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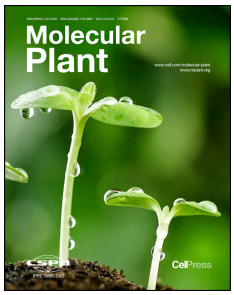

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Molecular Plant Spotlight

Lost memories of winter - Breaking the *FLC* silence

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Running title: Lost memories of winter - Breaking the *FLC* silence

Vernalization - the acquisition of competence to flower after extended cold treatment - has puzzled researchers throughout the last century. Great progress in understanding the molecular mechanisms of vernalization in Arabidopsis has been made in the course of the last 20 years, placing the flowering repressor-encoding gene *FLOWERING LOCUS C* (*FLC*) in the limelight (Bouché, et al. 2017). In vernalization-dependent accessions, FRIGIDA (FRI) ensures strong expression of *FLC* that delays flowering, and extended exposure to low temperatures such as during winter leads to stable repression of *FLC* which in turn clears the way for induction of flowering. The repressed state of *FLC* is stabilized by the activity of Polycomb Repressive Complex 2 (PRC2) and associated proteins which together induces the formation of a repressive chromatin state marked by histone H3 tri-methylation at lysine 27 (H3K27me3). Replication of the repressive chromatin structure at *FLC* ensures long-lasting mitotically heritable *FLC* downregulation for the rest of the plant´s life. With each new generation, however, *FLC* expression needs to be reset to prevent precautious temperature-independent flowering in the progeny of vernalized plants (Sheldon et al. 2008; Choi et al. 2009). The molecular mechanisms that reactivate *FLC* transcription and prevent the transmission of repressed *FLC* alleles into the next generation have long remained enigmatic. Reactivation of *FLC* occurs during embryogenesis (Sheldon et al. 2008; Choi et al. 2009; Crevillen et al. 2014; Tao et al. 2017). Several proteins that positively regulate *FLC* reactivation during reproduction were identified, including the polymerase associated factor (Paf1) complex or the SWR1 complex (SWR1c) (Choi et al 2009; Yun et al 2011; reviewed in

Crevillén & Dean 2011). Recently, Crevillen et al. showed that the H3K27 demethylase EARLY FLOWERING 6 (ELF6) is also involved in the activation of *FLC* (Crevillen et al. 2014), suggesting a function of active removal of repressive histone modifications to achieve full *FLC* reactivation. However, the requirement for ELF6 in *FLC* resetting might be more limited than originally thought (Tao et al. 2017). Nevertheless, key transcriptional activators of *FLC* during embryogenesis remained unknown until now.

In a recent issue of Nature, Tao et al. identify LEAFY COTYLEDON 1 (LEC1), the B-subunit of the NF-Y (nuclear factor Y-box) transcription factor complex, as the major *de-novo* activator of *FLC* expression during embryogenesis in Arabidopsis (Tao et al. 2017). LEC1 is an embryo-specific transcription factor and it is important for seed development (Pelletier et al. 2017). The authors show that *FLC* reactivation during embryogenesis in the progeny of both non-vernalized and vernalized plants is mitigated by a mutation in the *LEC1* gene. Thus, LEC1 is needed to *de-novo* activate *FLC* and to prevent transmission of the vernalized (repressed) state into the next generation. Furthermore, reactivation of *FLC* in progeny of non-vernalized plants and *FLC* resetting in progeny of vernalized plants are likely to share the same molecular mechanism (Tao et al. 2017). The partial *FLC* reactivation in some *lec1* mutant embryos suggested that additional genes are involved in the process. In a crucial experiment, Tao et al. showed that mutation or knockdown of four *LEC1* homologs in a *lec1* mutant background results in complete abrogation of *FLC* activity in seeds. Plants derived from these seeds further displayed early flowering without the need for additional vernalization. This demonstrated a pivotal role of the NF-YB transcription factors in transgenerational resetting of *FLC* expression and in preventing the transmission of the vernalized state of *FLC* into progeny.

