siting of veins. The regular positions of photosynthetic cells, stomata, internal air spaces, and supportive sclerenchyma and collenchyma relative to leaf veins suggests that they are subject to a common patterning system. Provascular (PV) cells and procambial cell files are generally the earliest visible markers of anatomical organization among ground cells in leaf primordia. The properties of PV cells are poorly understood, but their early appearance makes it plausible that they influence the differentiation of surrounding cells in patterns that assure the correct relationship to veins. Misexpression of Knl-class homeodomain genes causes a range of morphological aberrations at major leaf veins, suggesting that leaf PV sites may have properties in common with meristematic and organogenic sites. The steps from PV to differentiated vascular cells occur progressively along the forming vein path. How do PV cells form in continuous paths and how do they interact with neighboring non-PV cells?

We are using several approaches to identify the genes and mechanisms that influence the vascular pattern and cells complementary to it. First, we have recovered numerous *Arabidopsis* mutants with aberrant vascular patterns in cotyledons and/or leaves. Those characterized thus far correspond to genes for sterol pathways, auxin response, and receptor kinases. Second, we have identified genes expressed specifically or predominantly in PV cells. One such gene, VH1, encodes a PV-specific receptor kinase that resembles CLV1 and BRI1, including an extracellular LRR domain and an intracellular ser/thr kinase domain. Misexpression of VH1 causes an aberrant venation pattern and influences the differentiation of many leaf cell types; knockout causes premature senescence.

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Early genes in vascular development

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Hypothetical mechanisms underlying vascular pattern formation have to account for a number of conspicuous features of vascular differentiation (1, 2, 3, 4): a) sites of vascular differentiation are not rigidly specified, but adaptive to abnormal growth conditions. b) in a diversity of branching patterns, zones of vascular differentiation are always restricted to narrow, continuous strips of cells. c) vascular cells differentiate along a common axis and in reproducible spatial relationship to each other and to non-vascular cells. d) local auxin application can induce the formation of vascular strands (5). e) mutations in auxin signal transduction genes interfere with vascular differentiation (2, 6). f) leaf venation patterns are influenced by the auxin transport properties of the early leaf primordium (2, 7). Together, these observations implicate directional auxin signals in vascular patterning and suggest that genes in auxin signal transduction and auxin transport have central roles in the control of patterned vascular differentiation.

The pattern of vascular strands in developing organs is dependent on the auxin transport properties of the organ primordium (6). In leaves, critical periods can be defined, in which auxin transport inhibition results in alternative vein patterns. The characteristics of the observed pattern changes suggest vascular differentiation along preferred routes of auxin flow and the presence of auxin sources near the margins of early leaf primordia. This hypothetical auxin distribution profile is supported by the expression patterns of 'auxin response' reporter genes. Their expression patterns indicate that vascular differentiation is preceded and, under various experimental conditions, correlated to local auxin accumulation. The 'auxin response' transcription factor MONOPTEROS (MP) is critically required for promoting vascular differentiation as well as in other instances of cell differentiation aligned with the axis of auxin flow (7, 8).

Nevertheless, there is residual vascular differentiation in presumed mp null-mutants. Double mutant analyses show that the MP related transcription factor NPH4 contributes to vascular strand formation in the background of reduced MP gene activity and identify negative regulators of vascular differentiation among AUX/IAA gene products. Expression studies identify potential target genes of MP transcriptional regulation, whose expression is correlated to auxin induction as well as to MP gene dosage. These include members of the At PIN family of presumed auxin efflux carriers and early vascular transcription factors of the HD-ZIP family. These regulatory relationships could establish the link between a local, vascular-differentiation promoting influence of auxin and the regulatory circuitry controlling the actual differentiation events within vascular stands. To identify regulators of vascular development irrespective of their involvement in auxin signaling, we have used differential expression profiling in wild type versus mp mutant leaves as well as enhancer trap insertion. These strategies identify known landmark genes in vascular differentiation as well as large numbers of presently uncharacterized genes.

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Genetic analysis of stomatal density and distribution in *Arabidopsis*

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Stomata are non-randomly distributed, and their density is controlled by endogenous and environmental factors. The presence of a stomata-free region surrounding each stoma is the major and universal principle of order in the established stomatal pattern (Sachs, 1991). To gain insight into the molecular mechanisms regulating stomatal distribution, *Arabidopsis thaliana* mutants with altered stomatal characteristics were isolated and examined (Berger and Altmann, 2000). The *sdd1-1* mutant exhibits a two- to fourfold fold increase of stomatal density and the formation of clustered stomata (i.e., stomata that are not separated by intervening pavement cells), whereas the internal leaf architecture is not altered. Analysis of the developing leaf epidermis revealed a role of the *SDD1* gene in the regulation of three processes:
(1) The determination of the fraction of protodermal cells forming stomatal initials;
(2) The degree of higher order stomatal complex formation through control of meristemoid formation frequency by cells neighbouring primary guard cells (neighbouring cells) through asymmetric cell divisions;
(3) The control of cell division orientation in higher order meristemoids.

