Instituto Juan March
de Estudios e Investigaciones

150 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Plasticity in Plant Morphogenesis

Organized by

G. Coupland, C. Fankhauser and M. A. Blázquez

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INDEX

PAGE

PAGE

Introduction
M. A. Blázquez

A unique feature of plant development is that plants continue to produce and differentiate new organs during their whole life cycle, which contrasts with most other higher organisms. This peculiarity has resulted in two critical characteristics of plant development; first, many developmental decisions taken during post-embryonic life are subject to influence by the environment; and second, since plants cannot willingly change their location, they have developed a highly efficient strategy to adapt their architecture and physiology to changing environments.

Although we have reached a high level of description of signaling pathways, we still know very little about the mechanisms that integrate those external cues with the endogenous developmental status of the plant. This integration is extremely important since the same external stimuli are differentially interpreted in distinct organs or at different stages of development. Thus, to reach the next level of understanding, it is necessary to compare the different physiological mechanisms that allow plasticity in plant development, as well as to start building the connections between these different pathways to hopefully render a more integrated view of modulation of plant growth. This was the main goal of the workshop.

Recent progress in the study of how plants grow and differentiate –much of it presented at this meeting– is beginning to show the molecular mechanisms that underlie the tremendous plasticity in plant development. This plasticity has been achieved mainly through two strategies: first, a very sensitive machinery that perceives environmental conditions –such as light quality and intensity, daylength, temperature, and nutrients–; and second, the interweaving of multiple signaling pathways –light, hormones– that allows fine tuning of developmental programs as they proceed. How these two strategies combine with each other to allow plasticity is probably the next big question in plant development.

Miguel A. Blázquez

Session 1: Photomorphogenesis and circadian regulation
Chair: Jorge J. Casal

The Arabidopsis *SRR1* gene mediates phyB signaling and is important for normal circadian clock function

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Light affects most organisms throughout their life cycle. In plants, a number of photoreceptors including phytochromes, cryptochromes and phototropins are important to sense the light environment. Some of those photoreceptors also play a specific role in the light input pathway that resets the circadian clock. We have identified *SRR1* (sensitivity to red light reduced) which plays an important role both for phytochrome B mediated light signaling and regulation of multiple outputs of the circadian clock. *Srr1* and *phyB* mutants display a number of similar phenotypes such as early flowering in short days, reduced chlorophyll content and decreased sensitivity to red light specifically. Genetic analysis suggests that not all *SRR1* mediated functions require a functional phyB photoreceptor. In addition to those photomorphogenic phenotypes, *srr1* mutants have a short period in all clock-regulated genes tested and a short rhythm in leaf movement. Similar phenotypes have been found for the *elf3* mutant which plays a role in the light input to the clock, in phytochrome B signaling and physically interacts with phyB. We are currently testing if *ELF3* and *SRR1* work in the same pathway. The *SRR1* gene was identified and we showed that *srr1* is a null allele. The *SRR1* transcript is induced by light but not under circadian control. The SRR1 protein does not contain any domains with known functions except for a putative nuclear localization signal. SRR1-GFP fusion proteins are present both in the cytoplasm and the nucleus but excluded from the vacuole in stable Arabidopsis transformants. Interestingly SRR1 homologues are present as single copy genes in numerous eukaryotes. The function of these proteins is currently unknown. These results suggest that *SRR1* might be a regulator of the circadian clock that is conserved between plants and animals.

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The role of light in root development

Frances J. Salisbury, Claire S. Grierson and Karen J. Halliday

In the wild *Arabidopsis* is a pioneer species and in our towns it is found in uncrowded flower borders and quite frequently growing undisturbed at the edges of pavements between the cracks in paving slabs. Thus, when growing outside the laboratory, roots are more likely to be exposed, providing an opportunity for root photoreceptor activation. To investigate the role of light in root development we are using molecular genetics, genomics and micro-grafting techniques. This combined approach allows us to separate local and transmissible light signals. We have shown that phytochromes play a major role in controlling root development. This is achieved via phytochromes acting in the shoot and via phytochromes acting within the root. We present data that examines the role of HY5 in light-regulated root development. We also provide evidence of new roles for phyD and phyE in shaping root growth.

Genetics and models of the circadian clock

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Circadian rhythms in plants affect stomatal aperture, photosynthesis, several aspects of secondary metabolism and, by one measure, the expression of about 6% of *Arabidopsis* genes. The ubiquity of circadian rhythms among species is widely taken as evidence of their adaptive value: in contrast to fixed interval timers (e.g. an egg-timer started from lights-off), true clocks can allow great flexibility in timing, but is this a significant advantage? Photoperiodism enhances fitness through seasonal reproduction; photoperiodism requires a circadian clock, so the clock has adaptive value via this mechanism. We have recently demonstrated the importance of a well-adjusted circadian clock for vegetative growth of crops in the field and of Arabidopsis plants in laboratory competition experiments.

About 20 interacting genes have been shown to function in the core of the circadian system and the photoperiodic sensor, and this number would grow significantly if the light signalling genes that set the phase of the clock were included. We have taken genetic approaches to identify new components of the plant circadian clock, including a small protein, ELF4, which functions at the end of the day. The increasingly detailed results produced by molecular genetics do not necessarily lead to greater understanding of such a regulatory network. Mathematical modelling provides an invaluable, complementary approach, which can be powerfully applied to this scale of network. We are developing "complete" models for the plant clock and photoperiod sensor, which incorporate the molecular components in a realistic manner. Numerical simulations using such models should become directly relevant to molecular experiments. We have established a novel analytical method to assess the contribution of each component of the model (RNA or protein) at each phase of the cycle, which helps to understand their functions even when the models' complexity limits the scope for mathematical analysis. We are also developing mathematical understanding of classic circadian protocols, such as skeleton photoperiods.

Despite the enormous potential of mathematical analysis and simulation, their usefulness to the wider biological community is often under exploited, due to their apparent inaccessibility to those who are neither mathematicians nor computer programmers. Our aim is to model this system in a form that is accessible to experimental biologists. Thus we are developing user-friendly modelling software, which will simulate all common circadian and flowering time experiments. An intuitive interface allows the user to specify their input data and recover results in a user-friendly form. We will make our software widely available from

our website, with a variety of novel and published models for different species, on-going support and revisions in response to feedback from users and new experimental data. Ultimately our work will allow growers to predict floral initiation in the field, and suggest crop improvement strategies to evoke desired responses. Funded by BBSRC and DTI (UK).

The control of flowering by daylength in long and short-day plants

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A major developmental transition in the life cycle of plants is from vegetative growth to flowering. This transition is often controlled by environmental signals such as daylength and temperature. These responses are important in the adaptation of plants to growth in particular locations and produce characteristic seasonal patterns in flowering. We use the model species *Arabidopsis thaliana* to identify the molecular mechanisms that regulate flowering in response to environmental conditions, and are examining how this regulation is modified in other plant species to confer environmental responses not shown by Arabidopsis.

Flowering of Arabidopsis is triggered by long daylengths (or photoperiods) and by extended exposure to low temperatures (or vernalization). Both responses ensure that plants flower during spring or early summer, and are controlled by independent genetic pathways. We have identified and studied a class of mutants that disrupt the control of flowering by daylength, and have cloned the genes affected by the mutations. These genes act within a pathway that promotes flowering specifically in response to long-day conditions. The latest acting gene that is specific to this pathway is *CONSTANS*, which encodes a nuclear protein that regulates transcription of target genes including the flowering-time gene *FT*. Post-transcriptional regulation of CONSTANS by light appears to be required for *FT* activation, while control of *CONSTANS* transcription by the circadian clock ensures that it is expressed when plants are exposed to light only under long-day conditions. We will present our data on the transcriptional and post-transcriptional regulation of CONSTANS.

There is tremendous diversity in flowering behaviour both between and within species. In contrast to Arabidopsis, flowering of many plant species is promoted by exposure to short daylengths and inhibited by long days. *Pharbitis nil* is a classical short-day model species, that flowers rapidly after exposure to a single short day. We have cloned the *CONSTANS* and *FT* homologues from *Pharbitis nil*. Comparison of the regulation of these genes between Arabidopsis and Pharbitis suggests that differences in the mechanism of transcriptional control by the circadian clock may enable *CONSTANS* to trigger flowering in response to short days in Pharbitis. We will present our data on the expression of these flowering-time genes in Pharbitis.

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The role of DET1 in photomorphogenesis

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Phytochromes are the best characterized plant photoreceptors, responsible for a wide range of photomorphogenic events ranging from seed germination, de-etiolation, shade-avoidance responses, and flowering. New molecular methods have revolutionized our understanding of their precise mode of action. For example, some phytochromes have now been demonstrated to have serine/threonine protein kinase activity and to translocate from the cytoplasm to the nucleus in a light-regulated manner. Several phytochrome-interacting proteins have now been identified, most of which are nuclear localized and include transcription factors. To add to this knowledge, we are studying light hypersensitive mutants of *Lycopersicon esculentum*. Analysis of the *high pigment-2 (hp-2)* mutant has previously revealed that it is mutated in the *DET1* gene. The DET1 protein, first discovered by Joanne Chory's laboratory in *Arabidopsis thaliana*, has been suggested to play a role in specifying the correct developmental expression pattern of photoregulated genes in young seedlings. In line with *det1* mutant phenotypes, it has therefore been proposed to be a repressor of light-regulated genes. The precise function of DET1 has nonetheless remained a mystery. Although it has been localized within the nucleus, it does not exhibit any direct binding activity to DNA or to RNA polymerase. However, we have observed that it interacts with nucleosomes, more particularly with the amino-terminal tail of the core histone H2B both *in vitro* and *in planta*. Domain analysis of DET1 has identified two H2B binding sites, one present in the N-terminus and another in the C-terminus. Furthermore, binding experiments using synthetic peptides indicate that DET1 binds preferentially to the hypoacetylated form of H2B. These results suggest that DET1 may repress light-induced gene expression by influencing chromatin architecture via an interaction with non-acetylated tails of H2B, which are likely to be associated with transcriptionally-inactive chromatin domains.

