PNAS COMMENTARY

# A new negative link in flower development: Repression of ABC genes by Z factors—ZP1/ZFP8

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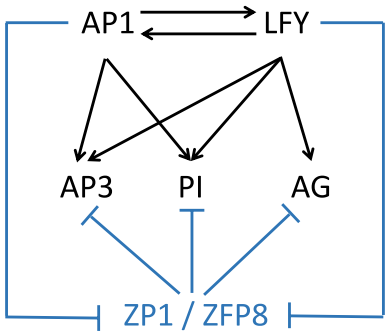
Ever since the discovery of homeotic mutations in Drosophila nd the subsequent identification and cloning of the corresponding homeotic genes, it has been clear that in addition to gene activation, the correct development of an organism is equally dependent on genes not being expressed in inappropriate cells and tissues or at inappropriate times. In flowering plants (angiosperms), the formation and development of flowers is controlled by a network of genes that determines first the identity of the corresponding meristems as floral (i.e., reproductive, as opposed to vegetative primordia), and then the identity and development of the different organs of the flower (sepals, petals, stamens, and carpels) (1–3). The key Arabidopsis floral meristem identity genes *LEAFY* (*LFY*) (4) and *APETALA1* (*AP1*) (5) act as integrators for the inputs of the different flowering time pathways, and their expression early in the incipient primordia promotes flower formation (1–3). LFY, a plant-specific transcription factor (TF), and AP1, a MADS-domain TF, subsequently activate the expression of floral homeotic genes in the floral primordia, in particular—but not only—of the B class genes *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) (up-regulated by both LFY and AP1) and of the C class gene *AGAMOUS* (*AG*) (up-regulated by LFY) (3, 6–9) [floral homeotic or organ identity genes are assigned to the A, B, C, D, or E classes depending on the type of organ whose development they control, and act in a combinatorial fashion, with B genes (*AP3* and *PI*) directing petal and stamen development and the C gene *AG* directing stamen and carpel formation (1–3)]. Importantly, the expression of floral organ identity genes is tightly regulated, and their misexpression or ectopic expression in vegetative tissues can lead to abnormal development (10–14). In fact, the identification and characterization of *CURLY LEAF* (*CLF*) revealed that *AG* (and *AP3*) transcription is negatively regulated during the vegetative phase by Polycomb Group Repressive Complex 2 (PRC2) (15–17). The correct development of the plant therefore requires of the active repression of floral organ identity genes in vegetative tissues and of the release of their silencing upon the formation of the floral primordia. Although evidence has accumulated on the epigenetic regulation of *AG* repression in vegetative tissues by PRC2 through various mechanisms (16), whether TFs directly participate in such silencing and how it is lifted in floral primordia is not well understood. In PNAS, Hu et al. (18) report the identification of specific C2H2 zinc finger TFs (ZINC FINGER PROTEIN1, ZP1, and ZINC FINGER PROTEIN 8, ZFP8) that are involved in the repression of *AG*, *AP3,* and *PI* in vegetative tissues and that are themselves down-regulated in developing flowers by LFY and AP1. That is, LFY and AP1, in addition to their role in directly activating in the floral primordia expression of the B and C class organ identity genes, would also up-regulate them indirectly: through the repression of their repressors, *ZP1* and *ZFP8*.

*ZP1* had been previously characterized as a negative regulator of root hair initiation and elongation (19), whereas *ZFP8* had been shown to play a role in trichome development (20). However, *ZP1* is also expressed in aerial parts of the plant, where its possible roles remained unknown. Hu et al. (18) found that *ZP1*, and *ZFP4*, *ZFP7*, and *ZFP8*, which also belong to the C1-1i subgroup of C2H2 TFs, were all down-regulated in four-wk-old inflorescences when compared to two-wk-old vegetative soot apex tissue by RNA-Sequencing and RT-qPCR. Conversely, in plants mutant for the floral meristem identity genes *LFY* (*lfy-1* allele) or *AP1* (*ap1-10*), expression of *ZP1* and *ZFP8* was up-regulated in early-stage floral primordia, as determined by in situ hybridization, altogether suggesting that *ZP1* and *ZFP8* could be downstream of (and repressed by) LFY and AP1 (18). Direct binding by both LFY and AP1 to the upstream region of *ZP1* and *ZFP8* had been detected in previous ChIP-Seq studies (21, 22), an observation that was confirmed by Hu et al by ChIP-qPCR (18).