The defect in the latter process caused the observed defects in stomatal patterning in the *sdd1-1* mutant, which in wildtype *Arabidopsis thaliana*, as in many other species, is established through control of the number and orientation of the cell divisions occurring during stomatal complex formation. Of the Arabidopsis *too many mouths* (*tmm*), and *four lips* (*flp*) mutants, which are also affected in stomatal pattern formation (Yang and Sack, 1995), *tmm* exhibits changes in the orientation of asymmetric divisions and the frequency with which neighbouring cells undergo asymmetric divisions that produce meristemoids similar to those of *sdd1-1* (Geißler et al., 2000). The *SDD1* gene was identified by map based cloning (Berger and Altmann, 2000). It encodes a subtilisin-like serine protease related to prokaryotic and eukaryotic proteins. Expression analysis through mRNA *in situ* hybridization and promoter-GUS fusions revealed activity of the *SDD1* gene predominantly in meristemoids / guard cell mother cells. SDD1-GFP fusions expressed in transgenic Arabidopsis plants accumulated at the extracellular surface associated to the plasma membrane. In analogy to the function of other eukaryotic subtilases, SDD1 is proposed to act as a processing protease involved in the creation of an extracellular signal emanating from meristemoids / guard cell mother cells that controls the development of cell lineages forming stomatal complexes. Overexpression of *SDD1* under the control of the CaMV35S promoter caused a two- to threefold reduction of stomatal density (probably due to repression of higher order stomatal complex formation) and premature arrest of stomatal precursor cells. The observed epistatic relationship between the *p35S-SDD1* gene and the *tmm* mutation revealed the action of SDD1 and TMM in a common signal transduction pathway.

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Light regulates stomatal development and pattern in *Arabidopsis*

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Photomorphogenesis is one of the most striking examples of how light shapes plant development. Stomata formation is also influenced by light, but very little is known on the links between photomorphogenesis and stomatal differentiation and pattern. We have studied the role of light on stomatal initiation and pattern establishment by taking advantage of the genetic tools developed for the study of photomorphogenesis. By applying cell lineage analysis to constitutive photomorphogenesis mutants, we have determined that light controls stomata initiation, and that genes involved in the repression of photomorphogenesis play a crucial role on stomatal pattern establishment in a light-independent manner. Remarkably, the developmental processes altered in the pleiotropic cop/det/fus mutants are almost identical to those described for mutants specifically altered in stomatal pattern.

Session 5: Other aspects of leaf development
Chair: Timothy Nelson

Genetic regulation of vegetative phase change in *Arabidopsis* and maize

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Flowering plants begin their post-embryonic development in a juvenile phase. This phase is characterized by a variety of distinctive vegetative traits and by the inability of the shoot to respond to floral inductive stimuli. Several genes required for the expression of the juvenile phase have been identified in maize and Arabidopsis by screening for mutations that cause the precocious expression of adult traits. Some of these mutations also affect the length of adult phase, whereas others primarily affect the duration of the juvenile vegetative phase. We have focused our efforts on this latter class of mutations.

Mutations of the *HASTY* (*HST*) gene in Arabidopsis have a pleiotropic phenotype that includes the precocious expression of adult vegetative traits and an increased sensitivity to the floral inductive effects of *35S:: LEAFY* (Telfer and Poethig 1998). Mutations of the *early phase change* gene in maize have essentially the same phenotype as *hst* mutations (Vega et al, 2002). *HASTY* encodes a protein that resembles members of the importin β family of nucleocytoplasmic transport receptors. Consistent with this structural similarity, HST is localized to the periphery of nuclear envelope and interacts with RAN, a small GTPase that plays a central role in nucleocytoplasmic transport. The maize orthologue of *HST* is *early phase change*. The genomic structure of *early phase change* (*ZmHST*) is unusual in that it is 60 kb in size and possesses several large introns with retrotransposon insertions. The closest relative of *HST* in yeast is *MSN5*, a gene that has been shown to be involved in the import and export of proteins involved in a variety of unrelated processes. In view of its pleiotropic mutant phenotype it is likely that HST also transports several different proteins. Candidate HST cargoes have been identified in yeast two hybrid screens and T-DNA insertions in several of these genes are being characterized.

Mutations of the *SQUINT* (*SQN*)gene in Arabidopsis interact synergistically with *hst* mutations, suggesting that these genes operate in parallel pathways. *SQN* is the Arabidopsis orthologue of Cyclophilin-40 (CyP40), a component of the HSP90 chaperone complex (Berardini, Bollman et al. 2001). In animals and yeast, this complex regulates the activity of several signal transduction pathways by keeping key factors in these pathways (e.g. steroid hormone receptors) in an inactive configuration under non-inductive conditions (Pratt 1998). Yeast two hybrid screens produced only one protein that interacts strongly with *SQN*. This protein contains a kinesin domain, supporting the hypothesis (Pratt, Silverstein et al. 1999) that cyp40/SQN mediates the interaction of the HSP90 complex with the cytoskeleton, and thereby facilitates the movement of this complex to the nucleus. Along with the observation that *HST* encodes a factor involved in nucleocytoplasmic transport, this result suggests that protein localization plays a major role in the regulation of vegetative phase change.

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Genetic control of leaf growth in *Arabidopsis*

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Leaf formation is a complex process in which pattern formation, growth and differentiation are overlapping rather than consecutive events. In dicotyledonous plants, including *Arabidopsis*, leaves are initiated post-germination at specific positions of the shoot apical meristem. The leaf primordia grow along three newly-formed axes into a leaf organ of a specific length, width and thickness. Growth is being determined by the number of cell divisions, the orientation of cell plates and the extent and polarity of cell expansion. Early growth processes interconnect cell division processes with dorsiventral pattern formation leading to dorsal and ventral identity of the leaf blade and lateral outgrowth of the lamina (Siegried et al., 1999; McConnell et al., 2001; Eshed et al., 2001). Later in organ formation cell expansion processes are predominate as shown by mutational analysis (Tsuge et al., 1996; Kim et al., 1998). Pattern formation in lateral growth results in the distinction between lamina and petiole (van der Graaff et al., 2000). Restriction of growth determines the final shape and size of the leaf organ (De Veylder et al., 2001).

