Flowering on time: genes that regulate the floral transition

Workshop on the molecular basis of flowering time control

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Received October 2, 2001; revised and accepted October 29, 2001

The workshop was held at the John Innes Centre, Norwich, UK, July 11–14, 2001. <http://www.jic.bbsrc.ac.uk/events/embo/>. Workshop Administrator: Dee Rawsthorne, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, Norfolk, UK (Tel: +44 1603 450527; Fax: +44 1603 450025; E-mail: dee.rawsthorne@bbsrc.ac.uk).

flowering time in response to environmental and endogenous cues (reviewed by Simpson *et al.*, 1999). Regulation occurs through a complex network of genetic pathways, with two main pathways mediating environmental responses (the long-day and vernalization pathways) and two pathways that function independently of environmental cues: the autonomous pathway, which promotes flowering under all conditions, and the gibberellin (GA) pathway, which is needed for flowering under non-inductive short-day conditions. These pathways converge in the induction of floral meristem identity genes and the floral transition (Figure 2). Many flowering-time genes have been cloned recently, and the primary aim of the EMBO workshop in Norwich, and this report, was to highlight the most recent advances in our understanding of the molecular mechanisms regulating the floral transition.

Introduction

Successful sexual reproduction in plants depends on the recognition of favourable environmental conditions and the integration of that information with endogenous developmental cues. Flowering in higher plants involves the transition of a vegetative meristem, producing leaves and stems, into a floral meristem, producing flowers. Most of our understanding of the regulation of the floral transition has been developed in the past 10 years using molecular genetic approaches in the model plant *Arabidopsis thaliana*, a rosette plant in which the initiation of flowering is followed by the elongation of the main stem. The two main environmental factors that promote flowering in *Arabidopsis* are long days (Figure 1) and exposure to low temperatures (vernalization). Genetic and physiological analysis of flowering time in *Arabidopsis* has led to the identification of a large number of flowering-time genes (>80) that regulate

Converging flowering pathways and the *FLC* floral repressor

Research by the Dennis (Canberra, Australia) and Amasino (Madison, WI) laboratories in the past 2 years has shown that a number of signals controlling flowering converge in the regulation of the *FLC* gene, which encodes a MADS-box transcription factor and represses flowering (Michaels and Amasino, 1999). Repression of *FLC* is caused by a set of genes that control the autonomous pathway. *FCA* is an important component of the autonomous pathway and its expression is characterized by complex regulation that includes alternative splicing. *FCA* encodes an RNA-binding protein that contains a WW protein-interaction domain. Recent work has shown that one protein that interacts with this WW domain in *FCA* is the one encoded by *FY*, another gene placed in the autonomous pathway based on genetic analysis (G. Simpson, Dean laboratory, Norwich,

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Fig. 1. Flowering in *Arabidopsis* is promoted by long-day conditions. *Arabidopsis* plants were grown under short-day conditions (left; 10 h light, 14 h dark) or long-day conditions (right; 16 h light, 8 h dark).

UK). Other genes in this pathway repress *FLC* independently of *FCA/FY* and include the recently isolated genes *FPA*, which encodes RNA recognition motifs (Amasino laboratory), and *FVE*, which encodes a WD40 repeat protein with similarity to proteins involved in transcription repression complexes (Zapater laboratory, Madrid, Spain).

Cold treatment (vernalization) is another effective repressor of *FLC*. In late-flowering ecotypes, exposure to cold temperatures at the seedling stage represses *FLC* and accelerates flowering. This repression is maintained after the inductive treatment ceases and is only reversed in the next generation, suggesting that vernalization is a typical epigenetic event. A very important finding is that *FLC* expression decreased during vernalization in two vernalization-insensitive (*vrn*) mutants, but increased again once the plants were returned to normal temperature (T. Gendall, Dean laboratory). Therefore, the *VRN* genes are needed for the maintenance of the epigenetic state, but not for the establishment of the repression. This is particularly interesting in view of the fact that *VRN2* encodes a nuclear-localized zinc-finger protein with similarity to polycomb-group proteins involved in the stable maintenance of the repressed state of chromatin in *Drosophila* and that *VRN1* encodes a β 3-domain putative DNA-binding protein.

