

Flowering Time: A Pathway that Begins at the 3' End

Dispatch

Richard M. Amasino

Flowering time control in plants involves integration of multiple signals. One of the signalling pathways in *Arabidopsis* involves a negative autoregulatory loop, in which the FCA protein together with FY promotes the choice of an alternative polyadenylation site within the FCA pre-mRNA to produce a transcript that does not encode a functional protein.

The control of flowering time has been intensively studied by genetic analyses in several plant species, particularly *Arabidopsis thaliana* [1,2]. These studies have revealed many of the genes involved in regulating flowering, but the biochemical processes in which the products of these flowering-time genes participate are largely unknown. Simpson *et al.* [3] have recently reported that two flowering-time regulatory proteins, FCA and FY, interact to control the choice of site of 3' mRNA cleavage and polyadenylation. Furthermore, a target of the FCA–FY complex is the pre-mRNA for one of the components of the complex: FCA, a promoter of flowering (the delayed flowering of an *fca* mutant is illustrated in Figure 1).

Precisely when to initiate flowering is a critical developmental 'decision' in a plant's life cycle. Annual plants typically initiate flowering only once and then die after setting seed. Thus, the timing of this decision in annuals such as *Arabidopsis* is extremely important. The pathways that control flowering in *Arabidopsis* have evolved to provide considerable flexibility. For example, there is a photoperiod pathway that promotes flowering when the days are long. The long days of late spring and early summer are usually an optimal time for *Arabidopsis* to complete its life cycle. In short days, flowering is delayed, but the *Arabidopsis* plant continues to grow as it awaits more optimal conditions.

Two other pathways, the vernalization and autonomous pathways, regulate flowering primarily by controlling the level of expression of *FLOWERING LOCUS C (FLC)* [1,2]. FLC is a repressor of flowering and a member of the MADS-domain family of proteins, which are known to act as transcriptional regulators [4–6]. The autonomous and vernalization pathways both promote flowering by repressing *FLC* expression, but they do so under different circumstances.

Vernalization is the promotion of flowering that results from the prolonged exposure to cold during winter [7]. Certain varieties of *Arabidopsis* known as winter-annuals typically begin growing in the fall, but are prevented from flowering before the onset of

winter by high levels of *FLC* expression. During winter the vernalization pathway represses *FLC* expression and thus permits flowering in the spring [7].

Another pathway that regulates *FLC* expression is known as the autonomous pathway because it appears to regulate flowering independently of environmental cues such as day-length and cold [1,2]. Six genes for components of this pathway have been identified, all of which affect flowering by repressing *FLC* [4]. Analyses of double mutants among pathway members, however, indicates that these genes are likely to operate in separate parallel pathways to regulate *FLC* [1,2,8]. One of these parallel pathways is defined by *FCA* and *FY*.

FCA was cloned by Caroline Dean's group, and they found that the *FCA* pre-mRNA is processed into four distinct transcripts [9]. They subsequently showed that only one of the transcripts (referred to as γ) is able to promote flowering; another major transcript (β) is generated by cleavage and polyadenylation at a site within the third intron [10,11]. This processing of the *FCA* pre-mRNA into active and inactive forms is regulated during development; this regulation is conserved in other plant species, and so is likely to be an important component of the developmental regulation of flowering time [10,11].

Recent studies have identified two of the factors involved in selection of the *FCA* pre-mRNA polyadenylation site. Quesada *et al.* [11] found that one of the factors is FCA — the FCA protein negatively regulates its own expression by favoring polyadenylation at the third intron site, which results in a transcript that does not make a functional protein.

How does FCA accomplish this negative autoregulation? An important clue was provided in the sequence of the FCA protein. Simpson *et al.* [3] noted that FCA has an RNA-binding domain and a specific type of WW domain that was predicted to interact with a Pro-Pro-Leu-Pro sequence. Such a (coding) sequence was



Current Biology

Figure 1. Phenotype of a wild-type *Arabidopsis* plant and an *fca* mutant in the Columbia accession.