A gain-of-function approach established the link between *ZP1* and *ZFP8* and the B and C class homeotic genes, *AP3*, *PI,* and *AG*. To specifically express *ZP1* and *ZFP8* in floral primordia, their coding sequences were fused to the *LFY*




**Regulatory interactions among the floral meristem identity genes *LFY* and *AP1*; the B and C class homeotic genes *AP3*, *PI*, and *AG*; and *ZP1* and *ZFP8*. Only a very reduced subset of factors and interactions involved in flower development are shown––those most relevant to the work by Hu et al. (18). Novel interactions are depicted in blue.**

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and *AP1* promoters; that is, in those lines, *ZP1* and *ZFP8* expression would be maintained in precisely the floral domain in which they would normally be repressed by LFY and AP1. Inflorescences and flowers from these plants showed phenotypes that resembled *lfy* and *bc* mutants (i.e., *ap3 ag* or *pi ag*), and the expression of *AP3*, *PI,* and *AG* was greatly reduced, despite *LFY* and *AP1* being themselves normally expressed (ectopic expression of *ZFP4* and *ZFP7* did not alter floral organ development). These results highlighted the importance of repressing the expression of *ZP1* and *ZFP8* in floral primordia. Neither single *zp1* or *zfp8* loss-of-function (but not null) mutants, or the double mutant *zp1 zfp8*, showed defects in flower development. However, *zp1 zfp8* could partially rescue petal development and *AP3* and *PI* expression in an *lfy-1* or *ap1-10* background. Importantly, although *AP3*, *PI,* and *AG* are not expressed in leaves, their expression—albeit at a low level—could be detected in leaf primordia of *zp1 zfp8* plants, demonstrating a repressive role for ZP1 and ZFP8 in vegetative tissue. Last, direct binding by ZP1 to *AP3*, *PI,* and *AG* regulatory regions was shown by ChIP-qPCR.

**The work by Hu et al. reveals an additional level of control for the expression of the B and C floral homeotic genes and adds new elements to the already intricate network that regulates flower development.**

Altogether, the results reported by Hu et al. (18) indicate that *ZP1* and *ZFP8* repress B and C homeotic gene expression, and that their downregulation by LFY and AP1 is necessary for the activation of *AP3*, *PI,* and *AG* in floral primordia and for proper petal, stamen, and carpel development. LFY and AP1 would therefore up-regulate B and C homeotic genes not only directly, but also in an indirect manner that depends on *ZP1* and *ZFP8*, which would act in a partially redundant manner with other factors.

The molecular mechanisms by which ZP1 and ZFP8 exert the repression of *AP3*, *PI,* and *AG*, as well as the identity of the additional factors that may be partially redundant with them, remain to be determined. It is possible that ZP1 and ZFP8 act through PRC2, because both proteins contain an EAR repression domain, and Arabidopsis TFs containing such domain have been shown to be involved in PRC2 recruitment to target genes for chromatin repressive marking (23). In addition, ZP1 and ZFP8 might repress *AP3*, *PI*, and *AG* through several (and different) mechanisms, not all dependent on PRC2, because *PI* ectopic expression is not detected in the vegetative tissue of mutants of the PRC2 component CLF (*clf* plants), in contrast to *AG* and *AP3* (15). In this respect, the EAR domain has been shown to mediate the interaction with TOPLESS/TOPLESS-RELATED corepressors, which couple TFs to class I histone deacetylases (HDACs) to repress the expression of target genes (24). Last, the results obtained by Hu et al. (18) indicate that further redundancy with *ZP1* and *ZFP8* exists, which could be caused by other members of the C1-1i subgroup of C2H2 TFs, although apparently not *ZFP4* or *ZFP7*.

In summary, the work by Hu et al. (18) reveals an additional level of control for the expression of the B and C floral homeotic genes and adds new elements to the already-intricate network that regulates flower development.


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