The genetic control mechanisms of growth are poorly understood. We study the genetic control of leaf growth by a mutational approach in *Arabidopsis*. Leaf mutants affected in growth along one or more of the axes are retained for gene cloning and phenotypic analyses. The mutants have been obtained from our own *Ds* tagging program or from an EMS mutagenized collection (Berna et al., 1999). Progress in the research on the tagged *drl1-2* mutant and the respective *DRL1* gene with a role in cell number control during lateral growth will be presented. A standardized method for map-based cloning has been developed based on a dense AFLP map (Peters et al., 2001), InDel and SNP markers. It is being applied to several EMS leaf growth mutants for gene cloning and a progress report will be presented. In addition an overview will be given of the growth defects in these mutants using a standardized method of imaging/ DIC optics and serial sectioning. The combined knowledge of gene sequence and mutant analysis will allow classifying the type of growth defect in each mutant: a molecular mechanism will be related to a cellular/morphological process. The *ron2* mutant and the *RON2* gene will be discussed in this respect. In addition, the genetic relationships between the novel gene function and other gene functions in leaf growth and development are being determined in several ways and will be exemplified for DRL1.

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ASYMMETRIC LEAVES1 reveals *knox* homeobox gene redundancy in *Arabidopsis*

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The shoot apical meristem comprises undifferentiated stem cells and their derivatives, which include founder cells for lateral organs such as leaves. Meristem maintenance and lateral organ specification are regulated in part by negative interactions between the myb domain transcription factor ASYMMETRIC LEAVES1, which is expressed in lateral organ primordia, and homeobox transcription factors (knox genes) which are expressed in the shoot apical meristem (1,2,3,4). The knox gene SHOOT MERISTEMLESS negatively regulates ASYMMETRIC LEAVES1 which, in turn, negatively regulates other knox genes including KNAT1 and KNAT2. Genetic interactions with a second gene, ASYMMETRIC LEAVES2, indicate it acts at the same position in this hierarchy as ASYMMETRIC LEAVES1. We have used a genetic approach to isolate mutations in KNAT1 and show that KNAT1 is partially redundant with SHOOT MERISTEMLESS in regulating stem cell function. Mutations in KNAT2 show no such interaction. Our studies provide a molecular framework for interactions between lateral organ development and meristems.

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Pea leaf development: old versus new

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Pea (*Pisum sativum L.*) leaves are compound as opposed to simple. Compound leaves are relatively common, and dispersed among Angiosperm orders, which suggests they evolved several times independently. This is supported by the identification of different fossilised compound leaves relatively late in the Cretaceous period when Angiosperms with simple leaves were already numerous1 It is thought that megaphylls, the large compound leaves of pre-Angiosperms, also originated several times independently, and it is apparent that a large, flat, pinnate megaphyll was the basic leaf type in the lineage leading to Angiosperms2. Thus compound leaves are either new "inventions" in the Angiosperms, by novel recruitment of a developmental pathway, or, they originated with the reinstatement of an ancient, pre-Angiosperm compound leaf developmental pathway. We have identified two genes involved in patterning the pea compound leaf. These are UNIFOLIATA3 and STAMINA PISTILLOIDA4, orthologues of the *Arabidopsis* floral meristem identity genes LEAFY and UNUSUAL FLORAL ORGANS. uni and stp mutants exhibit floral defects similar to the corresponding mutants, lfy and ufo in *Arabidopsis*, as well as leaf patterning defects. The identification of these genes suggests, either that parts of the floral developmental pathway have been co-opted to pea compound leaf patterning, or, that these floral developmental genes have roles that predate flowering plants. We have recently characterised a lfy mutant of *Medicago sativa*, thus, the role of LFY in compound leaf development is not limited to pea, and may apply more widely to legumes. The comparatively reduced role of LFY in tomato compound leaf patterning5 supports the hypothesis of multiple independent origins of compound leaves in Angiosperms. The identification of additional patterning genes will help us to determine whether legume leaves, and pea leaves in particular, are a novelty or an atavism.

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The development and evolution of compound leaves

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The Class I Knotted-like homeobox (KNOX 1) genes are highly expressed in the shoot apical meristem but not expressed in the emerging leaf primordium in tobacco, maize, or Arabidopsis. In tomato, KNOX1 expression (LeT6, TKN1) is seen in the early leaf primordium (Chen et al. 1997; Hareven et al. 1996). It is worth noting that tomato has compound leaves while the other organisms thus far tested have simple leaves. We have suggested that this early expression of KNOX 1 genes in the leaf primordium causes it to take on a compound fate in tomato (Sinha 1997). In order to thoroughly test this hypothesis we have completed an analyses at several different phylogenetic levels.

We have mapped the trait of compound leaves on the green plant evolutionary tree to identify genera in which compound leaves arose independently. This tree includes cycads, the monocot Aroids and Palms, and multiple independent origins in the dicot families. We have analyzed compound leaf producing shoot apices in all these clades except the monocots and found that in all instances except one (a derived clade in the Fabaceae) compound leaves always show expression of KNOX genes. In the derived pea clade the LFY/FLO gene regulates this function of generating leaf complexity.

