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Spotlight review on:

Hwang, K., Susila, H., Nasim, Z., Jung, J.-Y., and Ahn, J. H. (2019). Arabidopsis ABF3 and ABF4 Transcription Factors Act with the NF-YC Complex to Regulate SOC1 Expression and Mediate Drought-Accelerated Flowering. *Mol. Plant* Advance Access published 2019, doi:10.1016/j.molp.2019.01.002.

Title: The A-B-A of the floral transition: the to do list for the perfect escape

Running Title: Defining the regulatory events of drought escape

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The transition to flowering marks the initiation of reproductive competence in plants. After the floral transition the shoot apical meristem (SAM) ceases to produce leaf primordia and starts generating floral primordia, the reproductive organs of plants. To maximize reproductive success, the timing of this transition needs to be precisely aligned with the most favourable environmental conditions (Blackman, 2017). Daylength and cold temperature variations usually act as predictable seasonal signals to initiate flowering. However, due to the complexity of natural conditions, plants must gage a much wider array of information before committing to reproductive development. A striking example of this developmental plasticity is the drought escape (DE) response, where drought conditions experienced during vegetative growth cause an accelerated flowering time compared with optimal watering conditions (Fig 1A). Among the strategies to cope with drought stress, DE may provide ephemeral plants like *Arabidopsis* a simple "exit strategy" to anticipate seed set before drought conditions become incompatible with their survival.

Two signalling cascades are required for DE; the photoperiodic pathway and the drought-related phytohormone abscisic acid (ABA) signalling (Riboni et al., 2013; Hwang et al., 2019). The photoperiodic pathway allows plants to flower according to particular day length and in Arabidopsis it is activated upon exposure to long day conditions, typical of spring/summer in temperate climates. Key photoperiodic signalling genes are *GIGANTEA* (*GI*) and *CONSTANS* (*CO*). Light-stabilized CO protein cascades into the activation of the florigen genes *FLOWERING LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*) constituting a systemic flowering signal produced in the leaf vasculature and translocated in the SAM (Golembeski and Imaizumi, 2015). The florigen genes are necessary for DE activation and their transcript levels increase upon drought conditions through a poorly understood mechanism that involves the stimulation of GI function by ABA (Riboni et al., 2016). Major gaps thus exist in the definition of the molecular components that allow plants to integrate drought signals in the floral network. An exciting step towards this goal is described in a recent study, identifying the class of ABA-related bZIP transcription factors (TFs) ABRE-BINDING FACTORS (ABFs) as major drivers of DE (Hwang et al., 2019).

ABF3/4 integrate ABA signals into the floral network

Great progresses have been made in the definition of the core ABA signalling components. ABA is perceived by the PYR/PYL/RCAR (PYRABACTIN RESISTANCE1/PYR1-LIKE/REGULATORY COMPONENTS OF ABA RECEPTORS) family of soluble receptors which, upon ABA binding, indirectly stimulate the activity of the SnRK2 (SNF1-related protein kinases 2) kinase family. The SnRK2s activate a complex network of posttranslational modifications that affect virtually all biological functions. The ABFs are key targets of the SnRK2s-mediated phosphorylation and regulate the ABA gene expression network (Yoshida et al., 2014; Song et al., 2016). Phenotypic analyses indicate that besides being impaired in ABA responses, mutant combinations of *abf2*, *abf3*, and *abf4* are also late flowering (with *ABF3* and *4* playing a major role) (Yoshida et al., 2014; Hwang et al., 2019). Conversely, the ectopic expression of *ABF3* conferred an early flowering phenotype. *ABF3/4* transcripts accumulation was consistent with their positive role in flowering, increasing during the vegetative phase and preceding that of known floral markers. The pattern of *ABFs* expression was in sharp contrast with that of other ABA-related TFs known to act as floral repressors. Thus, while ABA can have both positive and negative effects in flowering regulation, the evidence presented points to a predominantly positive role, mediated by the ABFs (Hwang et al., 2019).

Defining *SOC1* as target of ABF3/4

A major observation of this study is that *abf3/4* mutants are impaired in DE, supporting the key role of ABA signalling in this process. The authors further elaborate on the definition of the MADS box type TF *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*) as *bona fide* floral target of ABF3/4. They compared datasets profiling the binding of 21 ABA-related transcription factors to chromatin and exploring the ABA-regulated gene networks to glean a short list of 12 targets. About half were identified as floral repressors and thus unlikely to activate DE. Among the other candidates, only *SOC1* survived further experimental validations, as its transcript levels were significantly down-regulated in *abf3/4* mutants as compared with the wild type under any watering conditions. Consistent with previous reports, *soc1* mutants

were impaired in DE and could completely suppress the early flowering phenotype conferred by the overexpression of *ABF3* (Hwang et al., 2019). These data led the authors to conclude that *SOC1* hyper activation, triggered by ABA-stimulated ABF3/4, has a major contribution to DE.

