

## P9—THE CONTROL OF FLOWER INITIATION

Organised by S. Jackson for the Plant Development group and sponsored by Bio-Rad

### P9.1—A thermosensory pathway controlling flowering time in *Arabidopsis*

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Flowering time is controlled by environmental signals such as light and temperature, and by endogenous cues such as hormone levels and sensitivity. Molecular-genetic studies in *Arabidopsis* have focused on photoperiodic regulation and vernalization. Yet, ambient growth temperature has been largely ignored. We have found that genes of the autonomous pathway, previously thought only to act independently of the environment as regulators of the floral repressor *FLC* are involved in mediating the effects of ambient temperature. In contrast to wild-type plants, and mutants in other pathways, *fca* and *fve* mutants 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*. In contrast, the exaggerated response to temperature of *cryptochrome2* mutants is probably caused by temperature-dependent redundancy with the phytochrome A photoreceptor.

### P9.2—Strategies for understanding the mechanisms of floral maintenance in an annual (*Impatiens balsamina*) and a perennial (*Fragaria vesca*) species

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Many plants flower more than once. Where this is the case, mechanisms must exist for reversing the state of floral induction, and controlling which meristems convert to flowers. With the long-term aim of understanding how this regulation is achieved, we are studying floral maintenance in *Impatiens balsamina* and the cycle of flowering in *Fragaria vesca*.

In *Impatiens* we have isolated putative homologues of *LEAFY*, *TERMINAL FLOWER 1* and *AGAMOUS*, genes that are candidates for a role in floral maintenance based on their function in *Arabidopsis* and *Antirrhinum*. Our strategy is to analyse their function by expressing these sequences in *Arabidopsis*, and altering their expression in transgenic *Impatiens*. Progress towards this goal will be described for *LEAFY*.

In the perennial *F. vesca* we are adopting a positional cloning strategy for isolation of the single gene whose dominant allele confers seasonality on flowering. In the presence of only the recessive allele plants flower continuously during the growing season. Measurements over several years suggest that individuals that pursue this strategy have a shortened lifespan, particularly when continuous flowering is combined with stolon production. This indicates an interaction between seasonality of flowering and the perennial habit. Progress towards isolation of the seasonality locus will be described.

### P9.3—Photoperiod response in long and short day plants is mediated by a conserved genetic pathway

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Flowering of *Arabidopsis* is triggered by long day-lengths (photoperiods) and by extended exposure to low temperatures (vernalization). Both responses ensure that plants flower during spring or early summer, and are controlled by independent genetic pathways. We have 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-days. 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 is 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*.

**P9.4—Irreversible expression of evocation genes in the shoot apical meristem of *Sinapis alba* during reversion from reproductive to vegetative morphogenesis: a clue to the plant memory of floral induction**

F. Bonhomme, A. Jacqumard and G. Bernier, Plant Physiology, University of Liège, Belgium

Although the cellular memory of a vernalization experience is a well-documented process in the flowering field, the question of the memory of a photoinduction episode has been much less investigated and is still a largely unsolved problem. During the life cycle, the SAM of the long-day plant *Sinapis alba* produces successively three metamer types: Type 1, vegetative; Type 2, cofilence-bearing; Type 3, flower-bearing. Physiological treatments were devised for causing the shift back from Type 3 to Type 1 metamers (reversion from reproductive to vegetative morphogenesis). In situ hybridisation was used to study expression of the evocation gene *SaMADSA*. This gene is upregulated in the *Sinapis* SAM early during floral transition and is the putative ortholog of the *Arabidopsis* *SOCI* gene (1). We observed that *SaMADSA* expression, once upregulated in the SAMs producing Types 2 and 3 metamers, remained high in vegetative reverted SAMs. On the other hand, these SAMs were found to differ by subtle histological features (bombing, precocious activation of axillary meristem sites, lack of rib meristem) from the SAMs of plants that were continuously kept vegetative since sowing, indicating that reverted SAMs, despite their return to vegetative morphogenesis, have kept a memory of their previous flowering experience. Irreversible activation of *SaMADSA* might be one mechanism responsible for this SAM memory. Funded by PAI 4/15.