Tomato has a large number of mutations that either increase or decrease the degree of compounding in the leaf. Mutations such as *w-3* and *w-6*, that severely disrupt any blade formation, also lead to radial leaflet-less structures, indicating that some blade and margin development in the leaf primordium is a prerequisite for leaflet formation. Mutations like *sf* and *c* that disrupt only margin differentiation do not disrupt the basic compound nature of the tomato leaf (Kessler et al. 2001). While *wiry6* has reduced expression of the MYB gene PHANTASTICA, this does not alter LeT6 expression as suggested in maize and Arabidopsis (Byrne et al. 2000; Timmermans et al. 1999; Tsiantis et al. 1999). Rather, TKN1, a different KNOX gene is misregulated. In plants overexpressing LeT6, PHAN levels are downregulated. Expression patterns of KNOX and PHAN in tomato are different from those seen in maize and Arabidopsis (Byrne et al. 2000; Timmermans et al. 1999; Tsiantis et al. 1999). While KNOX genes appear to be important for generating leaf complexity (except in a derived clade in the Fabaceae) we find that other genes like PHANTASTICA might play a role in determining the form of the compound leaf generated. Transgenic plants overexpressing antisense PHAN suggest that PHAN, by modulating dorsiventrality, has a role in regulating the number of leaflets and their placement in a compound leaf.

In addition to known genes like KNOX and PHAN, there must be role for as yet undiscovered genes in the generation of compound leaves. The genus *Lepidium* (Brassicaceae) has species that show great variation in leaf complexity. Two species are being examined: *L. hyssopifolium*, which has complex leaves; *L. oleraceum*, which has slightly lobed leaves. Leaf primordia in both these species show KNOX expression and

developmental analyses indicate that the primordium in *L. oleraceum* begins as a complex primordium but loses its complexity by secondary morphogenesis. In interspecific crosses the complex leaf phenotype segregates in $1/16^{th}$ of the population. We have initiated mapping of the two loci by using Arabidopsis RFLP markers.

In *N. aquatica* leaves with different morphologies are produced depending on environmental conditions. Simple leaves are produced under high-light terrestrial conditions while lobed/ compound or highly dissected leaves are produced under low-light terrestrial and underwater conditions, respectively. Experiments in our lab show that GA can induce the production of simple leaves on plants exposed to conditions that normally induce compound leaves (and Unaconalzole can lead to an opposite effect. With these experiments we hope to understand a basic problem in plant biology - why some derivatives from the shoot apical meristem are simple, while others can be compound, and how these alternate morphologies may have arisen in evolutionary time.

POSTERS

Phloem loading and the regulation of gene expression in minor veins

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Different vein classes in a leaf occupy distinct physiological niches. In growing leaves, nutrients are imported by, and unloaded from, the phloem of larger veins, while the smaller (minor) veins are still immature. As the leaf passes through the sink-to-source transition, the minor-vein phloem matures and begins loading photoassimilate from the mesophyll in preparation for export. The predominant role of minor veins in phloem loading is reflected in their anatomy. Recently, we established that a distinct genetic program operates in minor veins by demonstrating that a galactinol synthase promoter isolated from *Cucumis melo* is active exclusively in the companion cells of these veins (1). Galactinol synthase catalyzes the first committed step in the synthesis of the raffinose family of oligosaccharides, and is required in the intermediary cells (specialized companion cells) of species that phloem load symplastically via a polymer trapping mechanism. However, the galactinol synthase promoter also confers a highly specific expression pattern to companion cells that load sucrose from the apoplast, even though these plants do not synthesize detectable amounts of galactinol. This implies that the genetic program governing minor-vein expression is highly conserved among higher plants, irrespective of the actual loading mechanism. We are probing this unique genetic program to appreciate more fully the development of minor veins, minor vein function in phloem loading, and the translocation of nutrients and signaling molecules. We have identified elements within the galactinol synthase promoter responsible for minor vein expression by functional analysis in a heterologous host plant and by identifying conserved elements within the promoter by a cladistic approach. Currently we are using these elements to identify interacting protein factors, and other genes that are coordinately activated by this regulatory pathway.

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Ventral fate in lateral organs

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Asymmetry is a fundamental aspect of developmental biology. Early zygotes of many organisms approximate in shape to highly symmetrical spheres. Most begin to diverge in shape early in development, as new axes of asymmetry are specified and elaborated. Many of these axes must be co-ordinated to ensure the correct development of new structures in three dimensions. In plants, lateral organs, such as leaves and petals, initiate from meristems elaborating a proximodistal axis with a distinct pattern of asymmetry (petiole proximal and blade distal), and a dorsobentral axis that becomes apparent in the differences between adaxial and abaxial tissues. To explain how lateral growth of the leaf occurs in a plane perpendicular to the stem axis, it has been proposed that the interaction of dorsal and ventral cells is responsible for specifying a novel lateral axis of growth (Waites and Hudson, 1995). Candidate genes have been recently identified for both dorsal and ventral determinants. Despite this, almost nothing is known about how these genes interact, specify cell fate and promote lateral growth. We will investigate this using cloned genes required for dorsal and ventral identity, and mutants lacking dorsal or ventral cell fates. We will test genetic and molecular interactions exploiting petals as a model for lateral organs. To expand our studies we will initiate a search to identify further genes required for organ asymmetry.

