DISPATCH

Plant Biology: AHL Transcription Factors Inhibit Growth-Promoting PIFs

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How do plants respond to abiotic stresses such as drought, salt or cold? A new study in *Arabidopsis* reveals that the stress-responsive AHLs antagonize the function of the PIF transcription factors to restrict rosette growth and allow resource reallocation for stress-adaptive responses.

Plants have evolved many fascinating strategies overcome their lack of mobility. Thanks to their plasticity, plants can dynamically adjust their growth and development to variations and challenges in their immediate environment. In contrast to animals, plants continuously generate new tissues and organs such as leaves, which arise from undifferentiated stem cells called meristems. In *Arabidopsis*, new leaves emerge from the vegetative shoot apical meristem in a spiral pattern with very short internodes between them, to form a rosette optimized for light capture. Central to growth, the PHYTOCHROME-INTERACTING FACTORS (PIFs) are basic-helix-loop helix domain-containing transcription factors that promote growth throughout the life cycle of plants [1,2]. During leaf growth, PIFs promote petiole length, and consequently *pif* mutants display reduced petiole length and more compact rosettes [3–5]. PIFs directly regulate the expression of genes involved in growth regulation, including hormones and other signaling pathways [6,7], and PIF abundance and activity is highly regulated in accordance with the environment [8,9]. When exposed to stress, plants generally respond by reducing their growth to divert resources to stress-adaptive responses. Under biotic stress caused by pathogens and herbivores, this growth–defense tradeoff involves accumulation of the defense plant hormone jasmonate, which leads to an increase in DELLA protein levels [10]. DELLAs then interact with PIFs and block their action to inhibit growth [11–13]. Plants also restrict their growth when facing abiotic stresses such as cold or drought. However, in contrast to biotic stresses, how plants inhibit growth in response to abiotic challenges is not well understood. In this issue of *Current Biology*, Favero *et al.* [14] now describe that the abiotic stress-responsive SOB3/ AHL29, a member of the AT-HOOK MOTIF NUCLEAR LOCALIZED (AHL) family of transcription factors, restricts petiole elongation by antagonizing the growth-promoting PIFs.

In this paper, the authors observed that the *SOB3* mutant *SOB3-D,* with increased *SOB3* levels, had a short-petiole phenotype under long days (LD) at 22 ºC, while the dominant-negative *sob3-6* mutant had enhanced petiole growth. Studies using bright-field microscopy indicated that both cell division and elongation were affected in *SOB3-D,* while only cell division was altered in *sob3-6*. They then sought to identify genes directly downstream of SOB3 which could be implementing these petiole phenotypes. Favero *et al.* [14] analyzed LD-grown juvenile (14 day-old) rosettes at ZT4, approximately the time of day with maximum petiole elongation in these conditions [15], using a combination of RNA-seq and ChIP-seq. For ChIP-seq, a *ProSOB3::SOB3-GPP* expressing line in a SOB3-null background was used. These studies identified 1,386 genes differentially expressed between *SOB3-D* and *sob3-6* that were bound by SOB3, of which approximately half corresponded to SOB3-induced and half to SOB3-repressed genes, suggesting that SOB3 can act as both transcriptional activator and repressor. Enriched gene ontology terms in the repressed gene set included "regulation of cell size", and response to "auxin" and "brassinosteroid stimulus", while "response to abiotic stimulus" was enriched in the induced set. This result was in agreement with a role for SOB3 in growth repression and in the activation of stress responses. Motif analysis identified the TCP-binding-like motif GGHCCA as the most enriched cis-element, consistent with previous reports of TCP and AHL interaction and co-binding to DNA in the regulation of hypocotyl growth [16]. Interestingly, the second most enriched motif was CACRYG, resembling the PIF-binding motifs G- (CACGTG) and PBE- (CACATG) boxes [17–20]. Given the described role of PIFs as promoters of petiole elongation, this result was a hint that SOB3 might be regulating petiole growth by antagonizing PIFs. Moreover, a *pif4* mutant grown under LD conditions phenocopied *SOB3-D* with short petioles and reduced cell length and number.

Authors then re-analyzed previous PIF4 and PIF5 ChIP-seq experiments [6,19] to compare with their SOB3 ChIP-seq data. Interestingly, they found that PIF binding was enriched in the vicinity of SOB3 peak summits in co-bound loci. Subsequent analysis by RNA-seq of the PIF-regulated genes in juvenile rosettes of a *pif4 pif5 pif7* mutant grown under LD conditions, and comparison with SOB3-regulated genes and ChIP-seq data, identified a significant number of SOB3 and PIF4/5 co-bound genes that corresponded with genes induced by PIFs and repressed by AHL. Among them are growth and hormone-associated genes like *ATHB2*, *IAA19*, *PIN3*, *SAUR24*, *BRI1*, *ACS8* and *YUC8*, known to promote petiole growth. Some of them were validated for direct binding by PIF4 at ZT4 under the LD conditions. Based on these results, authors proposed that SOB3 might regulate petiole growth by directly antagonizing PIF activity in inducing hormone signaling pathways. A prediction from this was that the effect of SOB3

on petiole growth would therefore be dependent on PIF4. To test this possibility, authors next examined the genetic interactions between SOB3 and PIF4 by generating *sob3-6 pif4* double mutants, where *pif4* would be expected to be epistatic over *sob3-6*. Indeed, compared to *pif4*, no significant increase in petiole length was observed in *sob3-6 pif4*.