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Session 2: Hormones and light
Chair: Peter Doerner

Light signalling circuitry in plants

Jorge J. Casal

Plant development is controlled by environmental cues. Light and temperature play a crucial role in the definition of phase transitions and plant body form. This is possible thanks to the action of a complex network of signalling pathways. One of the emergent properties of this system is versatility. The same environmental change may have rather different consequences depending on the developmental context and on the status of other environmental cues. The aim of this talk is to show how this complexity can be analysed at different levels, ranging from the regulatory interactions between light and other signals to the signalling branches downstream a given photoreceptor.

The model plant *Arabidopsis thaliana* bears an extended repertoire of photoreceptors: Five phytochromes (phyA-phyE), two cryptochromes (cry1, cry2) and two phototropins. Seedlings of the wild type and of the *phyA phyB*, *cry1cry2* and *phyA phyB cry1cry2* mutants were grown in darkness and either transferred to white light for 1 or 3 h or left as controls in darkness. Affymetrix chips were used to investigate the changes in transcriptome as affected by the different conditions and genotypes. We have developed a new combination of algorithms to analyse the data. Noteworthy, we have observed that the genotypes could be grouped according to the presence or absence of phyA and phyB irrespective of light or darkness. This pattern was the result of the behaviour of five groups of genes. One of these groups contained a dominant proportion of genes related to the abscisic acid signalling network. The significance of these transcriptome changes is being investigated by using genetic tools. The data have also been used to evaluate the interactions between phytochromes and cryptochromes.

One level of connections is that relating phytochrome signalling with abscisic acid or cryptochrome signalling. However, complexity is also observed if the focus is placed on a single photoreceptor. phyA initiates two discrete photoresponses: The very-low-fluence response (VLFR) that saturates with infrequent excitation with red or far-red light, and the high-irradiance response (HIR) that requires sustained excitation with far-red light. A screening for Arabidopsis mutants lacking the HIR but retaining the VLFR has yielded the novel *phyA-302* alleles with missense mutations in the PAS2 domain of phyA. phyA-302:GFP fusion proteins migrate to the nucleus but show abnormal sub-nuclear localization (Yanovsky et al., 2002). The analysis of truncated phyA in transgenic seedlings indicates that the Ser-rich N-terminus represses VLFR signalling but enhances HIR signalling (Casal et al., 2002). Deletion and substitution analysis of a target gene promoter has revealed that a cis-acting factor is necessary for HIR but not for VLFR. Genetic analysis of VLFR and HIR has revealed that some elements that operate downstream phyA are common to both responses

while others are specific. Signalling via the VLFR pathway negatively regulates phyB signalling whereas signalling via the HIR enhances phyB-mediated responses.

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Mapping the relative sites of action of COP1, HY5, TIR1 and NAC1 in lateral root development

Peter D. Hare, Luisa-Maria Lois, Li-Fang Huang and Nam-Hai Chua

Previous studies indicated that COP1 appears to act upstream of HY5 (1) and that NAC1 acts downstream of TIR1 (2) in the regulation of lateral root development. Whereas the RING protein COP1, F-box protein TIR1 and transcriptional activator NAC1 are essential for maximal lateral root initiation, the transcription factor HY5 normally represses lateral root growth. The observation that transcripts encoding both NAC1 and TIR1 are elevated in hy5 but repressed in cop1 suggests that defects in lateral root formation in these mutants may arise at least in part from defective TIR1 and NAC1 expression. Analysis of lateral root initiation in crosses between hy5 or cop1 mutants and mutants or transgenic lines with the opposite lateral root phenotype resulting from altered TIR1 or NAC1 activities (cop1-6 x 35S::NAC1, cop1-6 x GVG::TIR1, hy5-1 x 35S::NAC1-AS, hy5-1 x tir1-1) was consistent with the four intermediates acting sequentially in the order COP1 ---| HY5 ---| TIR1 ---> NAC1 ---> lateral root initiation.

This interpretation was further validated by whole genome microchip analysis using RNA from roots of cop1, hy5, tir1 and NAC1 antisense plants. However, only a subset of genes affected in all four genetic backgrounds showed similar regulation in cop1, tir1 and NAC1 antisense roots with the opposite regulation in roots of hy5. Clustering of the genes misregulated in roots of these four lines indicates the complexity of the pathways that control lateral root development.

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GA regulation of LEAFY transcription

Ove Nilsson, Sven Eriksson, Henrik Böhlenius

We are interested in the mechanism for *Arabidopsis* flowering time regulation under non-inductive short-day conditions. In short days, gibberellins promote flower initiation in *Arabidopsis* by a gradual activation of the LFY promoter in the newly initiated leaf primordia. Therefore, one interesting possibility is that a gradual increase in GA and sucrose levels at the apex could be a way for the plant to monitor developmental time under non-inductive conditions. To address this question we have performed a detailed analysis over time of the changes in GA and sucrose metabolism in the very same tissues that express LFY, and have correlated these data to the expression levels of LFY and to flower initiation. We have also determined the sensitivity towards different GA species in the regulation of LFY activity. Based on these experiments we have determined what GA species is primarily responsible for regulation of LFY and, therefore, of flowering under non-inductive conditions. Also, we have studied in detail the mechanism for GA induction of LFY transcription. Here we have found that the induction of LFY by GAs is surprisingly quick, and does not require translation. Furthermore, our data suggests the involvement of a potent LFY-specific repressor activity.

Interactions between light and auxin signaling in Arabidopsis development

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Auxin regulates growth in part by regulating gene expression. Auxin Response Factors (ARFs) bind to Auxin Response Elements in promoters of regulated genes, and mediate gene expression responses to auxin. Auxin is believed to regulate ARF activity by increasing turnover rate of Aux/IAA proteins, which bind to ARFs and inhibit their activity. ARF and Aux/IAA proteins are encoded by multigene families, and different ARFs and Aux/IAAs probably have distinct functions.

We have characterized mutations in the Arabidopsis *ARF6* and *ARF8*genes encoding two closely related ARFs. *arf6 arf8* double null mutant flowers arrest as infertile closed buds with short petals, short stamen filaments, undehisced anthers that do not release pollen, and immature gynoecia. They have decreased gene expression response to auxin and less jasmonic acid than wild-type flowers. Exogenous jasmonic acid restored male fertility to *arf6 arf8* mutant plants. These results suggest that auxin, acting through ARF6 and ARF8, regulates maturation of multiple floral organs, in part by controlling jasmonate production. We are currently exploring whether environmental light conditions regulate flower maturation. *arf6* and *arf8* single mutants each have decreased self-fertility because of delayed stamen filament elongation and anthesis. Natural variation in this trait can influence rates of outcrossing, suggesting that ARF6 and ARF8 may be important in evolving plant mating systems.

Light and auxin each regulate seedling growth, and several findings suggest that light might act in part through Aux/IAA proteins. For example, auxin and light each regulate expression of *IAA* genes, and phytochromes can interact with Aux/IAA proteins. Auxin appears to regulate genes differently in light and dark-grown seedlings. However, we do not yet know the biochemical mechanism through which light regulates auxin-inducible genes.

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Regulation of brassinosteroid homeostasis by light

Michael M. Neff

Brassinosteroids are growth-promoting hormones likely to be involved in modulating plant plasticity in response to changes in the environment. Previous studies of brassinosteroids have concentrated on either the metabolic or perception pathways for these hormones. A cytochrome P450, *CYP72B1*, has recently been identified and hypothesized to be involved in brassinosteroid inactivation rather than biosynthesis or perception (Neff et al. 1999). Heterologous expression of CYP72B1 in yeast, coupled with brassinolide feeding experiments, demonstrates that CYP72B1 converts active brassinosteroids such as brassinolide and castasterone to 26-hydroxybrassinolide and 26-hydroxycastasterone respectively. Brassinosteroid feeding experiments with wildtype Arabidopsis, a *CYP72B1*-null mutant and a transgenic line over-expressing CYP72B1 demonstrate that CYP72B1 catalyzes this biochemical reaction in plants. Seedling growth assays demonstrate that 26-hydroxybrassinolide is an inactive brassinosteroid, demonstrating that CYP72B1 is responsible for steroid hormone inactivation, a mechanism similar to cytochrome P450-mediated carbon-26 inactivation of ecdysone in insects. *CYP72B1*-null mutant seedlings have a light-dependent, long-hypocotyl phenotype uncovering a role for this gene in photomorphogenesis. Together, these results demonstrate that CYP72B1 acts as a positive regulator of photomorphogenesis by inactivating the negative regulator, brassinolide.

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Session 3: Temperature control of developmental transitions
Chair: Ottoline Leyser

Regulation of flowering time by multiple environmental cues

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Flowering of the facultative long-day plant Arabidopsis is controlled by several endogenous and environmental factors, among them gibberellins (GAs), day length and ambient temperature. The promotion of flowering by long days involves an endogenous clock that interacts with light cues provided by the environment. We have used the short-period mutant *toc1* to investigate the role of the circadian clock in the control of flowering time by GAs and photoperiod, and we have found that the circadian clock regulates flowering through its interaction with *CONSTANS*, *SOC1* and *FT* expression, in a process that is independent of GA biosynthesis. On the other hand, changes in ambient temperature seem to be perceived by a mechanism that involves genes of the "autonomous pathway" for floral induction, such as *FCA* and *FVE*, since mutants for these genes flower at the same time regardless of ambient temperature. As with vernalization and photoperiod, ambient temperature ultimately affects expression of the floral pathway integrator *FT*, while information of the GA pathway seems to be integrated on the floral meristem-identity gene *LEAFY*.

Phytochromes: actions and interactions

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Light signals regulate all aspects of plant growth and development, and so play a crucial role in determining the architecture of plants. Red- and far-red light signals are perceived by the phytochrome family of photoreceptors. Higher plants possess multiple phytochromes and in *Arabidopsis thaliana* the phytochrome family comprises five members (phyA to phyE), the apoproteins of which are encoded by five discrete genes, *PHYA-PHYE*. The *Arabidopsis* PHYB and PHYD polypeptides are about 80% identical and are somewhat more related to PHYE than they are to either PHYA or PHYC (about 50% identity). The PHYB, PHYD and PHYE polypeptides are the most recently evolved members of the phytochrome family. Genetic approaches are revealing the complexity of phytochrome actions and interactions. Different phytochromes can have different or overlapping functions. The phytochromes can act redundantly and can interact with one another and with other photoreceptors or other regulatory systems, including other environmental cues. Through the identification and characterisation of phytochrome-null mutants, we are now able to determine the functions and interactions of every single member of the phytochrome family.