Floral development of the model legume *Medicago truncatula* : ontogeny studies to characterize homeotic mutations

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The ontogeny of *Medicago truncatula* flowers, like in many legume plant species, proceeds through a very different sequence of events from the same process in other model plant systems. Using scanning electron microscopy analysis, we have characterized the early developmental events of the wild type *Medicago* flower and selected morphological characters as markers to break it down into different developmental stages. The more remarkable features of the *Medicago* flower ontogeny were the abaxial-adaxial unidirectional initiation of organ primordia within each different floral whorl in contrast to the centripetal and sequential floral ontogeny in other plants species like *Antirrhinum* or *Arabidopsis*, the existence of four common primordia to petals and stamens and the early carpel primordium initiation. Organ differentiation within each of these common primordia appears to be a complex process that plays a central role in the ontogeny of many legume flowers. We also used these markers as tools to characterize early alterations in the flower development of a male-sterile *Medicago truncatula* floral homeotic mutant, the *mtapetala* (Penmetsa and Cook, 2000). This mutant displays a phenotype resembling those of class B mutations with homeotic conversions of floral organ whorls 2 and 3 into sepaloid and carpelloid structures respectively. Ontogeny studies of the *mtapetala* mutant showed similarities with the *stamina pistilloida* mutation previously described in pea (Ferrándiz *et al.*, 1999). Such a study could be very useful in the future to detect early defects in the pattern of floral development of other *Medicago* homeotic mutants, facilitating their subsequent characterization. The establishment of relationships with other related mutations described in different plant species will allow to elucidate how the B function works in legumes.

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The HVE gene is required for venation pattern formation

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We have undertaken the genetic analysis of venation pattern formation in the vegetative leaves of *Arabidopsis thaliana* as a model to study how plant vascular cells determine their position and differentiate accordingly. After examining the vasculature of 97 leaf shape mutants, each mutation affecting a different gene, we found that rotunda1 (ron1) and apiculata7 (api7) displayed abnormal venation patterns. The additive phenotypes of double mutants involving alleles of the HEMIVENATA (HVE) gene, which was first identified as a naturally-occurring variant responsible for an extremely simple vascular pattern, and other venation pattern genes, such as RON1, indicate that they act in independent pathways. In support of this notion, we have found that Hve plants are sensitive to auxins and auxin transport inhibitors, suggesting that HVE is not involved in auxin perception or transport. The fact that Hve plants display altered root waving points to a possible role of the corresponding gene in the metabolism of tryptophan and/or auxin.

Isolation and characterization of homologs of higher plant KNOX genes from the moss *Physcomitrella* patens

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Homeobox genes, which have been discovered in a range of eukaryotic species, determine cell fate by acting as transcriptional regulators of collections of developmental genes. KNOTTED1-LIKE homeobox (KNOX) genes can be subdivided into two classes based on sequence similarities and expression patterns (1, 2). Higher plant Class 1 KNOX genes appear regulate the formation or maintenance of meristematic tissue, while the function of Class 2 genes remains to be determined (3, 4, 5, 6). It has been postulated that factors such as the change in expression of KNOX genes have played a role in the evolution of plant leaf morphology and that Class 1 KNOX genes may have had a role in the acquisition of leaves in the earliest vascular land plants (7). Three independent genes named MKN1-3, MKN2 and MKN4 were cloned from a P. patens genomic library. Phylogenetic analysis indicates that MKN2 and MKN4 are Class 1 genes, and MKN1-3 is a Class 2 gene. Preliminary data suggests that the expression patterns of MKN1-3 and MKN4 are similar. Expression of both genes was observed in some, but not all, chloronemal apical and sub-apical cells. Often, cells that were stained were not stained homogenously. Using directed gene knockout, the function of MKN4 has been disrupted. MKN4 knockout phenotypes will be described. Deducing the function of moss KNOX genes may illuminate how the transition to three dimensional growth, characteristic of the Embryophytes, occurred.

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Tornado loci and leaf development: where does auxin comes in?

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The *TORNADO1* (*TRN1*) and *TRN2* genes are required for the radial and circumferential pattern in the *Arabidopsis* root (Cnops et al. 2000). Here we want to present our data on the anatomy of *trn1* and *trn2* leaves and we want to discuss the possible involvement of altered auxin concentration and auxin transport on the *trn* shoot phenotype. The analysis of the molecular marker line pDR5-GUS in a *trn1-1* and *trn2-1* background will be discussed in detail. Furthermore, auxin transport in *trn1-1* stems will be compared to the transport in wild type stems.

In order to elucidate the role of *TRN1* and *TRN2* in as well the root as the shoot we started a map based cloning approach to isolate the *TRN* genes. Both loci map to the bottom half of chromosome 5. Around 150 F2 mutants (derived from a cross of *trn2* (Col) with Ler) were used to map *TRN2* to a region of 250kb using AFLP (Peters et al. 2001) and INDEL (http://www.arabidopsis.org/Cereon/index.html) markers. An F2 population of approximately 2000 plants was screened with flanking INDEL markers for recombinants in the 250kb region. These recombinants were used to fine-map the *TRN2* region into an area of less than 30kb using INDEL and SNP markers. The latest data of the map based cloning of *TRN2* will be presented.

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The evolution of lateral organ identity in land plants

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Leaves can be classified into two types: microphylls, which have a single vascular strand; and megaphylls, which have a more complex, branching vasculature. Microphyllous leaves are typical of the Lycophytes, while megaphyllous leaves are typical of all other vascular plants (including monocots and dicots). It is thought that the two leaf types evolved independently, based on phylogenetic positioning of certain leafless fossils.

Studies in model dicot and monocot species have shown that a group of genes known as the *ARP* (**a***symmetric1/* **r***s2/***p***hantastica*) genes have a role in the development of at least some megaphyllous leaves. *ARP* genes from various species have similar expression patterns and the proteins that they encode show significant sequence similarity. They also have similar functions, i.e. they all repress ectopic *knox* gene expression in leaves.

The interaction between *knox* and *ARP* genes seems to have arisen before the divergence of monocots and dicots. The aim of this work is to study the evolution of this interaction. As it is required for normal leaf development (at least in angiosperms), it seems logical to study the evolution of the *knox/ARP* interaction in the context of leaf evolution. Therefore the Lycophytes represented a phylogenetically interesting point at which to start looking for *ARP* genes. So far, *ARP* genes have been isolated from two *Selaginella* species. These and other results will be presented.