(1) Bonhomme, Kurz, Melzer, Bernier and Jacqumard, Plant J. 24, 103, 2000.

**P9.5—The induction of flowering in *Impatiens balsamina***

F. Tooke, Department of Plant Sciences, Cambridge University; N.H. Battey, School of Plant Sciences, The University of Reading

*Impatiens balsamina* cv. Dwarf Bush Flowered displays a dependence on photoperiod for floral maintenance. In this plant flowering is induced by short days (SD), which must persist throughout the life of the plant if the flower is to be completed and reversion to leaf production prevented. Thus transferring the plants from SD to long days (LD) results in floral reversion.

We are studying photoperiodic responses of a reverting and non-reverting line (which is able to continue flowering when transferred from SD to LD), and progeny derived from crossing these two lines. Our aim is to understand how leaf-based signals are involved in controlling flower development at the meristem. We have found that once leaves of the non-reverting line are induced in SD, they can continue to promote flowering at the meristem subsequent to transfer to LD. This promotion may be achieved either by perpetuation of a signal initiated under SD, or by the production of a novel signal in LD. This appears not to be the case for the reverting line in which all floral promotion is lost with transfer to LD. The simplest interpretation of segregation ratios derived from F2, F3 and backcross populations suggests that reversion is controlled by a single major gene.

The underlying molecular basis of leaf signals involved in floral maintenance is being analysed by screening a subtractive cDNA library constructed using mRNA from leaves of reverting and non-reverting lines collected after transfer from SD to LD conditions.

**P9.6—Molecular basis of vernalization requirement and response**

C. Dean, L. Barrett, R. Bastow, P. Boss, S. Gazzani, T. Gendall, N. Geraldo, I. Henderson, R. Laurie, C. Lister, J. Mylne, N. Pauly, V. Quesada, G. Simpson. John Innes Centre, Norwich

A network of pathways has been identified that integrates the multiple environmental and endogenous inputs that control the timing of the transition to flowering. The Dean group has focused on the acceleration of flowering by a long period of cold temperature or 'winter', a process known as vernalization. Vernalization has been shown to reduce RNA levels of the floral repressor, *FLC*. In *vrn* mutants, *FLC* RNA is down-regulated normally in response to the cold but instead of remaining low *FLC* levels increase during the subsequent development of the plant in the warm. We are identifying the *VRN* genes required to cause the initial down-regulation of *FLC* and characterizing those involved in maintaining the cellular memory of vernalization.

We are also determining the molecular mechanisms that determine vernalization requirement. In the majority of *Arabidopsis* accessions active alleles at *FRIGIDA* strongly repress flowering until the plant has been vernalized. *FRIGIDA* function thus confers a dominant vernalization requirement ensuring that plants overwinter vegetatively. Loss-of-function mutations in genes of the autonomous floral pathway (eg. *FCA* and *FY*) have a late flowering phenotype that can be suppressed by vernalization. *fca*, *fy* mutations thus confer a recessive vernalization requirement. The mechanism of action of

FRIGIDA, FCA and FY is being investigated. We are also trying to establish the molecular basis for the changing predominance of these different floral pathways during seasonal progression.

### **P9.7—U Don't RING me flowers anymore!**

K. Morris, J. Holmes, L. Codrai (HRI, Wellesbourne), A. Huttly (IACR, Long Ashton), I. Carré (University of Warwick) and S.D. Jackson (HRI, Wellesbourne)

The transition to flowering is a complex process that is regulated by many different mechanisms. A fundamental component of any of these mechanisms is the link between environmental conditions and the molecular processes that promote flowering. We will introduce a novel late flowering mutant of *Arabidopsis*, *goliath*, which we have positioned on the long day photoperiodic promotion pathway. The disrupted gene in *goliath* codes for a RING zinc finger protein. Many RING finger proteins act as E3 ligases targeting proteins for ubiquitination and subsequent proteolytic degradation. The late flowering phenotype of this mutant has led us to hypothesize that GOLIATH targets a repressor of flowering for degradation. We will present data showing how the transcriptional regulation of *GOLIATH* may function in the transition to flowering.