Seed germination and seedling photomorphogenesis are regulated predominantly by phyA and phyB, operating via distinct action modes. In more mature plants, phytochromes B, D and E act redundantly to regulate plant architecture and flowering time (Franklin *et al.*, 2003). These three phytochromes act redundantly to perceive the low red:far-red ratio light signals that reflected from nearby vegetation. In response to this proximity signals the phytochromes mediate an increase in elongation growth, increase in apical dominance and eventually early flowering; the so-called shade avoidance syndrome. One of the most rapid facets the shade avoidance response, increased elongation growth, is gated by the circadian clock and involves rapid regulation of gene expression.

PhyB-deficient plants display a constitutive shade avoidance response, consistent with the view that this phytochrome plays the predominant role in mediating red:far-red ratio perception in wild type plants. The phenotypes *phyB* and of other phytochrome-deficient mutants are strongly affected by variations in growth temperature (Halliday *et al.*, 2003; Halliday and Whitelam, 2003). For example, the characteristic early flowering of *phyB* seedlings is not observed for plants grown at 16°C. These plants retain their elongated phenotype. Most dramatically, for *phyA phyB phyD* triple mutants, growth at 16°C leads to a very pronounced prolongation of the vegetative phase that is accompanied by marked

axillary meristem development. In these plants phyE acts as a strong floral repressor via regulation of the expression of the floral integrator gene, *FT*.

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Regulation of flowering by the autonomous promotion pathway

Victor Quesada, Gordon Simpson & Caroline Dean

The correct timing of the floral transition, critical for reproductive success in plants, is influenced by multiple environmental and endogenous cues. A key environmental cue is a long period of cold temperature (ie. winter) which promotes flowering in a process known as vernalization.

Three floral pathways (autonomous, FRIGIDA and vernalization) have been identified that regulate the requirement for and response to vernalization. These converge to regulate expression of the floral repressor, FLC. The autonomous promotion pathway down-regulates FLC RNA levels and several genes acting in this pathway have now been cloned (LD, FCA, FPA, FVE). FCA encodes an RNA-binding protein that contains a WW protein interaction domain and its expression is regulated through alternative processing of the FCA pre-mRNA. We have shown recently that FCA negatively autoregulates its own expression by promoting polyadenylation early in the transcript and this function requires an intact WW domain. FCA interacts with FY, another protein functioning in the autonomous promotion pathway, through the WW domain. The NH2-terminal half of FY is highly homologous to the yeast protein Pfs2p that functions in polyadenylation and cleavage complexes to regulate mRNA 3'end formation. FCA/FY therefore act together to regulate poly(A) site choice in the FCA message but it is not yet clear how they down-regulate FLC. To identify additional genes that function with FCA-FY to control FLC we have screened for suppressors of the early flowering phenotype caused by increased FCA levels and have so far isolated sof1 and sof2 (suppressor of over-expression of FCA).

Removal of introns from FCA bypasses the autoregulation and allows accumulation of higher levels of FCA protein in vivo. Higher levels of FCA overcome the repression of flowering normally conferred through the up-regulation of FLC by the FRIGIDA pathway. The negative autoregulation of FCA may therefore have evolved to limit FCA activity and therefore avoid precocious flowering. We have begun to analyze whether any variation exists in FCA autoregulation in natural *Arabidopsis* accessions.

Memories of winter: control of meristem competence to flower by vernalization

Rick M. Amasino

Certain plants, such as biennials or winter annuals, require relatively long periods of cold exposure to initiate flowering. Grafting studies have shown that cold exposure renders the meristem competent to flower, and this acquisition of competence is known as vernalization. A vernalization requirement ensures that flowering does not occur prematurely before the onset of winter. Molecular and genetic studies of vernalization in *Arabidopsis* have revealed that the state of expression of a gene, *FLOWERING LOCUS C (FLC),* is a major component of meristem competence. *FLC* encodes a MADS-domain protein that acts as an inhibitor of flowering: high levels of *FLC* expression prevent the shoot apical meristem from flowering. Exposure to cold causes an epigenetic switch of *FLC* to an unexpressed state and renders the shoot apical meristem competent to flower. The epigenetic switch is reset to the expressed state in the next generation. This is a unique example of a seasonally induced epigenetic switch of gene expression. *FLC* is also regulated by a developmental pathway known as the autonomous pathway. The interplay of the various pathways that control flowering time via *FLC* regulation will be discussed.

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Low temperature signaling in Arabidopsis: new pathways and old components

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During the past few years, substantial progress has been made towards understanding of how low temperature is perceived by plants and the signal is transduced to trigger the different responses originated by this stimuli. In the case of the cold-acclimation response, an adaptive process by which many plants are able to increase their freezing tolerance in response to low-nonfreezing temperaturers, perhaps the most important insight has been the discovery of a small family of transcription factors in Arabidopsis known either as CBF1, CBF2 and CBF3 (1, 2) or DREB1B, DREB1C and DREB1A (3), respectively. These factors belong to the AP2/EREBP family of DNA-binding proteins and bind the cold- and dehydration-responsive DNA regulatory element termed dehydration responsive element (DRE) (4) or C-repeat (CRT) (5). CRT/DRE elements contain the conserved CCGAC core sequence, which is sufficient to induce gene transcription under cold stress (4, 5) and is present in the promoters of many cold-inducible genes. Interestingly, the *CBF/DREB1* genes are themselves also induced by low temperatures, although not by related stresses as dehydration or high salt, and this induction is transient and precedes that of downstream cold-inducible genes that contain the CRT/DRE element (1-3). Ectopic overexpression of *CBF/DREB1* genes in Arabidopsis results in the constitutive expression of downstream cold-inducible genes and an increase in freezing tolerance (3, 6, 7), indicating they should play an important role in cold acclimation. In addition, overexpression of *CBF/DREB1* genes also enhances drought and salt tolerance (3, 7). Nevertheless, in spite of the extensive investigations carried out on these genes, how their expression is regulated in response to low temperature is an essential question that still await to be answered.

We have used both genetic and molecular approaches to shed some light on the cold-regulation of *CBF/DREB1* expression. Our results have revealed that they are subjected to an intricated regulation through positive and negative transduction pathways, including autoregulation, and that the proper functioning of this regulation is crucial for the accurate development of Arabidopsis tolerance to freezing and related stresses such as dehydration and high salt. Here we will present and discuss some of these data.

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Session 4: Abiotic and endogenous stimuli affecting tropisms and cell division
Chair: Christian Fankhauser

Guard cell signal transduction: From genomics to specificity in signaling

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Plant genomes include many large gene families compared to other organisms. Therefore often gene functions cannot be easily identified by conventional genetic screens due to redundancy (or lethality). To narrow down candidate signal transduction genes for functional characterization we have developed a "single cell functional genomics" approach using guard cells. Guard cells have been developed as a model system for dissecting early signal transduction mechanisms (Schroeder et al., 2001). To dissect new molecular signal transduction mechanisms, Genechip expression array experiments were pursued with purified *Arabidopsis* guard cells. Genechip experiments representing 8000 *Arabidopsis* genes show conditional expression of about 1500 genes and reveal guard cell expression of one or several defined members of otherwise very large gene families, allowing functional characterization.

We identified hyperpolarization-activated Ca^{2+}-permeable channels (I_{Ca}) as a component of ABA signaling (Pei al., 2000). ABA was shown to cause reactive oxygen species (ROS) production in guard cells. Furthermore ROS activate plasma membrane I_{Ca} Ca^{2+} channels in *Arabidopsis* guard cells. Data will be presented analyzing NADPH oxidase disruption mutations that suggest a central role for two redundant NADPH oxidase genes that function in ABA activation of I_{Ca} channels and stomatal closure.

Previous pharmacological research suggested that type 2A protein phosphatases (PP2As) act as both negative and positive regulators of ABA signaling. A T-DNA insertion allele in a guard cell-expressed PP2A gene, *rcn1*, was obtained and showed ABA insensitivity in stomatal movements and anion channel activation. Calcium imaging analyses show a reduced ABA sensitivity of ABA-induced cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$) elevations in *rcn1*, whereas mechanisms that are stimulated downstream of $[Ca^{2+}]_{cyt}$ increases show wild-type responses, suggesting that RCN1 functions upstream of ABA-regulated $[Ca^{2+}]_{cyt}$ increases. *rcn1* shows ABA insensitivity in ABA inhibition of seed germination and ABA-induced gene expression. The PP1/2A inhibitor, okadaic acid, phenocopies the *rcn1* phenotype in wild-type plants. These data show that RCN1 is a positive transducer of early ABA signaling. Further

examples of combined genomic and time-resolved dynamic signal transduction analyses will be presented.

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Phototropins; a new family of plant blue light receptors

John M. Christie, Michael Salomon, Trevor Swartz, Roberto Bogomolni
Koji Sakamoto, and Winslow R. Briggs

The phototropins (phot1 and phot2) are plant photoreceptors that have a classic serine/threonine protein kinase domain at their C-terminal end of two specialized PAS domains designated LOV domains at their N-terminal end. LOV domains are found in a wide range of proteins involved in detecting **L**ight, **O**xygen, or **V**oltage. PAS domains are known to be involved both in protein-protein interactions and ligand binding. When exposed to light in the presence of ATP, phototropins become heavily phosphorylated. Null mutants at the *NPH1* locus of Arabidopsis thaliana fail to respond to low levels of unilateral light by developing phototropic curvatures. These mutants have been shown to lack phototropin 1 (phot1). Using phot1 expressed in an insect cell/Baculovirus system, we demonstrated that photo1 itself was not only the kinase and the phosphorylation an autophosphorylation, but that phot1 itself was the photoreceptor. When the LOV domains themselves are expressed in E. coli, the resulting proteins bind FMN tightly and undergo a photocycle involving the transient formation of a C(4a) cysteinyl adduct [formation of a covalent bond between a cysteine in the LOV domain and the C(4a) position of the FMN]. This adduct decays back to the dark state over a period of seconds or minutes depending upon the LOV domain. In collaboration with the Japanese groups we have shown that like phot1, phot2 can mediate its own light-activated phosphorylation, and its isolated LOV domains can undergo the same photocycle involving formation of the cysteinyl adduct. We have also demonstrated that seedlings lacking phot1 still show curvature in response to high-intensity unilateral blue light, but that this response is dramatically reduced when both phot1 and phot2 are mutated. Phot 2 has been shown by a Japanese group to be required for the movement of chloroplasts to the side anticlinal cell walls to maximize self-shading under high light intensities. In collaboration with this same Japanese group, we have shown that chloroplast aggregation to the periclinal cell walls to maximize light interception in dim light is also lacking in the double mutant. Finally, another Japanese group has found that the phototropins redundantly mediate blue light-activated stomatal opening. Thus both photoreceptors play a role in phototropism, both play a role in chloroplast movement. However, phot2 mediates responses to far lower light levels than phot2. Both photoreceptors appear to be localized to the plasma membrane.