Characterization of a strawberry GAST-like gene

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A gene (FaGAST) was identified in strawberry. The deduced 106 amino acid sequence of FaGAST shares high similarity with previously characterised putative cell wall proteins of unknown function. These proteins are encoded by gibberellin inducible genes, namely GAST1 (for GA stimulated transcript) from tomato, GIP (for GA induced gene) in petunia, GASA (for GA-stimulated) in Arabidopsis, and the auxin inducible gene RSI-1 (for Root System Inducible-1) from tomato. The corresponding peptides display similar structural features: a putative signal peptide in the N-terminal domain, a highly divergent central region and a conservative 60 amino acid C-terminal domain containing 12 cysteine residues which defines a pattern not related to other known cysteine-rich motifs.

Northern blot analyses revealed an increased expression level of FaGAST transcripts during the ripening of strawberry fruit. Over-expression of FaGAST in Fragaria vesca produced dwarfism. This phenotype correlated with the expression level of the transgene. Similarly, ectopic expression of FaGAST in Arabidopsis thaliana produced small leaf size during development.

Our studies suggest that FaGAST plays an important role in GA-linked cell wall development.

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The roles of D-type cyclins in leaf growth and development

Walter Dewitte and Jim AH Murray

The generation of cells, differentiation and specialization are essential for organ formation. A primary event in the stimulation of cell division is the activation of cyclin D transcription by external signals, including sugars and hormones. Upon association with its CDK partner, D-type cyclins form active complexes that target phosphorylation of the Rb protein. During G1 phase, Rb is bound to a group of key transcription factors called E2F, and in doing so inactivate genes that are under E2F control. These include genes for cell growth, cell cycle progression and DNA replication. The phosphorylation of Rb in late G1 causes it to lose its association with E2F, driving cells into DNA replication and committing them to the cell cycle. Experimental data for the role of D-type cyclins in leaf growth and development will be presented.

Homeobox genes and leaf development: lessons from KNAT1, STM and WUS

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Leaf development is the result of a fine equilibrium between areas of the shoot apical meristem (SAM) where cells are undifferentiated and pluripotent (central meristem) and peripheral areas where cells become competent to form organ primordia.

Some genes, encoding proteins that belong to the knox (knotted-like) homeobox family of transcription regulators, play an important role in the formation and maintenance of shoot apical meristem (Barton and Poethig 1993, Endrizzi et al. 1996, Long et al. 1996, Williams 1998, Vollbrecht et al. 2000) together with other homeobox genes like WUSCHEL (Mayer et al 1998). Overexpression of knox genes affects leaf morphology and cell determination (Sinha et al. 1993, Lincoln et al. 1994, Chuck et al. 1996, Hareven et al 1996, Chan et al. 1998, Ori et al. 2000, Frugis et al. 2001). Misexpression of WUSCHEL has been shown to alter flower patterning (Lenhard et al. 2001, Lohmann et al. 2001), juvenile leaf formation (Hamada et al. 2000) and vegetative-to-embryonic transition (Zuo et al. in phase of publication).

At the present workshop, data regarding the overexpression of KNAT1 in lettuce (obtained in the laboratory of Prof. Domenico Mariotti), the overexpression of WUS in Arabidopsis and the overexpression and post-embryonic silencing of STM (obtained in the laboratory of Prof. Nam-Hai Chua) will be shown as a comparative analysis of the effects that meristem-specific homeobox genes misexpression have on leaf initiation and development. Moreover, hormone involvement and possible interactions between different homeobox genes in the control of leaf initiation will be discussed.

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Characterization of the dominant mutation *Wavy Auricle in Blade* (*Wab*)

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A maize leaf is patterned in a proximo-distal fashion with sheath tissue proximal to and encompassing the shoot and blade tissue distal to and lying out from the shoot. These tissue types are separated by an epidermal fringe of ligule tissue and two wedges of auricle tissue. Dominant *knox* mutations have been identified by shifts in this leaf pattern such that proximal tissue types such as sheath and ligule are found in distal postions in the blade. This defect in proximo-distal patterning is correlated with ectopic *knox* expression in the maize leaf [1] [2] [3].

Here we describe a dominant mutation where auricle, ligule and sheath tissue are found in distal positions in the blade. We have mapped this mutation to a novel position on chromosome 2L and, moreover, we have shown that this phenotype is not associated with ectopic *knox* expression in the leaf. Here we present data suggesting a link between proximo-distal and medio-lateral patterning in the maize leaf. The *Wab* blade is conspicuously narrow and contains less than half the number of lateral veins of a wild type blade. Double mutants between *Wab* and both *liguless1* (*lg1*) and *lg2* have extremely narrow leaves with very little blade tissue. We suggest that a signal produced at the blade/sheath boundary (perhaps LG1/LG2) is interpretted by the products of genes such as *Wab* in order to elaborate both medio-lateral and proximo-distal pattern in the maize leaf.

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Three closely-related AP2/ERF genes alter leaf/shoot interactions and induce axillary shoot growth from the base of the *Arabidopsis* leaf

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In *Arabidopsis*, all primordia initiated from apical meristems have both leaf and shoot identity. During development, however, they respond differentially to stimuli that act to promote or suppress various aspects of leaf and shoot development. We have identified three closely-related genes that promote the precocious outgrowth of shoots from leaf bases, when overexpressed under the control the constitutive 35S promoter. These three genes are members of the AP2/ERF-family of transcription factors. The AP2/ERF-family is unique to plants and contains members that are involved in development, responses to biotic and abiotic stress and responses to ethylene. These genes were isolated and overexpressed as part of a functional genomics program focused on the identification and analysis of transcription factors in *Arabidopsis*.