ABA activation of *SOC1*: NF-Y takes centre stage

An intriguing observation of this study concerns the mode of activation of *SOC1* by ABF3/4 (Hwang et al., 2019). At the *SOC1* promoter the ABF3/4-bound DNA region did not display the usual ABA responsive cis-element, but rather a non-canonical NUCLEAR FACTOR-Y binding element (NF-Y BE). NF-Y is a hetero-trimeric complex consisting of NF-YB and NF-YC histone-fold domain subunits and one NF-YA subunit responsible for NF-Y sequence specificity of CCAAT-box elements. NF-Y acts as pioneer transcription factor, promoting chromatin accessibility and thus binding of other TFs (Gnesutta et al., 2018). In plants, genes encoding the different NF-Y subunits are remarkably expanded and functional redundancy exists. Since NF-Ys control ABA-responses as well as flowering time they are prime candidates to take part to the DE mechanism (Song et al., 2016). In support of this idea, NF-YC3/4 subunits could physically interact with ABF3/4. Moreover, *nf-yc3/4/9* mutants were impaired in the drought mediated *SOC1* upregulation as well as DE, indicating that in the absence of NF-Y function, ABF3/4 activities alone were insufficient to trigger DE. CO directly contributes to *SOC1* activation in combination with NF-Y (Hou et al., 2014). However, under drought conditions CO was completely dispensable for *SOC1* activation, indicating that while different protein complexes may assemble at the *SOC1* promoter, some are specifically engaged by ABA, yet still licensed by NF-Y binding (Fig 1B).

Towards the definition of the DE regulatory events

In short, this study identifies the *ABFs*/*SOC1* module as key driver of DE, which begs the question of its connection with the florigen genes (Hwang et al., 2019). Histochemical analyses coupled with mis-expression studies indicate that the ABFs/SOC1 module operates in the leaf vasculature, where *FT* is activated, and not in the SAM. *FT* transcript levels were elevated in *ABF3* overexpressing lines, but again

reduced when combined in the *soc1* mutant background. Thus, the link between *ABFs* and *FT* is an indirect one, as neither ABFs nor SOC1 are enriched at the *FT* promoter. Several mechanisms may enable this cross-talk, as SOC1 directly represses a variety of well-known negative regulators of *FT* (Hwang et al., 2019). Thus, the *ABFs*/*SOC1* module may craft an accessible chromatin environment at the *FT* promoter for other activators to take over, among which GI, CO and the same NF-YB/C subunits that regulate *SOC1*, as CO (resembling the NF-YA subunit) promotes their assembly at specific cis-elements (Riboni et al., 2016; Gnesutta et al., 2018). These are still poorly understood layers of transcriptional regulations that contribute to bolster *FT* levels appropriate with the dry environment (Fig 1B).

Our increasing knowledge of DE improves our understanding of plants survival strategies in nature, as the balance between the duration of the vegetative and reproductive phases is usually a major driver of fitness (Kenney et al., 2014). The connection of DE with ABA has also practical consequences, as improvements to rice yields under certain irrigation scenarios may be achieved by reducing ABA responses (Miao et al., 2018). This is associated with an increased duration of the vegetative phase, likely caused by reductions in florigen expression (Du et al., 2018). These studies will offer opportunities to identify targets for a more precise breeding strategy for plants under suboptimal conditions.

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Figure Legend

Figure 1. A hypothetical model for DE regulation in *Arabidopsis*. (A) Low soil moisture elicits DE under long days, causing an early entry in the reproductive phase. (B) Drought stress triggers ABA production and signalling, causing changes in gene expression through the ABFs. In the leaf, ABA-stimulated ABFs engage the NF-Y complex at the NF-Y binding element (NF-Y BE) region to trigger *SOC1* transcriptional activation. The *SOC1* gene product acts as transcriptional repressor of several negative regulators of *FT*, thus facilitating its transcriptional activation (dotted arrow) by the CO-NF-Y complex which assembles at the CO-responsive element (CORE). ABA stimulates GI in an unknown manner which might relocate at the *FT* promoter in association with the CO-coordinated NF-Y complex.