Repressing flowering and altering chromatin structure

In contrast to many of the late-flowering mutants, which represent genes with relatively specific effects on flowering, early-flowering mutants show a large diversity of pleiotropic effects. This suggests that these genes may encode more general

repressors with a variety of functions and that these are recruited to control flowering time. The cloning of several of these genes is in agreement with this view. *ESD4* is homologous to a SUMO-specific protease (P. Reeves, Coupland laboratory, Norwich, UK). The genes corresponding to three other early-flowering mutants encode polycomb-group proteins (*EMF2*, R. Sung, Berkeley, CA; *TFL2*, K. Goto, Okayama, Japan) and a chromatin-remodelling protein (*EBS*, M. Piñeiro, Madrid, Spain). When this list is added to the previously described *CLF* (encoding a polycomb-group-like protein) and the *VRN2* genes, it appears that silencing mechanisms that involve chromatin remodelling are frequently used in the control of flowering.

Among the genes that are repressed in the vegetative phase are several MADS-box genes that encode floral organ identity proteins. These genes are ectopically expressed in early-flowering mutants such as *emf* and *clf*. Furthermore, overexpression of such genes often results in early flowering, despite the fact that the floral organ identity mutants do not have altered flowering time. A teleological explanation for the control of flowering time that is consistent with this observation and that takes into account the fact that MADS proteins generally act as multimers could be that there is an advantage in regulating different stages of flowering through transcription-factor complexes involving MADS proteins, whose composition varies with developmental stage (P. Huijser, Cologne, Germany).

Responding to day length: the plant circadian clock and flowering

Plants use their circadian clock (an endogenous oscillator that regulates rhythms with a period of ~24 h) in combination with

Fig. 2. A simple diagram showing the four major genetic pathways regulating flowering time in *Arabidopsis*. The two main pathways mediating environmental responses are the long-day and vernalization pathways. The two pathways thought to function independently of environmental cues are the autonomous pathway, which promotes flowering in all conditions, and the GA pathway, which is needed for flowering in non-inductive short-day conditions.

signals from photoreceptors to sense day length and to regulate photoperiodic flowering. In the last 5 years, genetic components of the day-length sensing and response mechanism have been identified in *Arabidopsis*, through mutations that affect both circadian-clock activity and flowering time. These genes include *CCA1* and *LHY*, which both encode transcription factors with single myb repeats. Their overexpression leads to arrhythmia, a hallmark of genes involved in the circadian clocks of other organisms, and to late day-length-insensitive flowering. The *LHY* and *CCA1* proteins can physically interact by forming homo- or hetero-dimers, and phosphorylation of *CCA1* is needed for its DNA-binding activity. A protein kinase, CK2, is proposed to phosphorylate *CCA1*. Consistent with this idea, the loss of CK2 activity resulted in a longer circadian period and late flowering, while overexpression of one of the kinase regulatory subunits causes the opposite phenotype (E. Tobin, Los Angeles, CA).

Another gene, *TOC1*, was also proposed as a key component of the plant clock, because in *toc1* mutants all circadian rhythms tested have a shorter period, regardless of light conditions. The *toc1-1* mutant allele also causes plants to flower early under short-day conditions in some ecotypes, and this has been shown to be due to their circadian defect. However, while the molecular details of the autoregulatory feedback loops that comprise the oscillators of other organisms, such as *Neurospora*, *Drosophila*, *Synechococcus* and the mouse have been known for some time, this has not been the case so far in plants.

At this meeting, the first molecular models for a plant-clock loop mechanism were presented (M. Yanovsky, Kay laboratory, San Diego, CA, and G. Coupland, Norwich, UK). The loop is proposed to begin in the morning with expression of the *LHY* and *CCA1* genes, which are light- and circadian-regulated and peak at dawn. The *LHY* and *CCA1* proteins then repress the

expression of evening genes during the day. *TOC1* is proposed as a key target for this repression. The repression of *TOC1* by *CCA1* and *LHY* may be direct, as these proteins bind the upstream 'evening element' (present in at least 31 evening-expressed genes) in the *TOC1* promoter and are essential for circadian regulation of the *TOC1* gene (Yanovsky). Once *LHY* and *CCA1* levels drop, repression of *TOC1* is relieved and it is expressed in the evening. Finally, the loop is completed by *TOC1* activating, directly or indirectly, the expression of *LHY* and *CCA1* (Yanovsky) (Albadini *et al.*, 2001). Genetic support for this model comes from the observation that the *cca1 lhy* double mutant flowers early, has very short period rhythms that dampen rapidly in constant light conditions, and expresses many evening genes in the morning (Coupland). This model raises the possibility of the existence of multiple parallel feedback loops, with *LHY* and *CCA1* repressing many evening-expressed genes during the day, and those, when finally expressed in the evening, positively regulating *LHY* and *CCA1* expression.