Finally, Favero *et al.* [14] began to examine the mechanistic nature of SOB3 inhibition of PIF function. Yeast-two hybrid assays failed to detect interaction between PIF4 or PIF5 and SOB3, which led authors to propose that PIFs and SOB3 are likely not part of the same DNA-binding complex, although direct PIF–SOB3 binding *in planta* or indirect binding through bridging factors cannot be ruled out. Next, authors tested whether binding of PIF4 to its target genes might be affected by SOB3. This was performed by ChIP–qPCR using a *ProPIF4::PIF4–myc XVE::SOB3* line, in which *SOB3* expression was induced by β-estradiol. A decrease in PIF4 binding to the PIF4–SOB3 co-bound *ATHB2* and *ACS8* regulatory sequences was observed compared to the control *ProPIF4::PIF4–myc*. Importantly, the binding decrease to these SOB3–PIF4 co-targets was more pronounced compared with the binding reduction to PIF4-only targets. This was significant because induction of *SOB3* in the *ProPIF4::PIF4–myc XVE::SOB3* line led to a drastic 70% reduction of PIF4–myc protein levels. This decrease in PIF4 levels would be sufficient to explain the reduction in PIF4 DNA binding in the presence of SOB3, but the specific enhanced decrease in binding to SOB3–PIF4 co-targets compared with PIF4-only targets supports an additional specific effect of SOB3 in restricting PIF4 binding to DNA. Moreover, these lines also showed a significant reduction of *PIF4* transcript levels of approximately 30%, and ChIP data showed that SOB3 binds both upstream and downstream of *PIF4*, suggesting that SOB3 might directly repress PIF4 transcription under certain conditions. However, this SOB3 regulation of *PIF* transcription was not detected in their RNA-seq data, and therefore it might only take place under specific conditions such as those used for the SOB3-induction experiment, which used light of very high intensity to compensate for the fact that the *ProPIF4::PIF4–myc XVE::SOB3* line contain extra copies of *PIF4*. Together, these data suggest that SOB3 might inhibit PIF4 at the transcriptional and protein levels, including PIF accumulation and binding activity. Future studies will be necessary to refine these findings under more physiological conditions and establish how SOB3 affects PIF abundance and activity. Interestingly, this work found that the transcription of *BIN2* and *RGA* were directly regulated by SOB3, and that SOB3 could directly interact with the transcription factor HY5. The possibility that SOB3 indirectly regulates PIFs through one or more of these factors, well known to be involved in the regulation of PIF degradation and activity [8], awaits to be investigated.

To summarize, the current study by Favero *et al.* [14] provides novel exciting insight into the inhibition of PIF-promoted rosette growth by the AHL family of transcription factors. This adds to a number of described factors that regulate PIF accumulation and activity in response to a variety of stimuli, like light, temperature, hormones or photoperiod, and include direct interaction of PIFs with phytochromes, circadian clock components, DELLAs, or transcription factors like HY5 and BZR1 [8]. Importantly, because AHLs are abiotic stress-responsive genes, the new work described by Favero *et al.* [14] establishes a novel link between abiotic stresses such as drought or cold and the modulation of growth. The balance between growth and abiotic stress responses could be seen as analogous to the growth–defense tradeoff in response to biotic stresses, and provides a new framework to understand how plants optimize resources to face abiotic environmental challenges like salt, cold or drought, which could be of increased relevance in the current scenario of climate change.

Figure 1. Schematic representation of the proposed model by Favero *et al.*

Top, under normal conditions, PIFs promote petiole elongation during vegetative development in *Arabidopsis* by directly inducing genes promoting petiole growth, such as *ATHB2*, *IAA19* or *YUC8*. ATH factors like SOB3, expressed at low levels, inhibit PIF function moderately. This is exemplified by the long petiole phenotype of the dominant-negative mutant *sob3-6*.

Bottom, under abiotic stress conditions, SOB3 is induced and represses PIF4 function. A loss in PIF4 function results in short petioles and more compact rosette. This is shown by the short petiole phenotype of the *pif4* mutant. Because the effect of SOB3 on petiole growth is dependent on PIF4, no significant increase in petiole length is seen in the double mutant *pif4 sob3-6* with respect to *pif4*. The mechanism by which SOB3 antagonizes PIF function is not well defined, but it does not seem to require direct ATH–PIF interaction and it likely involves promotion of PIF degradation and restriction of PIF binding to its target genes. Pictures are from Figure 4 in Favero *et al*. [14].

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In Brief

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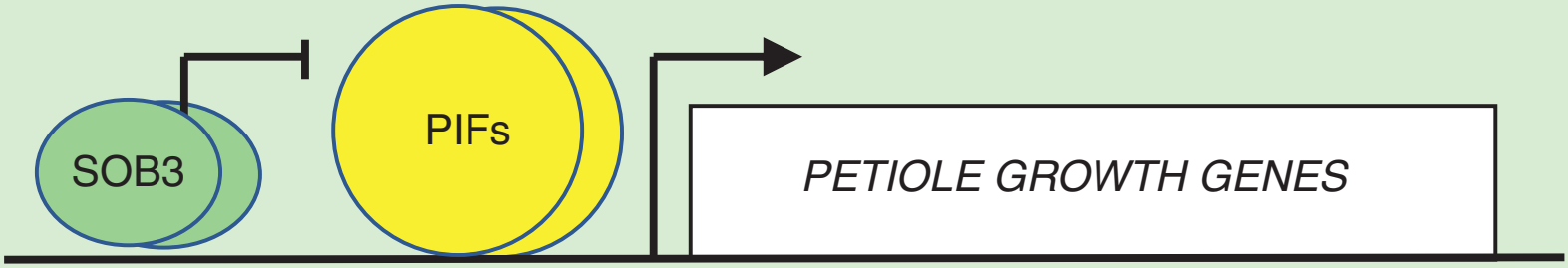
No stress



Abiotic stress