The NF-Ys can act as pioneer transcription factors binding DNA in a way that mimics H2A-H2B, rendering chromatin accessible for proteins introducing active chromatin modifications (Nardini et al. 2013). Tao et al. further proceeded in exploring the molecular mechanisms of *FLC* reactivation by LEC1. LEC1 binds to the *FLC* promoter recognizing CCAAT motifs - this is both required for FLC-dependent repression of flowering and sufficient for ectopic activation of *FLC* in a *LEC1*-inducible system. Activation of *FLC* is associated with increased abundance of the active histone modifications H3K4me3 and H3K36me3 and decrease of repressive H3K27me3 (Crevillén & Dean 2011). Reduced amounts of active histone modifications and increased levels of H3K27me3 along with reduced presence of the H3K36me3-histone methyltransferase EFS (SDG8) are found at the *FLC* locus in siliques of *lec1* mutants 5 days after pollination. Additionally, the chromatin remodelling complex SWR1c is epistatic to *LEC1* in the embryonic activation of *FLC*. Thus, LEC1 together with chromatin remodelling and modification complexes establishes a transcriptionally active chromatin state at the *FLC* locus during embryogenesis.

FLC, once activated in the embryo, remains active until its repression by vernalization later in vegetative development. Does the initial LEC1-induced *FLC* activation reflect its expression in post-embryonic vegetative tissue? It seems to be so, as the authors demonstrate by a series of experiments that utilize inducible LEC1 upregulation together with the incomplete penetrance of the impaired *FLC* reactivation phenotype in *lec1*. In vegetative tissue of 2, 3 and 5 day-old seedlings, *FLC* expression levels correlate with that in the embryo and is independent of the presence of embryo-derived *FLC* transcripts. The authors therefore propose that the *FLC* expression level depends on the chromatin environment at the *FLC* locus which, once established in the embryo, is inherited through mitotic cell divisions and maintained in the vegetative tissue despite the absence of its initial activator LEC1 (Figure 1). Whether inheritance of chromatin states is indeed sufficient for continuous *FLC* expression or whether a positive feedback loop that involves other transcriptional activators (perhaps directly or indirectly activated by LEC1) may be required for continuous *FLC* expression remains to be clarified. The efficiency of *FLC* reactivation was different between seeds from non-vernalized or vernalized parents in *lec1 FRI*. This observation indicates that vernalization may have a cumulative effect on the chromatin state at *FLC*. Importantly, the requirement for LEC1 in *FLC* resetting can explain the recently demonstrated lack of *FLC* reactivation during *in-vitro* plant regeneration from vernalized plants (Nakamura & Hennig 2017) during which *LEC1* expression is not induced. It will be of interest and considerable importance for plant biotechnology to determine whether vernalization memory in regenerated plants can be controlled by manipulating *LEC1* expression.

The NF-Y family of transcription factors are encoded by multiple genes with the NF-YA, B and C subunits interacting in different combinations (Petroni et al 2012). Similarly to the LEC1-containing NF-Y that activates *FLC*, gibberellin-induced NF-Y complex interacting with REF6 mediates H3K27me3 demethylation and activation of the flowering signal integrator *SOC1* (Hou et al 2014). It will be interesting to establish whether NF-Y transcription factors have a general function in resetting developmentally- or environmentally-induced epialleles.

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Figure legend

Figure 1. LEC1 resets *FLC* expression during embryogenesis. (A) Scheme of *FLC* (black) and *LEC1* (red) expression throughout the plant´s life cycle. Drawings below the chart illustrate major phases of plant development (germination/seedlings establishment, vegetative growth phase, reproductive phase, seed and embryo development). LEC1 can activate *FLC*. *LEC1* is strongly expressed during early embryogenesis. *FLC* transcription, which is repressed by cold during seedling development and vegetative growth, is reactivated in early embryogenesis. *FLC* expression is maintained even after *LEC1* transcription has ceased. *FLC* reactivation is impaired in plants lacking LEC1 (*lec1 FRI*) or both LEC1 and LEC1-like (*lec1 l1l FRI*) and completely lost in plants deficient in LEC1, L1L and three additional LEC1 homologs (RNAi *NF-YBs* in *lec1 l1l FRI*). (B) Heatmap of developmental expression patterns of *FLC* and genes involved in *FLC* reactivation (data from the AtGenExpress compendium). Expression of *EFS*, *SWC6, ELF6* and the NF-YBs is relatively uniform throughout the life cycle. In contrast, *LEC1* and *L1L* are preferentially expressed during early seed development.