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Cell cycle regulation, cell growth and morphogenesis

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A large number of cell cycle regulators have been identified in *Arabidopsis* and several links exist between cell cycle control and maintenance of the differentiated state. The correct balance between cell proliferation and differentiation is crucial for organogenesis. Therefore, one major challenge ahead is to understand how the cell cycle regulators integrate with the developmental and differentiation processes. Increasing evidence support the notion that modulation of cell proliferation may have profound consequences at the cellular level, e.g., nuclear ploidy, cell size and cell shape among others. As a consequence, organogenesis and plant architecture as a whole is also modified.

Reactivation of cell division (the G0ÆG1 transition) and the G1ÆS transition are crucial checkpoints that integrate intra- and extracellular signals. The targets responsible for such a fine are the retinoblastoma(RBR)/E2F and the initial events of DNA replication. We are concentrating in analyzing plants that mis-express some of these targets.

The E2Fc gene is expressed in both dividing and differentiated cells. E2Fc protein availability is regulated by the ubiquitin-dependent proteasome pathway through a SCFSKP2 complex. E2Fc is abundant in arrested, dark-grown hypocotyl cells and it is rapidly degraded shortly after light-stimulation of cell proliferation. Overexpression of a stable form of E2Fc negatively affects cell division and cell morphogenesis as revealed by the reduction in cell number, concomitant with a compensatory increase in cell size, both in palisade and epidermal cotyledon cells.

However, cotyledon size is not dramatically modified. One of the likely mechanisms mediating these effects is the repression of CDC6 gene expression, a gene which is expressed in proliferating and endoreplicating cells. Interestingly, plants overexpressing CDC6 modify the endocycle program of developing leaf cells. Consequently, most of these cells have an 8C DNA content, instead of the normal 4C DNA content. Similar results are obtained by overexpressing CDT1, a CDC6-interacting protein, whereby CDT1-overexpressing cells also have an increased ploidy level (8C).

However, none of them shows gross changes in leaf size and shape, suggesting that compensatory mechanisms must exist and that changes in the ploidy level can be necessary but not sufficient to overcome them. These results will be discussed in the light of our current understanding of plant cell cycle regulation.

Nutrient sensing pathways and cell division control

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Plants adapt their growth rates and patterns to changes in their environment. Ultimately, this is mediated by changes in cell proliferation in meristems or developing organ primordia. We are interested in the mechanisms that mediate changes in proliferation rates. We are taking two approaches to address this question: We aim to identify the mechanisms involved ion perception and transduction of a mitogenic signal to cue the growth machinery and second we are dissecting the mechanisms involved in changing cell division rates. We are using phosphate sensing and the regulation of mitotic cyclin expression as paradigm for these aims, respectively.

Arabidopsis responds to phosphate starvation by coordinately activating responses to mobilize endogenous reserves, increase its ability to acquire phosphate and reduce internal demand [1]. To establish a framework for the phosphate starvation syndrome we examined the temporal pattern of adaptive responses. We find that there are three phases that we can distinguish by changes in growth behavior. We can correlate this with coordinated responses leading to increased expression of molecular markers for phosphate starvation. To date we have observed only one apparent regulatory circuit which activated systemically, suggesting one major pathway involved in co-ordinating the plants response.

We have so far not found any evidence for differential regulation of individual cyclins in response to changes in phosphate nutrition. However, reduced levels of expression of mitotic cyclin B1;1 correlates with prolonged starvation. We are currently mapping promoter elements responsible for this response.

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Control of cell proliferation and differentiation in the plant cell cycle

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CycD3;1 expression is associated with proliferating tissues such as meristems and developing leaves but not with differentiated tissues in *Arabidopsis*. CYCD3;1 is a highly unstable protein degraded by the proteasome pathway. Constitutive overexpression of *CycD3;1* in cultured cells and in shoot apices leads to a shift of cells from G1 (2C) to G2 (4C) DNA content. In transgenic plants development is retarded, and leaf architecture is radically altered, with a failure to develop distinct spongy and palisade mesophyll layers. We observe hyperproliferation of leaf cells, and in particular the epidermis consists of large numbers of small, incompletely differentiated polygonal cells. As a consequence, cell division largely replaces cell expansion as the primary mechanism for leaf growth, although the expression of *AINTEGUMENTA* which is proposed to control leaf cell number is unchanged. Other defects in leaf and stem tissues are consistent with the continued proliferation of many cell types preventing their complete differentiation. Endoreduplication, a marker for differentiated cells that have exited from the mitotic cell cycle is strongly inhibited in *CycD3;1* overexpressing plants. These results demonstrate that cell cycle exit in the G1 phase is required for normal differentiation processes during plant development, and show that *CycD3;1* controls cell proliferation and cell differentiation in leaves likely acting downstream of *AINTEGUMENTA*.

Dispersed plant suspension cultures allow cell proliferation and growth to be analysed in the absence of developmental processes. We have recently established synchronisation procedures for Arabidopsis cell lines and used these to analyse cell cycle regulated gene expression by microarrays and Massively Parallel Signature Sequencing (MPSS) of cDNAs on immobilised microbead arrays. To extend the utility of the analysis, we have now also analysed gene expression in suspension cultures re-entering the cell cycle and during normal growth from sub-culture to stationary phase. Analysis of results shows that around 1100 genes show significant cell cycle regulation, and these are involved in a wide range of cellular processes. Identified genes include those already known to be cell cycle regulated, genes previously known to be cell cycle regulated in other eukaryotes and genes for novel plant-specific processes. We conclude that cell cycle regulation is an important mechanism in controlling cell division and reflects the many interfaces by which cellular processes impinge on the cell cycle. These analyses establish that cell suspension cultures can be used to analyse transcriptional regulation of a wide range of cellular processes.

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Session 5: Nutrient and hormonal control of organogenesis
Chair: George Coupland

Coupling of plant signaling pathways with the proteasome

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Phosphorylation-dependent ubiquitination is a common mechanism controlling proteasomal degradation of regulatory proteins in eukaryotes. Phosphorylation by protein kinases provides a signal for recognition of substrates by SCF (Skp1-Cullin-F-box protein) E3 ubiquitin ligases that mediate polyubiquitination of regulatory factors controlling transcription, DNA replication, cell cycle and signal transduction cascades (Deshaies, 1999). Studies of molecular interactions between protein kinases, SCF enzymes and the 26S proteasome thus offer a novel insight into the regulation of essential cellular pathways.

Our studies focus on the analysis of signalling functions of AMP-activated protein kinases (AMPKs) in the model plant *Arabidopsis*. AMPKs represent one of the most conserved classes of protein kinases that show a remarkable structural and functional conservation in eukaryotes. Yeast Snf1, a prototype of AMPKs, and its plant and animal homologues perform analogous functions in the control of energy homeostasis, glucose de/repression, gluco/neo/genesis, lipolysis, mitochondrial and peroxisome biogenesis, transcription, and cell cycle (Hardie *et al.*, 1998). AMPKs are trimeric enzymes consisting of catalytic α/Snf1, substrate targeting β/SIP/Gal83, and activator γ/Snf4 subunits. Our data show that the catalytic subunits of plant type I Snf1-related protein kinases (SnRKs) also form a dimeric complex with an unusual SNF4 βγ subunit, which carries a kinase-interacting sequence (KIS domain) found normally only in AMPK β-subunits (Lumbreras et al. 2000). Combinatorial assembly of subunits appears to determine the cellular location and function of AMPKs. The *Arabidopsis* genome encodes at least two functional α (AKIN10 and AKIN11), two β (AKINβ1 and AKINβ2) and two gamma (AtSNF4 γ and βγ) SnRK subunits (Bhalerao *et al.*, 1999; Kleinow *et al.*, 2000). Coimmunoprecipitation and copurification of AKINβ2 and AtSNF4γ with either AKIN10 or AKIN11 indicates that various SnRK isoforms can assemble from the different subunits *in vivo* (Ferrando *et al.*, 2001). Protein interaction studies show that the regulatory domains of *Arabidopsis* SnRK α subunits can also recruit different signaling factors (Bhalerao *et al.*, 1999). These include the common SKP1 subunit of SCF E3 ubiquitin ligases, the PRL1 kinase inhibitor WD protein, and the α4/PAD1 subunit of 26S proteasome.

Our studies demonstrate that SnRKs coimmunoprecipitate and copurify with the 26S proteasome and SCF complexes that carry SKP1 and cullin CUL1 subunits. The SKP1, CUL1

and proteasome α4/PAD1 proteins show nuclear colocalization in all cell types examined, and are detected in association with the mitotic spindle and phragmoplast during cell division. SKP1 facilitates the interaction of SnRKs with α4/PAD1 indicating that SnRKs play an important role in targeting SCF E3 enzymes to the 20S cylinder of the proteasome. By contrast, the kinase inhibitor PRL1 protein competes for SnRK-binding of the SKP1 and α4/PAD1 proteins suggesting that PRL1 may negatively control the association of SnRK protein kinases with SCF-proteasome complexes (Farrás *et al.*, 2000). Inactivation of PRL1 by a knockout mutation result in pleiotropic defects characterized by derepressed transcription of glucose regulated genes, altered leaf development, arrested root elongation, ectopic root hair development; and hypersensitivity to glucose, sucrose, and several plant hormones, including abscisic acid, ethelene, cytokinin and auxin (Németh *et al.*, 1998). These observations suggest that PRL1-mediated control of SnRKs' activity and molecular interactions may play a role in the regulation of several signaling pathways. In fact, the SnRK-binding SKP1 protein has been identified in SCF complexes with different F-box proteins implicated in the control of diverse hormonal and developmental pathways. These F-box proteins include TIR1, controlling auxin signaling; COI1, regulating jasmonate responses; UFO1, involved in the control of flower organ identity; EID1, affecting phytochrome A signaling and several others (for review see Hellmann and Estelle, 2002). Therefore, our data suggest that binding of SnRKs to SKP1 may modulate the phosphorylation and F-box protein-mediated recognition of different SCF substrates. Identification of substrates and subunits of SnRK-binding SCF enzymes is therefore an important goal of further studies, which address the question how AMP-activated protein kinases modulate signaling by participating in the phosphorylation-dependent ubiquitination and proteasomal degradation of regulatory proteins in plants.