The plants were grown in long days which are inductive for flowering. Both plants are shown at the time of initiating flowers and thus the *fca* mutant in which flowering is delayed is chronologically much older than the wild type. (Photograph by Scott Michaels.)

found in a gene in the chromosomal interval in which *FY* resides, and subsequent experiments showed that this gene was in fact *FY*. The *FY* protein is similar to the yeast polyadenylation factor *Psf2p*. Simpson *et al.* [3] went on to show that *FCA* and *FY* interact via *FCA*'s *WW* domain.

The interaction of an RNA-binding protein with a polyadenylation factor suggests a possible biochemical mechanism for *FCA* autoregulation (Figure 2). The *FCA* protein might bind to the *FCA* pre-mRNA and, via its association with *FY*, direct the cleavage and polyadenylation machinery to the processing site in the third intron to favor the formation of the β transcript.

One intriguing aspect of this work is that both the budding yeast *Saccharomyces cerevisiae* and the plant *Arabidopsis* have only a single copy of the *FY/Pfs2p* factor. In yeast, mutation of *Pfs2p* is lethal [12]. Why are *fy* mutations not lethal in *Arabidopsis*? One possibility, as noted in Simpson *et al.* [3], is that the two *fy* alleles tested so far are not nulls; complete loss of *FY* function may be lethal. Another possibility is that *FY* is reserved for processing of specific regulated mRNAs. Unlike yeast, in which *Pfs2p* is the only protein of this type in the core cleavage and polyadenylation machinery, *Arabidopsis* also has three genes encoding proteins similar to mammalian *CstF50* which might have the same role as *FY/Pfs2p*. *CstF50* is related to *FY/Pfs2p* in domain organization and has been proposed to be the functional equivalent of *Pfs2p* in mammals [12]. Moreover, the mammalian ortholog of *Fy/Pfs2p*, *WDC146*, has not been found in the core cleavage and polyadenylation machinery. Thus, as noted [3], in *Arabidopsis* and mammals perhaps *CstF50*-type proteins participate in most 3' mRNA end formation, whereas *FY* and *WDC146* participate in regulated 3' mRNA end formation.

These results suggest many future experiments. Does the *FCA* protein actually bind to *FCA* pre-mRNA as predicted? Is *FY* involved in the processing of certain pre-mRNAs independently of *FCA*? The fact that *fy fpa* double mutants are lethal, whereas *fca fpa* double mutants are not [8], indicates that *FY* has roles in addition to flowering that are not shared with *FCA*. How do *FCA* and *FY* down-regulate *FLC* expression? There is no direct evidence that *FLC* is a direct target of a *FCA-FY* complex or that the *FLC* pre-mRNA can be processed at alternative polyadenylation sites, but, as noted [3], alternative polyadenylation sites may be difficult to detect if non-functional *FLC* transcripts are rapidly degraded.

Regardless of whether or not *FLC* pre-mRNA is a direct target of the *FCA-FY* complex, *FCA* and *FY* acting together must somehow regulate other flowering time genes in addition to *FCA*, and a likely candidate is *FLC* (Figure 2). This is because the *fy* mutation suppresses the early flowering effect of increased production of the active *FCA* protein translated from the γ mRNA. If the only function of the *FCA-FY* complex was to negatively regulate *FCA*, which is a promoter of flowering, *fy* mutants would be earlier flowering rather than delayed in flowering.

