The Plant Cell, Vol. 31: 1190–1191, June 2019, www.plantcell.org © 2019 ASPB.

EDITORIAL: REFLECTIONS ON *THE PLANT CELL* CLASSICS

FLOWERING LOCUS C Isolation and Characterization: Two Articles That Opened Many Doors[OPEN]

These two classic articles from *The Plant Cell* archive **(Michaels and Amasino, 1999; Sheldon et al., 1999)** were tremendously satisfying in providing a first glimpse of the mechanism by which seasonal flowering of Arabidopsis (*Arabidopsis thaliana*) is induced by winter cold (vernalization) through *FLOWERING LOCUS C* (*FLC*). However, their significance has been much broader than could have been imagined when they were published, as *FLC* became a paradigm for defining the effects of histone modifications on plant gene expression and allelic variation at *FLC* explained many phenotypic differences observed among *A. thaliana* accessions and Brassicaceae species. Here, I briefly summarize the origins, content, and influence of these landmark articles.

In 1999, our genetic understanding of how plants control flowering was still in its infancy. Many *A. thaliana* mutants showing late flowering had been identified and placed in parallel pathways based on epistasis and physiological analysis. Mutants affecting the photoperiodic pathway were only late flowering under inductive long days. By contrast, mutants in the autonomous pathway flowered later under long and short days, and therefore impaired a pathway required for flowering under both conditions. Strikingly, the flowering delay of these autonomous pathway mutants could be corrected by exposure to vernalization, whereas that of photoperiodic pathway mutants could not. Independent work on natural genetic variation among accessions also identified flowering time loci. In work that predated the mutant analysis, the dominant *FRIGIDA* (*FRI*) locus was shown to delay the flowering of late-flowering accessions, but its effect could be overcome by vernalization (Napp-Zinn, 1979), indicating some similarity between FRI action and the autonomous pathway. Furthermore, elegant genetic analysis demonstrated that a second dominant locus was required for *FRI* to cause late flowering (Koornneef et al., 1994; Lee et al., 1994), and the authors gave this locus the rather enigmatic name *FLC*. They located *FLC* to a region on chromosome 5, creating the opportunity to isolate it by map-based cloning.

The articles in *The Plant Cell* described the isolation of *FLC* by independent routes. Sheldon et al. (1999) identified a dominant mutation causing late flowering due to a T-DNA insertion. They also showed that this caused increased expression of an adjacent gene, and demonstrated that a transposon insertion in the adjacent gene suppressed the late-flowering phenotype. They concluded that this gene encodes a repressor of flowering, which they initially called *FLOWERING LOCUS F* (*FLF*), but also mentioned that its chromosomal location was similar to *FLC* and they might therefore be allelic. By contrast, Michaels and Amasino (1999) isolated *FLC* by map-based cloning and then confirmed its isolation by molecular complementation and molecular analysis of an *FLC* allelic series. Their article confirmed that *FLC* and *FLF* were indeed the same gene based on sequence comparisons. Both articles show that *FLC* encodes a MADS box transcription factor, demonstrating what was at the time a novel function for this class of protein in repressing floral transition rather than contributing to floral development. In addition, they demonstrated that *FLC* is hardly expressed in early-flowering accessions such as Columbia but is highly expressed in the presence of active *FRI* or in autonomous pathway mutants, and this high expression correlates with late flowering. Exposure of these genotypes to vernalization caused them to flower early and reduced *FLC* transcript levels. Therefore, FRI and the autonomous pathway are linked, as they both act upstream of *FLC* to regulate flowering, placing *FLC* in a central position in the flowering network. Michaels and Amasino (1999) also discuss that when plants are exposed to vernalization and returned to normal temperatures, *FLC* expression remains repressed through mitosis, while after meiosis in the following generation its expression is reset to levels observed before vernalization. Thus, regulation of *FLC* transcription mimics many of the classical physiological characteristics of vernalization.

In the decades since the publication of these articles, *FLC* regulation has proven to be a powerful system to define general mechanisms of plant gene regulation (Whittaker and Dean, 2017). Deciphering the mechanism by which *FLC* is repressed during vernalization revealed the importance of histone modifications in plant gene regulation (Bastow et al., 2004; Sung and Amasino, 2004). FRI activates *FLC* transcription before vernalization by recruiting histone methyl transferases and histone acetyl transferases to the *FLC* promoter. This causes the trimethylation of Lys-4 of histone 3 (H3K4me3) and the trimethylation of Lys-36 of histone 3 (H3K36me3) that are associated with active chromatin. By contrast during vernalization, polycomb repressive complex2 is recruited to *FLC*, leading to trimethylation of Lys-27 of histone 3 (H3K27me3) that is correlated with repression of transcription. Such studies revealed the key roles of histone modification in the mitotic stability of the vernalized state and its resetting at meiosis. However, more generally, slight increases in *FLC* mRNA levels are sufficient to confer late flowering on early-flowering accessions such as Columbia. Therefore, in these accessions, *FLC* acts as a canary in the coal mine, so that many mutations with general effects on histone modification or gene regulation were identified as late-flowering plants due to slightly elevated *FLC* transcript levels. This is exemplified by the autonomous pathway mutants, which proved not to define a dedicated flowering pathway but a set of genes with important roles in general gene regulation such as 3′-end processing, RNA binding proteins, spliceosome subunits, and additional proteins with important roles in chromatin regulation.

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www.plantcell.org/cgi/doi/10.1105/tpc.19.00325

The early work of Napp-Zinn (1979) on variation in FRI activity and vernalization response among accessions of *A. thaliana* proved to be the tip of the iceberg. Systematic genetic and genomic analysis revealed extensive allelic variation in *FRI*/*FLC* that was proposed to explain a large fraction of the phenotypic variation in flowering time among Arabidopsis accessions (Weigel, 2012). Many alleles of varying strength were described at both *FLC* and *FRI*, and variation at *FLC* was proposed to be adaptive, for example in altitudinal clines of Arabidopsis accessions. In addition, perhaps surprisingly, variation at *FLC* was also implicated in traits other than flowering time, some of which are associated with seasonal life history, such as seed germination and regulation of circadian rhythms, but others are apparently unrelated, including age-related changes in leaf morphology and water use efficiency. Allelic variation at *FLC* also contributes to flowering variation in other Brassicaceae, including *Arabidopsis lyrata* and more distantly related members of the family such as *Arabis alpina* and *Brassica oleraceae*. Some of these are perennials, in which the resetting of *FLC* transcription was found to occur soon after vernalization in vegetative tissues, not only during meiosis as in annual *A. thaliana* (Wang et al., 2009). This reactivation of *FLC* contributes to the perennial life cycle determining the duration of flowering and maintaining vegetative axillary branches that flower the following year.

These articles appeared in *The Plant Cell* 20 years ago and contributed to a wave of discoveries in plant biology, but the analyses of precisely how *FLC* is regulated and how it contributes to the regulation of the developmental transition to flowering continue apace into a third decade.

George Coupland
Max Planck Institute for Plant Breeding Research
50829 Cologne, Germany
coupland@mpipz.mpg.de
ORCID ID: 0000-0001-6988-4172

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*References highlighted for the 30th Anniversary of *The Plant Cell*.

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George Coupland

Plant Cell 2019;31;1190-1191; originally published online May 2, 2019;
DOI 10.1105/tpc.19.00325