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Auxin regulates organ initiation and phyllotaxis

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Leaves and flowers are initiated at the periphery of the shoot apical meristem (SAM). The arrangement of leaves and flowers, phyllotaxis, is determined by the pattern of organ initiation at the SAM. Organ primordia are always initiated at predictable sites with characteristic divergence angles relative to preexisting primordia, leading to regular phyllotactic patterns such as alternate (distichous), opposite (decussate) or spiral. Inhibition of polar auxin transport, as well as mutations in the auxin transport protein PIN1 block organogenesis at the SAM. However, meristem perpetuation and stem growth are not affected, resulting in the formation of pin-like shoots (pins). Local application of exogenous auxin triggers organ formation on such pins. Based on this finding, we have proposed that local auxin peaks in the meristem dictate the pattern of organ formation. To test this hypothesis, we have initiated a detailed analysis of the distribution of the auxin transport proteins AUX1 and PIN1 in the meristem. We conclude that auxin is transported into the meristem and becomes redistributed within the meristem by the youngest primordia which act as auxin sinks. This mechanism leads to local accumulation of auxin at characteristic distances from the preexisting primordia, by that determining regular phyllotactic patterns.

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Iron transport and signaling in plants

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Iron homeostasis at a cellular level and in the whole organism must be balanced in order to supply enough iron for the plant growth and development, and to avoid excessive, toxic levels. In order to perform iron uptake from the environment, iron distribution to various organs and tissues, and iron intracellular compartmentalization, various membranes must be crossed by this metal. An integrated regulation of the transport systems required for iron trafficking in the whole organism is therefore necessary. However, our knowledge of the iron transporters in pluricellular organisms is limited, and even less is known about their coordinated activity. This prompted us to characterize plant transporters involved in iron homeostasis, through a combination of reverse genetics, cellular, molecular and physiological approaches.

Grasses and non grasses use different strategies to acquire iron from the soil in response to deficiency conditions. In *Arabidopsis*, iron deficiency induces synthesis of FRO2, a ferric-chelate reductase (1), leading to Fe(II) generation, which is taken up accross the root plasma membrane by specific transporter(s). We will present our results demonstrating that IRT1 (2) is the major root iron uptake system under iron deficient conditions, and that it is essential for plant growth and development (3). Expression of the IRT1/FRO2 system is subject to a diurnal regulation. It requires iron itself as a local inducer and is also under the control of systemic signal(s) related to the iron status of the shoots. The characterization of IRT2, a gene highly related to IRT1 that also encodes an iron transporter expressed in root epidermal cells (4), will also be presented and discussed.

In contrast to *Arabidopsis*, iron deficiency in maize induces the secretion by the roots of deoxy-mugineic acid (DMA), which is synthesized from nicotianamine (NA), a structurally related precursor found in all plants (5). Then, DMA binds to soil Fe(III) into the rhizosphere. The resulting complex is recognized and transported across the root plasma membrane by an uptake system. The maize *ys1* mutant has been extensively studied. It carries a monogenic recessive mutation, responsible for a defect in the transport of the Fe(III)-mugineic acid through the root plasma membrane. In this mutant, mugineic acid synthesis and secretion is normal. In collaboration with Pr Walker (Amherst, University of Massachusetts) we have recently cloned and characterized the maize *ys1* gene, and expressed it in the *fet3fet4* yeast mutant strain, in order to demonstrate that it codes for a Fe(III)-mugineic acid transporter (6). An intriguing output of this work was the discovery by sequence database mining that eight *Arabidopsis* genes share important sequence similarities with maize *ys1*, although

Arabidopsis does not produce mugineic acids despite the fact it contains NA. These genes have been called *ys*-Like (*ysL1* to 8), and we will present new data demonstrating the biological role of one of them in *in planta* iron homeostasis, likely through a role in iron compartmentalization and / or long distance signaling of the leaf iron status to the roots.

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Hormonal control of shoot branching in Arabidopsis

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Plant development is continuous, with new organs being produced throughout the life cycle. It is the continuous nature of plant development that allows environmental responsiveness and gives plants their characteristically plastic morphology. This is strikingly illustrated by the wide variety of shoot system architectures that can be adopted by plants of a single genotype. A major component of this variation is in the degree of shoot branching. Shoot branches are usually derived from secondary meristems laid down in the axils of leaves. These axillary meristems can remain dormant or they can activate to produce a branch. This decision is influenced by a wide range of factors, including the position of the axil along the primary shoot axis, the developmental stage of the plant, the availability of nutrients and the proximity of neighbouring plants. Little is known about the way in which multiple environmental and developmental signals are integrated to regulate plant development. However, it is clear that plant hormones play a central role. Hence the regulation of shoot branching provides an ideal system in which to study the role of hormones in integrating environmental and developmental signals to control plant development.

There is a long history of experimental data demonstrating a role for auxin in the control of shoot branching. Removal of the primary shoot apex results in outgrowth of lateral branches. If auxin is applied to the decapitated stump, the outgrowth of the lateral shoots is suppressed. These classical experiments gave rise to the concept of apical dominance:- that auxin exported from the primary shoot apex, and transported down the plant in the polar transport stream, inhibits the growth of axillary buds. However, the mechanism by which auxin regulates bud growth is unclear and this is compounded by the fact that auxin appears to act indirectly because radio-labelled auxin applied to the decapitated stump can inhibit bud outgrowth, but very little label accumulates in the bud (1). In order to understand better the mechanisms by which auxin regulates bud development, in recent years we have been developing tools to study shoot branching in Arabidopsis. We have primarily been concerned with two questions

1. What is the site of auxin action in the inhibition of bud growth?

We have used the auxin response mutants of Arabidopsis to determine the site of action of auxin in inhibiting bud growth. Loss-of function mutations at the *AXR1* locus of Arabidopsis result in reduced sensitivity to auxin, a highly banched shoot and buds resistant to the effects of apically applied auxin (2). In order to determine the site of auxin action, we generated chimeric plants where some tissues were wild-type for *AXR1* and others mutant (3). We used a hypocotyls grafting technique to show that the primary site of auxin action is in the shoot. To pinpoint the site further, we fused the wild-type *AXR1* cDNA to a variety of tissue-specific promoters and introduced these constructs into the *axr1-12* mutant background. We found that expression in xylem-associated cells was sufficient to restore wild-type branching to the

axr1-12 mutant and auxin-responsiveness to its buds. In contrast, expression in the phloem or in the epidermis was unable to restore the wild-type branching pattern. These data strongly suggest that the auxin signal is perceived in xylem-associated cells and is subsequently relayed into the bud.

2. How is the auxin signal relayed into the bud?
Several theories have been proposed to explain how the auxin signal is relayed into the bud, mostly involving a hormonal second messenger. To test these ideas, we developed an assay to characterise the response of individual Arabidopsis buds to added hormones (4). Isolated cauline nodes including a single cauline leaf with its associated bud are excised and inserted between 2 agar slabs held in a petri dish. The growth of the bud can be readily monitored in the days following excision, and hence the effects of either basally or apically supplied hormones can be determined. The bud assay was used to test hypotheses about the nature of possible second messengers for auxin. Abscisic acid and ethylene resistant mutants were found to respond normally to apical auxin, indicating that neither of these hormones is required to mediate the auxin signal (4). Basally applied cytokinin was found to overcome the inhibitory effect of apical auxin. This observation is consistent with one model for auxin action, which proposes that auxin regulates the synthesis and/or export of cytokinin from the roots (5), and possibly also from more apical tissues.

A second mechanism of auxin action is suggested by analysis of the *rms* mutants of pea, which are characterised by a bushy phenotype accompanied by reduced stature and rounder leaves (reviewed in 6). Mutant *rms* buds are resistant to the inhibitory effects of auxin applied to the shoot stump following decapitation, suggesting that the *RMS* genes could act downstream of auxin in mediating apical dominance. Grafting experiments performed using the *rms* mutants has implicated a novel graft transmissible signal in mediating apical dominance. When wild-type root stocks are grafted to *rms1* or *rms5* scions, a wild-type branching phenotype is restored to the shoot. This suggests that *RMS1* and *RMS5* are required for the production of a graft-transmissible signal that can move from up the plant to inhibit bud activity. The signal is unlikely to be cytokinin because, despite the increased branching, the cytokinin levels in the root saps of *rms1* and *rms5* are dramatically reduced compared to wild-type. Mutations at *rms3* and *rms4* cannot be rescued by grafting to wild-type root stocks, suggesting that they may be involved in the perception of the graft transmissible signal.

Our screen for new bushy mutants has revealed a closely analagous system in Arabidopsis. We identified four loci, named *max1-max4* for More AXillary growth, recessive mutations at which confer a suite of phenotypes (7). Apart from increased branching, all the mutants have somewhat reduced stature, rounder leaves, shorter petioles and slightly reduced root growth. Double *max* mutants are no bushier than the single mutants. These observations are consistent with the idea that the *MAX* genes act in a single pathway. The *MAX* genes appear to act downstream of auxin because *max* mutant buds are auxin resistant in the isolated node assay (8) and double mutants between *axr1-12* and the *max* mutants are no more bushy than the single mutants. Similarly, the non-branching phenotype of the *axr3-1* auxin over-responding mutant is partially suppressed in *max* mutant backgrounds (9). However, the putative *max* pathway is, most likely, only partly responsible for the effects of auxin, since

our data show that *max* mutant buds are more responsive to auxin than *axr1-12* buds, and that the non-branching phenotype of *axr3-1* plants is only partly suppressed in *max* mutant backgrounds.