### **P9.8—Differences in the circadian clockwork between long day plants and short day plants**

P.J. Lumsden, University of Central Lancashire

Photoperiodic time measurement is determined by a circadian rhythm in sensitivity to light, the photoperiodic response rhythm (PRR). In short-day plants this is set by the transition from light to dark, and is observed as either the rhythmic flowering response to increasing durations of darkness, or, more usually, the rhythmic flowering response to the time of a light interruption of an extended dark period. An external coincidence model, in which light controls the phase of the oscillator and interacts with the observed rhythmic process, accommodates the basic components of a circadian system: rhythmic output, light input, and underlying oscillator. Incorporating a limit cycle model to describe the behaviour of the oscillator also explains at least some of the reported evidence supporting the existence of a semidiurnal rhythm in *Pharbitis nil* and for its involvement in time measurement. This evidence will be reviewed.

Flowering in long-day plants (LDP) requires (or is accelerated by) exposure to long photoperiods, characterised by a changing responsiveness to R and FR during the daily cycle. While physiological experiments into rhythmicity in LDP are difficult, significant advances have been made in *Arabidopsis*. Using data from

mutants such as *elf3* (with altered flowering response) the behaviour of the *Arabidopsis* photoperiodic oscillator will be compared to that operating in short-day plants.

### **P9.9—Genetics and Models of the Photoperiodic switch**

A.J. Millar, A. Hall, P.E. Brown, Biological Sciences; J. Locke, M.S. Turner, Physics; B. Shulgin and D.A. Rand, Mathematics, University of Warwick

About 20 interacting genes have been shown to function in the core of the circadian system and the photoperiodic sensor, excluding light signalling genes. We have identified new components of the plant circadian clock, including a small protein, ELF4, which functions at the end of the day. Increasingly detailed results from molecular genetics do not necessarily lead to greater understanding of such a regulatory network. Mathematical modelling provides an invaluable, complementary approach. We are developing 'complete' models for the plant clock and photoperiod sensor, which incorporate the molecular components in a realistic manner. 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 in very complex models. We are also analysing classic circadian protocols, such as skeleton photoperiods. Numerical simulations using such models have enormous potential relevance to molecular experiments but are rarely used in the biological community, due to their apparent inaccessibility. Our aim is to model the flowering control circuit in a package that is accessible to experimental biologists. Thus we are developing user-friendly modelling software based on the most widely-used spreadsheet programme, which will simulate all common circadian and flowering time experiments. This will be freely available from our website, with ongoing support and revisions. Ultimately such work may help growers to predict floral initiation in the field, and suggest crop improvement strategies to evoke desired responses. Funded by BBSRC and DTI.

### **P9.10—New components in the autonomous pathway of flower induction in *Arabidopsis***

J.M. Martinez-Zapater, I. Ausin, C. Alonso-Blanco, J.A. Jarillo and L. Ruiz-Garcia (CNB, Madrid)

Abstract not supplied

### **P9.11—Elucidating the ubiquitin mediated proteolysis of a floral repressor**

J. Holmes, K. Morris, L. Codrai (HRI, Wellesbourne), A. Huttley (IACR, Long Ashton), I. Carré (Warwick) and S.D. Jackson (HRI)

*Goliath* is a novel flowering time mutant of *Arabidopsis* that has been positioned on the long day (LD) photoperiodic promotion pathway. The response to photoperiod is determined by a complex set of interactions between specific photoreceptors and the circadian clock. Sequence analysis has revealed that *GOLIATH* encodes a novel RING-finger protein. RING-finger proteins act as components of the ubiquitin proteasome pathway which targets specific proteins for degradation via the 26S proteasome. *Goliath* flowers at the same time as WT plants in short day (SD) conditions due to the induction of flowering through the autonomous pathway. However the late flowering phenotype of *goliath* in LDs suggests that it is defective in a photoperiodic inductive signal normally seen in LDs. It is hypothesized that *GOLIATH* targets a repressor of flowering for degradation in inducing LD conditions. We will present evidence that positions *GOLIATH* downstream of the circadian clock on the LD photoperiodic promotion pathway. We will also present an approach to identify the putative repressor of flowering by epitope tagging *GOLIATH* and performing pull down experiments in planta followed by mass spectrometry to identify purified proteins.