Two major physiological models have been proposed to explain how day length regulates photoperiodic flowering. The 'external coincidence' model proposes that the coincident timing of light exposure (the external signal) with an internal circadian-regulated phase promotes flowering in long-day plants (such as *Arabidopsis*) and delays flowering in short-day plants (such as rice). The 'internal coincidence' model, on the other hand, proposes that two circadian rhythms coincide to promote flowering under floral-inductive conditions.

Several *Arabidopsis* genes that promote flowering are cyclically expressed and circadian-regulated and may correspond to the internal circadian-regulated factors predicted by the models. These include *CO*, whose phasing to the light period at the end of long days may be needed to activate a key target gene, *FT*, and hence to promote flowering (Suarez-Lopez *et al.*, 2001). Consistent with this, a *CO* transcript was rephased into the light in short days in the early-flowering *toc1-1* mutant (Yanovsky). Surprisingly, while *CO* appears to be activated by light, the *CO* protein is more stable in the dark than in white light, although it does accumulate to high levels in plants exposed to blue light (Coupland). Differential stability of a second floral protein, the blue light receptor *CRY2*, also appears to regulate flowering time (M. Koornneef, Wageningen, The Netherlands, and Chentao Lin, Los Angeles, CA). The presence of *CRY2* protein under long-day conditions correlates with rapid flowering, but the protein is unstable on non-inductive short days. The exception to this is the *EDI* allele of *CRY2*, which causes early flowering on short days and increases protein stability of the photoreceptor.

Response to day length in *Arabidopsis* and rice

How well does the *Arabidopsis* model hold up in rice? Molecular characterization so far indicates that the genes controlling photoperiodic flowering in *Arabidopsis* also control it in rice. Great progress has recently been made in the isolation of rice genes that correspond to quantitative trait loci (QTLs) for flowering time (called 'heading date' in rice). Three of these QTLs, *Hd1*, *Hd3a* and *Hd6* have recently been cloned and found to encode similar proteins to the *Arabidopsis* genes encoding *CO*, *FT* and the CK2 alpha subunit, respectively

(M. Yano, Fukuoka, Japan). A rice gene similar to the *Arabidopsis* flowering-time gene *GI* has also been characterized and found to be circadian-regulated and to promote *CO* expression as it does in *Arabidopsis* (Ryousuke Hayama, Shimamoto laboratory, Ikoma, Japan). However, there must be differences in regulation of some of these flowering-time genes, since rice and *Arabidopsis* respond quite differently to day length. The explanation may lie in *CO* regulation of *FT*. Under long-day conditions, *CO* appears to repress flowering in rice via repression of *FT*. The opposite is true in *Arabidopsis*, where *CO* upregulates *FT* under long-day conditions and promotes flowering.

Hormonal regulation of flowering

Alteration in hormonal and nutrient balance has long been known to affect flowering time. However, proof that some of these compounds are indeed involved in the determination of flowering time has been obtained only in recent years.

In terms of the role of hormones, the GAs, which regulate multiple aspects of plant vegetative growth, have been shown to be essential for flowering under non-inductive short-day conditions (Wilson *et al.*, 1992), and the mechanisms by which they influence flowering are beginning to be unravelled. Under short-day conditions, GAs activate downstream molecular targets such as the floral meristem identity gene *LFY* through a signalling pathway that is independent of the long-day pathway (Blazquez and Weigel, 2000). Newly identified components of the pathway include *PPF1* (S. Melzer, Zurich, Switzerland) and *FOG1* (M. Blázquez, Valencia, Spain), whose characterization has unveiled interactions between these long-day- and GA-dependent flowering pathways. A dominant mutation at the *FOG1* locus accelerates flowering under short days, even in the absence of GAs, and increases GA sensitivity during germination and hypocotyl elongation, solidifying the role of this protein in the GA-dependent pathway. On the other hand, expression of the long-day pathway gene *FT* and the *FLC* floral repressor is altered in the *fog1* mutant. This indicates that the *FOG1* pathway feeds into the long-day pathway, and that the *FT* and *FLC* genes may serve as integration points for many different floral-inductive signals.

Even more exciting than the idea of a connection between the long-day- and GA-dependent flowering pathways is the hypothesis, based on physiological observations from the Bernier and other laboratories, that GAs and nutrients act through a common signalling pathway to promote flowering. Careful analysis of the concentrations of sucrose and GA₄ (the most active endogenous GA that promotes flowering in *Arabidopsis*) in plants growing under short-day conditions reveals that both compounds start accumulating at the apex of the plant, shortly before flowering occurs (O. Nilsson, Umeå, Sweden). Interestingly, GAs and sucrose synergistically activate the *LFY* promoter.