We have used hypocotyl grafting to identify the sites of action of the *MAX* genes (8,10). The shoots of *max1*, *max3* and *max4* mutants can be restored to wild-type by grafting to wild-type root stocks, indicating that these genes are required for the production of a graft-transmissible inhibitor of shoot branching. Reciprocal grafting experiments have led to a model in which MAX1 acts downstream of MAX3 and MAX4 in the synthesis of this inhibitor. The *max2* mutant shoot phenotype cannot be rescued by a wild-type root stock, suggesting that this gene may be involved in perception of the inhibitor.

We have cloned the *MAX* genes. Consistent with their proposed role in the production of a mobile signal, MAX3 and MAX4 encode divergent members of the carotenoid cleaving dioxygenase family. Using the Arabidopsis *MAX4* sequence, a gene homologous to *MAX4* was identified in pea and shown to map to the *RMS1* locus, demonstrating the othology of the MAX and RMS systems (9). *MAX2* was found to encode an F-box protein (7). F-box proteins are found throughout the eukaryotes and have been implicated in regulating a wide variety of processes, such as cell cycle progression and plant hormone signalling (11), by targeting specific regulatory proteins for degradation.

Taken together, these data have led to a model in which the auxin signal is carried into buds by a combination of down-regulation of Ck and up-regulation of the as yet unidentified RMS/MAX signal.

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POSTERS

CCA1 and LHY activities are critical in keeping circadian rhythmicity in *Arabidopsis*

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Plants have developed a circadian system that allows them to adapt to the dynamic changes in their environment, mainly in light quality and quantity. The environmental information must ultimately modulate the activity of the components of the molecular mechanism that generates the circadian rhythms, in order to keep it in synchrony with the seasonal day-night cycle. CCA1 and LHY code for two highly similar transcription factors, each containing a single-MYB domain, involved in a negative feedback loop important for the functioning of the *Arabidopsis* circadian oscillator. The similar circadian phenotypes of lines overexpressing either CCA1 or LHY have suggested that the functions of these two transcription factors are largely overlapping. cca1-1 plants, which lack CCA1 protein, show a short period phenotype for the expression of several genes when assayed under constant light conditions. This suggests that LHY function is able to only partially compensate for the lack of CCA1 protein. It was, therefore, of great interest to obtain plants with a simultaneous loss of both functions, in order to determine the extent of the redundancy between CCA1 and LHY and to assess their collective role in the molecular clock mechanism. We have applied RNAi technology to obtain plants lacking both activities, cca1-1 lhy-R, and show that these plants are unable to maintain sustained oscillations in both, constant light and constant darkness. However, these plants exhibit some circadian function in light/dark cycles, showing that the *Arabidopsis* circadian clock is not entirely dependent on CCA1 and LHY activities.

Gene expression during early fruit development and ovary senescence in *Arabidopsis* revealed by DNA microarray analysis

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It is known that certain plant hormones trigger fruit-set and early fruit development. Application of gibberellic acid (GA3) to young developing ovaries induces the transformation of the unpollinated ovary in a seedless parthenocarpic fruit. In contrast, ovary development ends in a programmed senescence process that is activated several days after anthesis, depending on the species. *Arabidopsis* unfertilized ovaries fully respond to GA3 only during the first 3 days after anthesis, while senescence begins 7-8 days after anthesis. We have used DNA microarrays containing more than 11,000 *Arabidopsis* cDNA clones, corresponding to 7,000-8,000 genes, to characterize changes in gene expression associated to early fruit development induced by GA3 and ovary senescence. Functional genomic approaches such as DNA microarrays allowed us to characterize global changes in gene expression, to identify marker genes, and to discover new gene functions. More than 500 *Arabidopsis* genes whose expression, at the mRNA level, is altered during fruit development and/or ovary senescence have been identified. Several of these genes correspond to putative or hypothetical proteins. Therefore, this approach might allow us to assign new gene functions. We have selected a subset of genes based on their gene expression patterns and sequence information. Expression of several genes has been confirmed by Northern blot analysis. To determine the function of these genes during early fruit development and ovary senescence, we are carrying out several experiments aimed to alter their gene expression by over-expression, T-DNA insertion, or RNAi.

The relationship between IRES (Internal Ribosomal Entry Sites) and microRNAs in Arabidopsis.

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Among the targets found for microRNAs in Arabidopsis thaliana, Rhoades *et al.* (2002) describe many genes that are similar or identical to genes reported to contain IRES in the databases (Auxin response factors, Myb proteins and other transcription factors,...). This led us to investigate the relationship between IRES and microRNAs.

As a result of the search in the UTR database for entries containing Arabidopsis and IRES, a non-redundant list of 203 genes was obtained and analysed. In general, IRES containing genes encode proteins related with cell signalling and differentiation.

BLAST analysis with these UTR sequences was done to identify other sequences related among Arabidopsis genes, being either in the IRES database or not.

Members of the scarecrow gene family share nucleotide sequence identities with many IRES containing genes; Squamosa-promoter binding protein genes were retrieved in searches with four IRES containing genes. Thus, some genes that are microRNA targets contain canonical IRES, but others share sequences with IRES.

Among the IRES containing genes that show similarity in nucleotide sequences, some encode pairs of proteins that may operate in alternative pathways including transcription factors, a protein of the nuclear envelope with a C2 domain-containing protein, probably involved in transport through the nuclear membrane and other examples.

Our results indicate new connexions between microRNAs and IRES: IRES containing genes may be preferential targets for microRNAs and we would like to suggest that they could also be involved in microRNA production as a means to regulate gene expression and cell differentiation in the cell environment. Recent work in root development shows that scarecrow gene is responsible for quiescent center identity and blocking cell differentiation in the stem cells surrounding the quiescent center (VanderBerg *et al.*, 1997; Sabatini *et al.*, 2003). The model here presented indicates a way by which scarecrow genes may compete with and inhibit translation of IRES containing genes.

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Function and regulation of the cell cycle transcription factor AtE2Fc

Carlos del Pozo, Beatrice Boniotti and Crisanto Gutierrez

E2F transcription factors regulate the expression of cell cycle and differentiation genes depending on the developmental context and the environment clues. The positive or negative transcriptional activity these factors depend on their interaction with RB proteins. The components of RB/E2F pathway, as well as other cell cycle regulators, have been identified in plants. To understand the role of AtE2Fc we generated transgenic plants that express a truncated AtE2F2, lacking the regulatory N-terminal region. Our results show that both the cell shape and cell length are affected in these transgenic plants. We also found that some cell cycle genes containing E2F-site in their promoter are down-regulated.

In vitro assays showed that AtE2Fc directly interacts with a plant RB, suggesting a repressor role for AtE2Fc. In addition, *Arabidopsis* E2Fc is regulated by the ubiquitin-proteasome pathway, implicating the function of the E3 ubiquitin-ligase SCFAtSKP2. Using the GUS reporter protein fused to the N-terminal of E2Fc we have found that this region is sufficient to drive the ubiquitin-mediated proteolysis of AtE2Fc in cycling cells and in light-stimulated seedlings. Furthermore, phosphorylation of AtE2Fc by an AtCDC2a/CycA complex is required for interaction with the F-box protein AtSKP2. *Arabidopsis* contains two genes AtSKP2, which showed high homology.

However, expression analysis of both genes revealed that they are likely functioning in different processes. Interestingly, the auxin response mutant axr1-12, in which the modification of CUL1 with RUB1 is impaired, shows increased AtE2Fc protein levels, suggesting a dysfunction in the control of AtE2Fc stability. Taken together, these data suggest that AtE2Fc functions as a repressor of gene transcription cycle genes and its availability is regulated by ubiquitin-mediated proteolysis, either in cycling cells and in response to light stimulus. At present, we are developing mutant plants for AtE2Fc and AtSKP2 using the RNAi technique.

These results will be presented and discussed at the meeting.

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HFR1, a putative bHLH transcription factor, mediates both phytochrome A and cryptochrome signalling

Paula D. Duek and Christian Fankhauser

Plants are very sensitive to their light environment. They use cryptochromes and phytochromes to scan the light spectrum. Those two families of photoreceptors mediate a number of similar physiological responses. The putative bHLH (basic Helix Loop Helix) transcription factor HFR1 is important for a subset of phytochrome A (phyA) mediated light responses (1). Interestingly hfr1 alleles also have reduced de-etiolation responses, including hypocotyl growth, cotyledon opening and anthocyanin accumulation, when grown in blue light. This phenotype is particularly apparent under high fluence rates. The analysis of double mutants between hfr1 and different blue light photoreceptor mutants demonstrates that, in addition to its role in phyA signalling, HFR1 is a component of cryptochrome 1 (cry1) mediated light signalling. Moreover HFR1 mRNA levels are high both in blue and far-red light but low in red light. These results identify HFR1 as a positively acting component of cry1 signalling and indicate that HFR1 integrates light signals from both phyA and cry1.

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Genetic and environmental control of floral size in Antirrhinum

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We are studying how floral size is controlled in Antirrhinum using a genetic approach. We have characterized mutants that show smaller flowers that include compacta (co), compacta ähnlich (coan), ktana (kta), muscoides (mus), Nitida (Ni) and unilabiata (un) and mutants that have bigger flowers including formosa (fo), Grandiflora (Graf), largiflora (larg) and splendida (sple). Floral size seems to be controlled at different levels with mutations that affect the whole flower (kta, mus and Ni) whereas others affect only the perianth (co, coan and un). Furthermore kta, mus and un affect overall growth whereas the rest show normal mass and shape. We have analyzed the environmental effects on body and floral size using different growth conditions. Crowding experiments show that increased plant density has a strong effect on body size but not on floral size that remains unchanged irrespective of the phenotype. Leaf size decreases in all genotypes with increased crowding and the number of flowers per plant also decreases. Our hypothesis is that there is a system that controls the minimal size of a floral meristem that is independent of the mutations we have been using, this clock or size controller is not active during leaf development and might be the result of the floral program on the SAM.

The role of phytochrome C in *Arabidopsis* photomorphogenesis

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Light signals regulate plant growth and development through the action of specialised photoreceptors, the red/far-red light (R/FR) –absorbing phytochromes and the UVA/blue light (B) -absorbing cryptochromes and phototropins. In *Arabidopsis*, 5 discrete apophytochrome-encoding genes, PHYA-PHYE have been isolated and sequenced (Mathews and Sharrock 1997).

PhyB, D and E are the most recently evolved members of the phytochrome family and form a distinct subgroup, while phyC is most closely related to phyA (about 50% identity).