### **P9.12—Cell-to-cell communication in the shoot apical meristem of *Sinapis alba* during floral transition**

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Previous studies showed that the tunica of vegetative shoot apical meristems (SAM) is symplasmically subdivided into a central and a peripheral field, which restricts symplasmic diffusion of metabolites and signalling molecules to the cells inside the field boundaries (1). The fields keep their position at the SAM, even though cells are continuously displaced towards the periphery, showing that they are dynamically maintained. The resulting separation of SAM centre and periphery might safeguard the distinct roles of these areas in primary development. As during the floral transi-

tion SAM function is changing, the symplasmic subdivision might also be subject to change. We investigated whether such alteration occurs during the floral transition in *Sinapis alba*, a plant that is induced by exposure to a single long day. Iontophoretic microinjection experiments, using Lucifer Yellow CH (a fluorescent non-toxic membrane impermeant probe of 457 Da), into single superficial cells showed that, in the induced SAM, the size of the central symplasmic field (CSF) increases not only in absolute terms but also in proportion to SAM size. In addition, the CSF shape changes from triangular to circular. The size and shape changes, detectable already during the first short day after the long day, may serve the re-balancing of the transitional SAM, thereby supporting production of a novel type of appendages in an altered phyllotactic pattern (2).

Funded by PAI 4/15.

(1) van der Schoot and Rinné, Trends Plant Sci. 4, 31, 1999.

(2) Ormenese, Havelange, Bernier and van der Schoot, Planta 215, 67, 2002.

### **P9.13—Modelling the Biological Clock of *Arabidopsis***

James Locke, Matthew Turner, Andrew Millar, Depts of Biological Sciences and Physics, University of Warwick

The molecular mechanisms of the circadian clock in *Arabidopsis*, although less well understood than that in other organisms, e.g. *Neurospora* and *Drosophila*, are fast being revealed through gene-chip and luciferase data experiments. A computational model of the circadian clock will allow the prediction of the most valuable experiments to carry out, and allow us to differentiate between several equally viable models. We are now producing simple mathematical models, including biochemically realistic components, for the central oscillator.

### **P9.14—The Control of Flower Initiation – The Development of User-Friendly Circadian Modelling Software for the Biological Community**

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Approximately 20 interacting genes have been shown to function in the circadian clock and photoperiodic system

of *Arabidopsis*. Mathematical modelling provides an invaluable tool for understanding such complex systems. We are developing 'complete' models for the plant clock and photoperiod sensor, incorporating molecular components in a realistic manner. Numerical simulations should become directly relevant to molecular experiments. We have established a novel analytical method to assess the contribution of each model component at each phase of the cycle, helping 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 such systems, 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 accessible to experimental biologists. Thus we are developing user-friendly modelling software to simulate all common circadian and flowering time experiments. An intuitive interface allows users to specify 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 and new experimental data. Ultimately our work will allow prediction of floral initiation in the field, and suggest crop improvement strategies to evoke desired responses. Funded by BBSRC and DTI (UK).

### **P9.15—Circadian Clock Mutants in *Arabidopsis***

M.M. Southern, A. Hall, M. Eriksson, V. Ravenscroft, S. Hanano, S.J. Davis & A.J. Millar, Dept. of Biological Sciences, University of Warwick

The circadian clock allows anticipation of and co-ordination with the Earth's daily cycles of light and temperature. In plants the circadian clock controls leaf and flower movement, growth, photosynthesis and ~2–6% of *Arabidopsis* transcripts. The clock is required to measure the photoperiod, detecting the changing seasons, and partially determining when the plant flowers. The molecular mechanisms of the circadian clock in plants are less well understood than those of animals or fungi. To unravel its workings we use two different genetic approaches in the model higher plant *Arabidopsis thaliana*. To find mutant individuals we screened EMS and T-DNA mutagenised *Arabidopsis* for circadian defects using a luciferase reporter gene assay, imaging in fully automated Topcount 96-well plate counters and low-light video cameras. We found many mutants, and