Certainly, GAs are not the only hormones involved in the control of flowering time. As reported by S. Davis (Millar laboratory, Warwick, UK), brassinosteroids (BRs) become limiting for flowering in certain genetic backgrounds. Davis reported that mutations that prevent BR synthesis or perception severely enhanced the late-flowering phenotype caused by loss of function in an autonomous pathway gene called *LD*, raising the

possibility that BRs and GAs share signalling elements in the control of flowering.

The final players: meristem identity genes

The decision to flower involves a dramatic change in plant architecture, which is governed by the interplay of genes that promote shoot identity, such as *TFL1* and *TFL2*, and those that promote floral identity, such as *LFY* and *AP1*. Mutations in these genes affect both processes. For instance, *tfl* mutations confer early flowering and premature consumption of the shoot meristem into a flower, and a reduction in *LFY* activity causes delayed flowering and inability to construct normal flowers. The similar phenotypes of *tfl1* and *tfl2* can now be easily explained, as both mutants show increased expression of *FT*. Similarly, *AP1*, but not *LFY*, is expressed precociously in *tfl2* mutants (K. Goto, Okayama, Japan), an effect that is in agreement with the observation that *AP1* is genetically downstream of *FT*.

Understanding how floral meristem identity genes activate flower development programmes is a key issue that will require the identification of elements that link floral identity specification with floral patterning. For instance, it will be important to determine how *LFY* activates floral homeotic genes in particular regions of the floral meristem if *LFY* itself is expressed throughout the primordium. The hypothesis that floral-patterning programmes 'recruit' shoot-patterning elements would be one explanation, and this idea has gained strong experimental support recently. *WUS*, a gene required for the organization of the central zone of the shoot meristem, acts as a coactivator of *LFY* in the transcriptional regulation of *AG* in the centre of the flower meristem (D. Weigel, San Diego, CA).

Perspectives

The molecular data reported at Norwich have strengthened the existing complex genetic model that has been developed and continuously refined in *Arabidopsis*. The involvement of chromatin-remodelling factors and epigenetic changes in the control of flowering time is an important emerging theme. However, many of the details of how environmental signals are integrated with endogenous regulatory systems such as the circadian clock, photoreceptors and plant hormones remain to be elucidated, as do the biochemical functions of some of the cloned genes. The rapid progress made in the monocot model rice, especially by Japanese research groups, was impressive and in fact showed that many components of the floral transition are similar to those used for the same purpose in *Arabidopsis*. The next important question is to what extent the complex molecular mechanisms that govern flowering in *Arabidopsis* can be generalized to other plant species with different reproductive habits in order to allow manipulation of reproductive traits.

Acknowledgements

We thank the organizers, Caroline Dean, Tony Gendall, Gordon Simpson and Dee Rawsthorne for organizing a great meeting, which included almost all of the research groups in the exciting

meeting report

field of molecular genetics of flowering. We thank participants for their openness in communicating data and allowing these to be reported, and we apologize to those not mentioned here because of the limitations of space and theme. We thank George Coupland for the photograph in Figure 1.

References

- Albadini, D., Oyama, T., Yanovsky, M.J., Harmon, F.G., Mas, P. and Kay, S.A. (2001) Reciprocal regulation between *TOC1* and *LHY/CCA1* within the *Arabidopsis* circadian clock. *Science*, **293**, 880–883.
- Blázquez, M.A. and Weigel, D. (2000) Integration of floral inductive signals in *Arabidopsis*. *Nature*, **404**, 889–892.
- Michaels, S.D. and Amasino, R.M. (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell*, **11**, 949–956.
- Simpson, G.G., Gendall, A.R. and Dean, C. (1999) When to switch to flowering. *Annu. Rev. Cell Dev. Biol.*, **99**, 519–550.
- Suarez-Lopez, P., Wheatley, K., Robson, F., Onouchi, H., Valverde, F. and Coupland, G. (2001) *CONSTANS* mediates between the circadian clock and the control of flowering in *Arabidopsis*. *Nature*, **410**, 1116–1120.
- Wilson, R.N., Heckman, J.W. and Somerville C.R. (1992) Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. *Plant Physiol.*, **100**, 403–408.

The authors and organizers of the workshop (from left to right): Jo Putterill*, Caroline Dean, Gordon Simpson, Tony Gendall, Maarten Koornneef* and Miguel Blázquez**

DOI: 10.1093/embo-reports/kve254