The analysis of phytochrome-deficient mutants has revealed different phytochromes to play both distinct and overlapping roles throughout plant photomorphogenesis. Until recently, the absence of a phyC mutant has precluded functional analysis of this phytochrome. Over-expression studies in both *Arabidopsis* and tobacco have suggested roles for phyC in leaf development (Quail et al 1997, Halliday et al 1997). The recent isolation of a phyC mutant and the subsequent creation of mutants, deficient in multiple phytochrome combinations have revealed functional roles for phyC throughout *Arabidopsis* photomorphogenesis. Such work is supported by the parallel analysis of a quadruple mutant, deficient in phytochromes A, B, D and E. Despite operating as a weak red light sensor in isolation, phyC performs a significant role in the modulation of other photoreceptors. phyA and phyC act redundantly to modulate the phyB-mediated inhibition of hypocotyl elongation in R and act together to regulate mature leaf morphology. In addition, phyC performs a significant role in the modulation of blue light sensing, possibly through interaction with cryptochrome 2 (cry2).

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The *Arabidopsis* Athkt1 mutant produces a pleiotropic effect in the shoot due to over-accumulation of Na

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The AtHKT1 protein belongs to a family of K^+ transporters present in eukariotes and prokariotes. However, electrophysiological studies in *Xenopus laevis* oocytes revealed that AtHKT1 functions as a K^+-independent highly selective Na^+ transporter (1). Mutations in the AtHKT1 gene can supress the Na^+ hypersensitive phenotype of sos3-1 mutants (2), thus suggesting that AtHKT1 is an important Na^+ influx system in plant roots.

During an *Arabidopsis* transformation experiment looking for plants with reduced gibberellin content, we isolated a T-DNA insertion mutant that showed phenotypic effects not associated with the transgene inserted (a ribozyme against the CPS gene, which encodes copalyldiphosphate synthase, a gibberellin biosynthesis enzyme). Adult rosette leaves of the mutant were shorter and had accelerated senescence compared to wild type (Columbia) leaves. Inflorescence shoots were shorter and thinner, and near 90% of the ovaries failed to produce fruits in the mutant. This phenotype could not be rescued by gibberellin aplication. Sequencing of T-DNA flanking regions and co-segregation analysis were used to determine that the described phenotypic effects were due to a disruption of the AtHKT1 gene.

The Athkt1 mutant does not show any phenotype (in the roots nor in the shoot), and its roots are not more tolerant to external Na^+ at early stages of development. The mutant is not more sensitive to lower concentrations of K^+ in the media than wild type plants. When Na^+ and K^+ concentrations were measured in the leaves, the Athkt1 mutant showed higher amounts of Na^+ and lower amounts of K^+. All together, these data suggest that AtHKT1 functions as a Na^+ transporter in *Arabidopsis*, but that it is probably involved in Na^+ transport from the shoot to the root rather that in Na^+ acquisition from the soil. The phenotype observed is probably due to the higher amount of Na^+ accumulated in the mutant shoots.

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A GTPase system in chloroplast protein import

Andreas Hiltbrunner

Most of the about 2000 chloroplast proteins are synthesised in and imported from the cytosol (1). Protein translocation across the outer and inner chloroplast membrane in *Arabidopsis thaliana* is facilitated by translocon complexes in the respective membrane (2). The Toc complex in the outer membrane consists of three major components. Toc75 is deeply embedded in the outer membrane and provides a protein conducting channel (3) whereas Toc33 and Toc159 were identified as integral membrane proteins sharing highly homologous GTP-binding domains (4, 5). However, Toc159 also exists in a soluble cytosolic form, which is able to bind to Toc33 (6). This suggests that Toc159 may act as soluble receptor for precursor proteins in the cytosol and that Toc33 might provide a docking site for cytosolic Toc159 at the outer chloroplast membrane (7).

By transient expression of GFP fusion constructs in Arabidopsis protoplasts we demonstrate that the GTP-binding domain of Toc159 is sufficient for targeting to the chloroplast membrane (8). Furthermore we show that GTP binding is essential for targeting and membrane insertion of Toc159 *in vivo* and *in vitro* and that a Toc159 version impaired in GTP binding is not able to complement a Toc159 T-DNA insertion mutant (8, 9).

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Molecular characterization of the auxin-resistant6 gene

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The auxin-resistant6 mutations cause pleiotropic defects in plant growth and development, including alterations in embryonic pattern formation and vascular development, altered lateral root growth, and altered response to gravity. All the defects are likely to be due to a general reduction in auxin sensitivity in axr6 mutant plants (Hobbie et al., 2000). We now show that the AUXIN-RESISTANT6 gene is identical to the gene that encodes cullin1. This protein is a component of the SCF complex involved in targeting various proteins for degradation by the ubiquitin-proteasome pathway. Knockouts of cullin1 were previously shown to be lethal at a very early embryonic stage (Shen et al., 2002). The semidominant axr6 mutations result in single amino acid c hanges in the cullin1 protein that appear to alter SCF complex formation. Thus, these results strongly support a model in which SCF-mediated protein degradation is a crucial step in auxin signal transduction.

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Characterization of a narrow leaf mutant of Arabidopsis, angustifolia3, suggests the presence of a mechanism controlling cell division orientation in the leaf-width direction

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Leaf is a flat organ and its shape flexibly changes in response to various environments and physiological conditions. To understand such flexible nature of leaf shape control, basic mechanisms involved in the determination of leaf shape should be elucidated. We have proposed that the two-dimensional growth of leaf is controlled, at least in part, by polar cell expansion. ANGUSTIFOLIA (AN) and ROTUNDIFOLIA3 (ROT3) genes, encoding a CtBP-like protein and a cytochrome P450, are involved in the control of polar cell expansion in leaf-width and leaf-length directions, respectively.

On the other hand, contribution of cell division orientation in the determination of leaf shape has been unclear. To further understand the molecular basis of leaf morphogenesis, we characterized an additional leaf-shape mutant, an3. Wild-type leaves and an3 leaves were similar in length, while the width of an3 leaves was narrower than that of wild-type plant. Leaf cell shape (ratio of cell length versus cell width) in an3 mutant is not significantly different from that of wild-type plants. In contrast, the number of leaf cells was reduced preferentially in the leaf-with direction when compared to that of wild-type plant. These observations suggest that the cell division orientation in the leaf-with direction and the leaf-length directions are controlled by different mechanisms. In addition, separate pathways regulate the leaf cell expansion and the cell division along the leaf-width direction since an an an3 double mutant exhibits an additive phenotype. Progress of positional cloning of AN3 gene will also be presented.

GOLIATH, a novel gene controlling flowering time

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GOLIATH (GOL) is a novel gene involved in the photoperiodic induction of flowering in Arabidopsis. The goliath mutant flowers later than WT in long days (LD) but at the same time as WT in short days (SD), resembling the flowering phenotype of the constans mutant. The gol mutation is a recessive mutation caused by insertion of a Ds element, the effects of gol and co are not additive in the double mutant suggesting that they are acting in the same pathway. Genomic sequences flanking the Ds insertion were cloned by inverse PCR, analysis of these sequences revealed that GOLIATH encodes a RING finger protein, the Ds element having inserted in the promoter just downstream of the TATA box. An independent SALK insertion mutant was obtained that has an insertion in the RING finger domain of GOLIATH, this SALK line is also late flowering in LD.

RING finger proteins are E3 ligases which direct the ubiquitination of specific proteins thus targeting them for degradation. As GOLIATH is essential for the photoperiodic induction of flowering in LD, it is possible that it targets a repressor of flowering for degradation in inducing LD conditions. In the goliath mutant this repressor would not be degraded in LD, resulting in late flowering.

A regulatory role for salicylic acid in the transition to flowering in *Arabidopsis*

Cristina Martinez and Jose Leon

Salicylic acid (SA)-deficient plants, known to be defective in pathogen-activated defenses, exhibit late-flowering phenotypes and high levels of FLC transcript both under long- and short-day photoperiods.

Exogenous application or enhanced production of SA by UV-irradiation partially rescued the late-flowering phenotype of SA-deficient sid mutants but not of transgenic nahG plants unable to accumulate SA. Short-days grown SA-deficient genotypes are all responsive to vernalization. Under long-days, the late-flowering phenotype of SA-deficient plants correlates to a lower expression levels of photoperiod-dependent flowering time genes CO, FT and SOC1 and higher expression of FLC than Columbia wild-type plants. We have generated a double mutant transgenic flc3 nahG plant, which is null for FLC gene and allowed us to conclude that photoperiod-dependent and SA-mediated transition to flowering is independent of FLC under long-days. However, SA-mediated transition to flowering in short-days grown plants may proceed both through FLC-dependent and FLC–independent pathways.

Light controls shoot apical meristem activity and leaf development in *Arabidopsis*

Enrique López-Juez, Edyta Dillon and Laszlo Bögre

One dramatic difference between the 'etiolated' programme of growth of seedlings, that happening while in darkness, versus that in the light is that in many species, including *Arabidopsis*, leaves and other aerial organs only form in the light. The shoot apical meristem is formed during embryogenesis, but it obviously remains repressed during seedling growth in the dark. Surprisingly little is known about how does this dark-repression or subsequent photoreceptor de-repression take place. In an attempt to understand this, we have monitored the morphology of the meristem in wild type *Arabidopsis* seedlings, upon transition from dark to white light. Changes in the meristem and leaf primordia become externally apparent between 24 and 48 hours after the transfer. To test photoreceptor-dependence we have generated a triple mutant containing the mutations hy1 (which almost completely blocks the synthesis of the chromophore for all phytochromes) cry1 and cry2 (for the two cryptochromes). Both this triple mutant and a quadruple phyA phyB cry1 cry2 (provided by J. Casal, Univ. Buenos Aires) show a dramatic slow-down in meristem derepression in the light, which hy1 alone shows only to a small extent and cry1 cry2 does not show. This indicates a co-requirement of phytochrome and cryptochrome in a redundant manner. We have also confirmed that, as previously reported, meristem derepression does happen in absence of light in a de-etiolated 1 (det1) mutant or in a wild type grown on vertical sucrose-containing plates (with direct sucrose access to the apex). We are currently undertaking an analysis of the expression of cell cycle regulators and meristem marker genes during this transition, in collaboration with the group of J. Murray (Univ. Cambridge). Progress in this analysis will be presented.