I am characterising and cloning some of these. One new *gigantea* allele has long period *CAB2* expression rhythms and short period leaf movement, like *gi-2*, though its flowering time is close to wild type, separating *GI*'s circadian and flowering functions. Wild-type *Arabidopsis* flower later under short days than long days. Early flowering mutants may be insensitive to photoperiod, possibly due to an aberrant circadian clock. Therefore, the lab has screened early-flowering lines for circadian phenotypes, which I am characterising.

I will present data on these genes. Although many genes involved in the *Arabidopsis* circadian clock are known, the screens are not yet saturated. More knowledge of the molecules involved is needed to fully understand how they interact to form a clock.

### **P9.16—Locus replication and the floral transition in *Brassica***

G.R. Teakle, E.P. Kop, McClenaghan and G.J. King (HRI)

A predictable transition from vegetative to floral growth is of key importance in the production of many crops. This transition has been extensively characterised in *Arabidopsis* and a complex picture of interacting environmental and endogenous signals has emerged that converge on genes that control floral meristem identity. *Arabidopsis* has a simple diploid genome whereas a large number of crop species are polyploid or derived from ancestral polyploids. *Brassica oleracea* is in the latter category with an average triplication of its genome originating from a hexaploid progenitor. Its close relationship with *Arabidopsis* makes it an excellent model to study the effect of increased gene copy number on the potential for increased regulatory complexity in a crop species. *B. oleracea* is morphologically diverse and includes varieties which arrest at a range of stages through the progression to flowering. Cauliflower curds arrest at the earliest stage of the transition and consist of a highly proliferated inflorescence meristem which represents a valuable source of this normally limiting tissue. We are using a combination of approaches to identify and characterise the individual roles of replicated regulatory genes associated with the floral transition in *B. oleracea*. These include the molecular genetic analysis of curd development, an analysis of the genes expressed in curds and an analysis of the promoters of replicated paralogues of the *FRUITFULL* MADS-box gene and the regulation patterns they confer. These studies will contribute to our understanding and hence our ability to control the floral transition in brassicas.

**P9.17–FY, a 3'-end processing factor performs both flowering-time and essential functions**

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The 3'-end processing machinery is highly conserved throughout eukaryotes and functions to catalyse the cleavage and polyadenylation of pre-mRNA. We have identified the flowering time gene *FY* as encoding a protein with strong homology to 3'-end processing factors such as yeast Pfs2p. *FY* is unique relative to these homologs in that it has acquired a novel C-terminal domain. Through this domain *FY* interacts physically with the novel RNA binding protein FCA to repress the floral repressor *FLC*.

To investigate *FY* function we have characterised an allelic series of mutations in this gene. We demonstrate that mutations affecting the C-terminal domain are late-flowering and viable. However, a null *fy* mutation terminating the WD repeats is lethal, revealing the late-flowering alleles as hypomorphic. Hence, *FY* performs dual flowering-time and essential functions. This essential function likely reflects a conserved role for the *FY* WD repeats in mRNA processing, while acquisition of the C-terminal domain appears to have facilitated a novel role in the floral transition.

**P9.18—Using the lcycler to measure the effect of altered t-cycles on CO expression**

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Daylength is used by plants as an indicator of the time of year and this is important in deciding when to flower. Plants measure daylength through the use of an internal clock (the circadian clock), which controls the rhythmic expression of a large number of genes including a key gene involved in the control of flowering called *CONSTANS (CO)*. Light has to interact with high levels of expression of *CO* for flowering to be induced. In *Arabidopsis* this normally happens in long days when flowering is induced, but not in short days which are non-inductive for flowering. We demonstrate that by changing the rhythm of the circadian clock using different t-cycles, the rhythm of expression of *CO* is altered so that flowering can be induced in certain t-cycles even in short days.