Xcl1 causes delayed cell divisions in developing maize leaves leading to cellular differentiation by lineage instead of position

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Plant cells have been shown to differentiate by position dependent mechanisms rather than lineage. Molecular mechanisms controlling cell division and differentiation patterns have proven difficult to elucidate in plant model systems. However, the predictable patterns of cell division and differentiation in the maize leaf have allowed for the identification of mutations such as crinkly4(1), tangled1(2) and warty1(3) which affect these processes. The maize Extra cell layers1 (Xcl1) mutation affects both cell division and differentiation. Oblique, periclinal divisions in the leaf protoderm, producing an extra layer of large, highly vacuolate cells between the mesophyll and epidermal cell layers. Mutant kernels also have several aleurone layers instead of one, suggesting that Xcl1 plays a role in controlling cell division orientation in cells that divide predominantly in the anticlinal plane. The extra cell layers produced in leaves and kernels differentiate according to lineage and not position. In developing maize leaves, cell divisions occur mainly at the base of the primordium, while differentiation occurs in a basipetal gradient from the tip of the leaf down (4). Dosage analysis of Xcl1 revealed that the mutant phenotype is caused by the overproduction of a normal gene product which shifts the division gradient upward and allows cells that have already received differentiation signals to continue to divide in aberrant planes. Therefore, in leaf development, the timing of cell division determines the differentiation pathway that will be followed.

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Ab-adaxiality and leaf development: the role of PHAT in leaf morphogenesis

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The upper leaf surface (adaxial) is structurally different from the lower (abaxial) surface. Ab-adaxial organization is important for formation of the leaf lamina in simple leaved species.(1, 2, 3, 4) Compound leaves have regions where lamina formation between leaflets is interrupted. Recently, several genes have been studied for their roles in establishing ab-adaxiality and compound leaf development. (2, 5, 6, 7, 8, 9, 10) In nature, compound leaves are classified into two forms: pedate and peltate. The relationship between ab-adaxiality and these compound leaf forms is largely unknown.

To dissect the function of ab-adaxiality in compound leaf development, four tomato wiry mutants (wiry, wiry3, wiry4 and wiry6) were examined. In these mutant plants, both ab-adaxiality and leaflet formation are defective and symmetric needle-like leaves without leaflets are made. The ab-adaxial defect can be seen from as early as cotyledons and persists throughout development. Tomato PHANTASTICA (PHAT), YABBY and KNOX gene expression are altered in these mutants, suggesting that abnormal ab-adaxiality and reduced compoundness are due to the failure of normal activities of these genes.

To understand the relationship between ab-adaxiality and different leaf forms (pedate vs.peltate), ab-adaxial features of leaves from various species were also examined. The adaxial domain was significantly reduced in peltate leaves, suggesting that the extent of the adaxial domain is important for final leaf morphology. Antisense PHAT transgenic tomato plants often produce peltate leaves, instead of the normal pedate leaves. These results indicate that leaf ab-adaxiality is important for the placement of leaflets on leaves, and that PHAT is involved in these developmental processes.

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Isolation and characterization of a new allele of *aba2* locus in *Arabidopsis thaliana*

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In higher plants, glucose is an essential signaling molecule that controls a wide variety of developmental and physiological processes such as embryogenesis, germination, cotyledon greening and expansion, leaf and root morphogenesis, flowering, senescence and wounding/ pathogen responses, in addition to gene expression. However, the components in the glucose-signaling pathway are mostly unknown. Isolation and characterization or glucose response mutants would allow us dissection of the glucose signal transduction pathway and will provide important information about novel cross-talk between sugar and other signaling pathways mediated by phytohormones, nitrogen and light. For example, glucose-specific up-regulation of ABA (abscisic acid) levels is a prerequisite of glucose signaling during seedling development, as shown by the ability of the ABA-deficients mutants to grow in the presence of high glucose concentrations. On the other hand, ABA-deficient and ABA-insensitive mutants can germinate in the presence of paclobutrazol, a GA (gibberellin) biosynthetic inhibitor.

We have identified three Arabidopsis mutants, *gin* (glucose-insensitive) A, *gin* B and *gin* C, in which glucose and paclobutrazol repression of cotyledon greening and expansion was impaired. Moreover, these mutants present an adult wilty phenotype similar to that caused by ABA deficiency mutations. Endogenous ABA level analyses have revealed that one of this mutants, *gin* A, lacks in ABA biosynthesis. Allelism test and T-DNA localisation in the genome have showed that *gin* A is a new allele of the recently cloned gene, *aba2*.

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Drl1, a putative transcriptional regulator, important for leaf growth

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Leaf growth is determinate and is defined along three axes. A pattern of growth along the length and width direction is responsible for the formation of the petiole and the blade, growth in the thickness direction is very restricted resulting in the typical sheath-like structure of the blade. The last few years many factors involved in growth along these three axes were defined. We propose that DRL1 (DEFORMED ROOT AND LEAF 1) plays a role in linking the pathways for lateral growth and dorso-ventrality in *Arabidopsis thaliana* (Eshed *et al.* 2001).