A conserved functional module controlling distinct photoperiodic responses in different plant species

Jaime F. Martínez-García, Ariadna Virgós-Soler and Salomé Prat

Photoperiod controls several responses throughout the plant life cycle, like germination, flowering, tuber formation, onset of bud dormancy, leaf abscission and cambium activity. From these processes, flowering has been most extensively studied, specially in *Arabidopsis thaliana*. Photoperiod sensing by the function of photoreceptors and the circadian clock appears to regulate flowering time via CONSTANS (AtCO), a putative transcription factor that accelerates flowering in response to long-days (LD). In contrast, the genetic factors controlling plant photoperiodic responses other than flowering, like tuberization, are little known. In the wild species of potato Solanum tuberosum spp. andigena tuberization is strictly dependent on photoperiod for tuberizing: under LD (16h light/ 8h darkness) plants do not tuberize, whereas under short-days (SD, 8h light/ 16h darkenss) plants produce tubers.

Grafting experiments demonstrated that leaves from potato plants induced to tuberize caused non-induced stocks (that contain the stolons) onto which they were grafted to tuberize, indicating that tuber-inducing signals (tuberigen), like the flower-inducing ones (florigen), are produced in the leaves. On the other hand, interspecific grafts between tobacco shoots (from LD, day-neutral and SD cultivars) and potato stocks resulted in tuberization only when the resulting chimeras were growing under the corresponding inductive photoperiod for the tobacco part (reviewed in Jackson, 1999), demonstrating that the florigen and tuberigen signals are functionally exchangeable. To investigate the possible existence of common genetic factors and mechanisms controlling both photoperiodic flower and tuber formation, the AtCO gene has been overexpressed in potato. Under inducing (SD) conditions, AtCO overexpression impairs tuberization; AtCO overexpressing lines require prolonged exposure to SD to form tubers. Grafting experiments using these lines indicated that AtCO exerts its inhibitory effect on tuber formation by acting in the leaves. Our data suggest that a conserved photoperiodic functional module may be involved in controlling distinct photoperiod-regulated "evocation" responses in different species. This module would involve the action of CONSTANS in the production of the elusive and long-distance acting florigen-tuberigen signal(s) in the leaves.

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Genetic analysis of natural variations in the architecture of *Arabidopsis thaliana* vegetative leaves

José Manuel Pérez-Pérez, José Serrano-Cartagena and José Luis Micol

To ascertain whether intraspecific variability might be a source of information as regards 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. A total of 188 accessions from the *Arabidopsis* Information Service collection were grown and classified into 14 phenotypic classes, defined according to petiole length, marginal configuration, and overall lamina shape.

Accessions displaying extreme and opposite variations in the above-mentioned leaf architectural traits were crossed and their F2 progeny was found to be not classifiable into discrete phenotypic classes.

Furthermore, the leaf trait-based classification was not correlated with estimates on the genetic distances between the accessions being crossed, calculated after determining variations in repeat number at 22 microsatellite loci. Since these results suggested that intraspecific variability in *Arabidopsis thaliana* leaf morphology arises from an accumulation of mutations at quantitative trait loci (QTL), we studied a mapping population of recombinant inbred lines (RILs) derived from a Landsberg erecta-0 x Columbia-4 cross. A total of 100 RILs were grown and the third and seventh leaves of 15 individuals from each RIL were collected and morphometrically analyzed. We identified a total of 16 and 13 QTL harboring naturally occurring alleles that contribute to natural variations in the architecture of juvenile and adult leaves, respectively.

Our QTL mapping results confirmed the multifactorial nature of the observed natural variations in leaf architecture.

Repression of flowering under short days. The role of EBS

Manuel Piñeiro, Concepción Gómez-Mena, George Coupland and Jose Miguel Martínez-Zapater

Mutations in the EARLY BOLTING IN SHORT DAYS (EBS) gene of *Arabidopsis* cause an acceleration of flowering, especially under non-inductive photoperiods (short days-SD). Genetic analyses have demonstrated that the early flowering phenotype of ebs mutants requires both the functional product of FT gene and gibberellic acid (GA) biosynthesis. In addition to early flowering, ebs mutants show increased expression of floral organ identity genes such as AGAMOUS, APETALA3 and PISTILATA within the flowers (Gómez-Mena et al., 2001).

We have identified the EBS locus, and the predicted aminoacid sequence of the protein suggests that EBS could be part of a protein complex involved in the repression of gene expression by modulating chromatin structure.

Since EBS is likely to act as a transcriptional repressor we have analysed the expression of flowering time genes in ebs mutants. These analyses have demonstrated that FT gene is prematurely expressed in ebs mutants grown under SD, whereas the expression of other genes involved in the control of flowering time and GA biosynthesis is not affected in ebs mutants. These results indicate that the repression of flowering by EBS is mediated by its effect on FT expression. In addition, we have analysed the effect of overexpressing EBS on the flowering time of Arabidopsis; as for the loss of function ebs mutant alleles, 35S:EBS plants display early flowering, and this phenotype appears to be mediated also by FT. The early flowering phenotype of the EBS overexpressing lines is consistent with the hypothesis that EBS could be part of a protein complex, and the accumulation of EBS product in 35S:EBS plants could disrupt the proper formation or function of the complex. Our results indicate that EBS is required to delay the onset of flowering repressing FT expression under SD. Progress in understanding the molecular mechanism of EBS function will be discussed.

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AtBRM, an ATPase of the SNF2 family, controls shoot development and flowering in *Arabidopsis thaliana*

Sara Farrona, Lidia Hurtado and Jose C. Reyes

Activation and repression of gene transcription is mediated by changes in the structure of the chromatin. Ones established, cell type-specific pattern of gene expression must be stable over many cell generations and this is accomplished by labeling the chromatin (mostly DNA and histones) with epigenetic marks. ATP-dependent chromatin remodeling machines are involved in these processes and its role in plant development is an exciting new field. We are investigating the role of AtBRM an *Arabidopsis thaliana* homolog of Brahma, the DNA-dependent ATPase of the *Drosophila* SWI/SNF complex. AtBRM contains the characteristics domains of the SNF2 subfamily of proteins: a DNA-dependent ATPase domain, a bromodomain and an AT-hook able to bind DNA inspecifically. AtBRM is a nuclear protein mostly expressed in inflorescences and in leaves. We have generated by RNAi *Arabidopsis* lines with reduced levels of AtBRM. The atbrm plants present a pleiotropic phenotype characterized by abnormal plant architecture, organ development and flowering time.

Cloning of constans homologues from potato

Paula Suárez-López, Nora García, Jaime F. Martínez-García and Salomé Prat

Flowering and tuberization are reproductive processes regulated by photoperiod. Flowering of *Arabidopsis thaliana* is accelerated by long days and delayed by short days, whereas potato tuberization is promoted by short days and delayed by long days. In *Arabidopsis*, the gene CONSTANS (CO) is required in long days to accelerate flowering and its overexpression causes photoperiod-insensitive early flowering.

Overexpression of *Arabidopsis* CO in potato delays tuberization under short day conditions, suggesting that CO homologues might be involved in the photoperiodic control of tuber formation in potato. To identify CO homologues in potato, the *Arabidopsis* CO sequence was used to search in the EST databases. Several ESTs corresponding to two potato genes showing homology to CO were identified. Additionally, several PCR products were amplified from a potato genomic library using degenerate primers deduced from the conserved domains of CO. Sequencing of these PCR products identified a third CO-like gene. Full-length cDNAs corresponding to these three genes have been cloned. We are currently generating potato plants that overexpress these genes to study their function.

Control of stomatal development by the pleiotropic COP/DET/FUS genes

Javier Torres-Contreras and Carmen Fenoll

Most of the gas exchange between a plant and its environment is performed through small epidermal structures known as stomata. Both endogenous and environmental signals modulate stomatal opening and, thus, they continuously control gas exchange. Stomata density and distribution also influence overall gas exchange, and therefore, stomatal production and stomatal patterns in epidermal surfaces are important. To understand how light influences stomata development, we analysed the stomatal phenotype of the cop/det/fus mutant class, because these mutants represent a convergence point of multiple light signal transduction pathways. We have found that COP/DET/FUS genes contribute to at least two pathways: a light-dependent one, involved in the repression of stomatal production, and a light-independent one that contributes to the repression of both stomatal production and formation of stomata in direct contact. Both pathways can be genetically dissected, because some cop/det/fus mutants show alterations only in the first pathway but others show alterations in both of them. While the light-dependent pathway acts just at the entry into the stomatal pathway (the acquisition of meristemoid mother cell fate), the light-independent pathway acts at this point but also at subsequent steps: the acquisition of guard mother cell fate, the orientation of asymmetric divisions during stomatal development, and the response to cell communication events between different epidermal cell types and adjacent pre-formed stomata. The relationships between both pathways in the context of the role of COP/DET/FUS genes as both light switches and general developmental regulators will be discussed.

Global gene expression changes indicate distinct perception and signaling mechanisms in response to UV-B irradiation

Roman Ulm, Alexander Baumann, Attila Oravecz, Markus Funk, Eberhard Schäfer, Edward Oakeley and Ferenc Nagy

During evolution plants have optimized their ability to collect solar radiation, the vital source of biological energy. Part of the incident light encompasses a fraction of the UV-B region (280-320 nm) that, in contrast to solar UV-C (<280 nm), is not entirely absorbed by the ozone layer in the stratosphere of the earth. This fraction of the solar radiation that inevitably reaches the sessile plants is not merely an environmental stress but can cause morphogenetic effects through molecularly yet unidentified UV-B photoreceptor(s) as well, both influencing the architecture and performance of plants. The UV-B mediated changes include, for example, reduced whole plant biomass, hypocotyls growth inhibition and cotyledon expansion. These phenotypic alterations pose three major, mostly unanswered questions at present: (i) what are the photoreceptors that mediate UV-B action, (ii) what are the signalling components that transduce the photomorphogenic UV-B signal and (iii) what is the interplay of the UV-B responses with other environmental cues that results in an optimal adaptation of the organism to the complex natural environment.

To address these questions, we have performed global transcriptional profiling comparing responses of Arabidopsis to different UV-B wavelength ranges. This analysis identified interference between different UV-B ranges that was not anticipated before. Results postulating the presence of partly distinct, but interfering UV-B perception mechanisms, and possible signaling components will be presented.

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