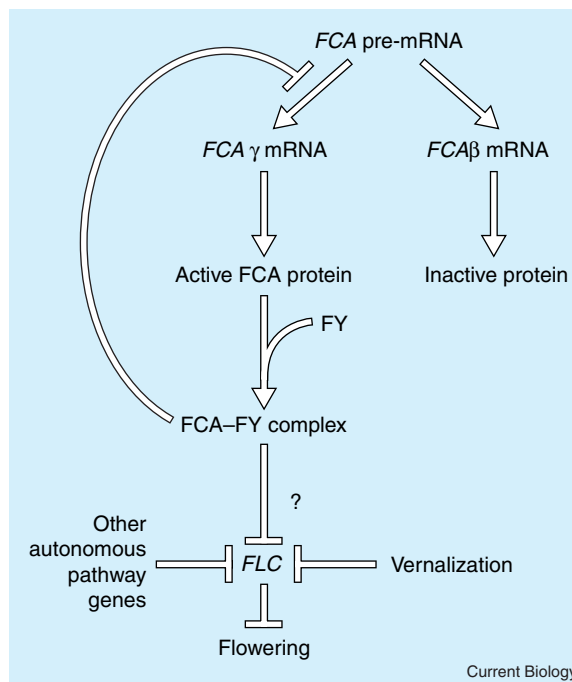


Figure 2. Model for *FCA* auto-regulation and the regulation of flowering time.

Negative autoregulation of *FCA* results when *FCA* and *FY* proteins together promote the formation of the β form of the *FCA* mRNA. The β form results from cleavage and polyadenylation at a site in the third intron and cannot produce an active protein. *FCA* and *FY* also co-operate to lower the levels of *FLC* mRNA and promote flowering. The '?' between *FCA-FY* and *FLC* indicates that the mechanism of this interaction is not known and may not be direct.

Raising many questions for future research is, of course, a sign of an exciting advance. The new work of Simpson *et al.* [3] is significant not only because it is a major advance in our understanding of flower-time regulation, but it is also the first example of a specific factor that regulates the choice of the cleavage and polyadenylation site of a specific gene.

References

1. Koornneef, M., Alonso-Blanco, C., Peeters, A.J.M., and Soppe, W. (1998). Genetic control of flowering time in *Arabidopsis*. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 345-370.
2. Simpson, G., Gendall, T. and Dean, C. (1999). When to switch to flowering. *Ann. Rev. Cell Dev. Biol.* 15, 519-550.
3. Simpson, G.G., Dijkwel, P.P., Quesada, V., Henderson, I., Dean, C. (2003). *FY* is an RNA 3' end-processing factor that interacts with *FCA* to control the *Arabidopsis* floral transition. *Cell* 113, 777-787.
4. Michaels, S., and Amasino, R. (2001). Loss of *FLOWERING LOCUS C* activity eliminates the late-flowering phenotype of *FRIGIDA* and autonomous-pathway mutations, but not responsiveness to vernalization. *Plant Cell* 13, 935-941.
5. 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.
6. Sheldon, C.C., Burn, J.E., Perez, P.P., Metzger, J., Edwards, J.A., Peacock, W.J., and Dennis, E.S. (1999). The *FLF* MADS box gene: a repressor of flowering in *Arabidopsis* regulated by vernalization and methylation. *Plant Cell* 11, 445-458.
7. Michaels, S. D., and Amasino, R.M. (2000). Memories of winter: vernalization and the competence to flower. *Plant Cell Environ.* 23, 1145-1154.

8. Koornneef, M., Alonso-Blanco, C., Blankestijn-de Vries, H., Hanhart, and Peeters, A.J.M. (1998). Genetic interactions among late-flowering mutants of *Arabidopsis*. *Genetics* 148, 885-892.
9. Macknight, R., Bancroft, I., Page, T., Lister, C., Schmidt, R., Love, K., Westphal, L., Murphy, G., Sherson, S., Cobbett, C., and Dean, C. (1997). FCA, a gene controlling flowering time in *Arabidopsis*, encodes a protein containing RNA-binding domains. *Cell* 89, 737-745.
10. Macknight, R., Duroux, M., Laurie, R., Dijkwel, P., Simpson, G., and Dean, C. (2002). Functional significance of the alternative transcript processing of the *Arabidopsis* floral promoter FCA. *Plant Cell* 14, 877-888.
11. Quesada, V., Macknight, R., Dean, C., and Simpson, G.G. (2003). Autoregulation of the site of 3'-end formation in FCA pre-mRNA prevents precocious flowering. *EMBO J.* 22, 3142-3152.
12. Ohnacker, M., Barabino, S.M.L., Preker, P.J., and Keller, W. (2000). The WD-repeat protein Pfs2p bridges two essential factors within the yeast pre-mRNA 3'-end-processing complex. *EMBO J.* 19, 37-47.