The *drl1-2* mutation was obtained in a *Ds*-transactivated F2 population. The mutant consisted of rosette and cauline leaves of which the lamina is severely reduced in the width direction. Anatomical analyses of the leaf lamina showed that it was a defect in cell division rather then a defect in cell expansion, which caused the narrow leaf lamina in the *drl1-2* mutants. Moreover, the leaf blade was thicker compared to wild type and it seemed that the palisade cell layer was replaced by the spongy parenchyma. Based upon these data, double mutant analysis with *angustifolia* (Tsuge *et al.*, 1996) and the analysis of dorso-ventral marker lines (in collaboration with John Bowman) might enable us to position DRL1 in the pathways for lateral growth and dorsoventrality. The *DRL1* gene was cloned and encoded a putative transcriptional regulator, containing an ATP/GTP-binding domain as well as a calmodulin-binding domain. *DRL1* promotor activity is restricted to specific domains of the soot apical meristem and leaf primorodia.

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Genetic analysis and positional cloning of the *ultracurvata* genes of *Arabidopsis thaliana*

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To better understand the genetic mechanisms underlying plant leaf development, we have performed a large-scale screening for *Arabidopsis thaliana* mutants to identify those displaying abnormally shaped or sized leaves. One of the stronger mutant phenotypes found was that of the ultracurvata (ucu) mutants, whose vegetative and cauline leaves are spirally rolled downwards and show a reduced expansion along the longitudinal axis, in contrast to wild type leaves, which are flattened organs. We have identified six ucu lines, whose genetic analyses indicate that they fall into two complementation groups, UCU1, which includes one recessive and two semidominant alleles and UCU2, with three recessive alleles. Several organs in the ucu2 mutants are helically rotated along the apical-basal axis, a trait that is more pronounced in roots, pistils and mature siliques. Following a map-based strategy, we have cloned both the UCU1 and UCU2 genes. UCU1 was found to encode an intracellular kinase closely related to GSK3/SHAGGY, one of the components of the Wingless/Wnt animal signalling pathway, whereas UCU2 encodes a peptidyl-prolyl cis-trans isomerase of the FKBP (FK506-binding protein) family of proteins, whose animal homologues are known to be involved in cell signalling, protein trafficking and transcription. The responses of ucu1 mutants to exogenous plant hormones and the genetic analysis of double mutants involving ucu1alleles indicate that UCU1 is a key component in several signalling pathways controlling cell expansion and overall plant growth, including those of auxins and brassinosteroids.

DAG genes: A family involved in plastid development

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The dag mutant in *Antirrhnum majus* is defective in leaf pigmentation due to the complete arrest of chloroplast development in the mutant areas. In mutant sectors, the palisade cells fail to develop and expand properly. The development of the etioplasts in dark is also affected. DAG is required for the expression of plastid encoded genes, very early during plastid development.

DAG belongs to a gene family unique to plants, whose products show a transit peptide and a highly conserved N-terminal region. There are ten DAG genes in *Arabidopsis*. We have cloned cDNAs for 8 of these family members, including AtDAG1, the orthologue to the *Antirrhinum* DAG gene.

The dal1 mutant of *Arabidopsis* has a T-DNA insertion which blocks the expression of AtDAG3. In these plants, chloroplasts fail to develop, although a low level of chlorophyll may be synthesised. Genes encoding subunits of the plastid encoded polymerase (PEP) are expressed, unlike in the dag mutant of *Antirrhinum*.

We have isolated insertion mutants in AtDAG1, AtDAG2 (from *Arabidopsis* KO facility at the University of Wisconsin) and AtDAG5 (from the collection at the University of Gent), but we have been unable to identify homozygous lines. Gaps observed in the siliques of the heterozygous plants suggest that the mutations are seedling lethal. We are crossing heterozygous plants with plants carrying a dominant visible marker linked to each one of the three genes to test the lethality.

We will transform the heterozygous plants with a construct containing each gene cloned after a double ABI3 promoter, which allows expression during embryogenesis. We will also silence DAG genes using hairpin RNAi vectors. A third approach to rescue the DAG phenotype would be transforming heterozygous plants with constructs that include the gene of interest between lox sites.

The effect of the mutations on plastome expression will also be investigated. We have amplified by PCR all the 87 proteins, 4 rRNAs and 37 tRNAs from the plastid genome in *Arabidopsis*. These PCR products will be spotted onto filters, providing a tool to assess the effect of DAG genes on plastome transcription.

These and other experiments will shed light on the DAG family function in plastid and plant development.

Supracellular control of the orientation of the guard mother cell division plane

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More than 100 years ago, plant biologists formulated a general rule of cell division: cells divide in a plane perpendicular to their long axis. Thus, cells know their shape and this shape dictates the precise orientation of the cell division plane. Here we show that the stomatal precursor, the guard mother cell (GMC), in *Arabidopsis* does not follow this general rule. GMC has an oval shape, but it divides in a plane parallel to its long axis. This result indicates that a supracellular control prohibits that cell shape guide the orientation of the cell division plane, and it dictates a strict and shape-independent cell division plane alignment. The nature of this mechanism is unknown.

Genetic analysis of a new uni-like mutant in *Vicia faba* L

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A new unifoliata mutant (typical compound leaf reduced to a terminal single leaflet) with abnormal flowers (cauliflower-head type) and sterile have been found in a germplasm collection of faba bean. Genetic analysis of heterozygous plants showed a 3 wild type: 1 unifoliata mutant segregation ratio. Three genes, un-a, un-b and un-c expressing the unifoliata character have been described in Vicia faba (Sjodin 1964). Allelism test showed that the new mutant is not allelic to the un-a gene however segregation data of crosses with the un-bc1 mutant (U4) showed that the new unifoliata might be allelic to either un-b or un-c genes but it was not possible to distinguished which. Determination of homology with the flo/lfy gene is underway.

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