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IN BRIEF

The AREB1-ADA2b-GCN5 Complex Regulates Gene Expression during Drought Stress

Abiotic stresses constitute a global threat to agricultural crop production and natural ecosystems. One of the most prominent abiotic stresses is drought, which dramatically alters plant physiology and morphology. Studies in model organisms have shed light into how plants respond to drought stress, including transcriptional reprogramming of genes responsive to the hormone abscisic acid (ABA) (Song et al., 2016), and epigenetic modifications, like histone acetylation (Kim et al., 2008). Results by Li et al. (2018) further illuminate our understanding by mechanistically linking plant drought response with ABA-responsive gene activation through differential histone modifications in the tree species *Populus trichocarpa*.

To gain an understanding of the global histone acetylation landscape in *P. trichocarpa* during drought stress, the authors performed ChIP-Seq of well-watered and drought-stressed plants using antibodies against acetylated histone H3 at lysine 9 (H3K9ac), which is associated with an open chromatin and is a known activation mark for gene expression. Using the ChIP-Seq results vis-a-vis parallel RNA-Seq analyses, they found that genomewide changes in both H3K9ac profiles and transcript levels were heavily associated with ABA signaling pathway-related genes. In these genes of interest, there was significant enrichment of the ABA-Responsive Element (ABRE) promoter motif that could be bound by the AREB1 (ABA-Responsive Element Binding 1)-type transcription factor (Fujita et al., 2005).

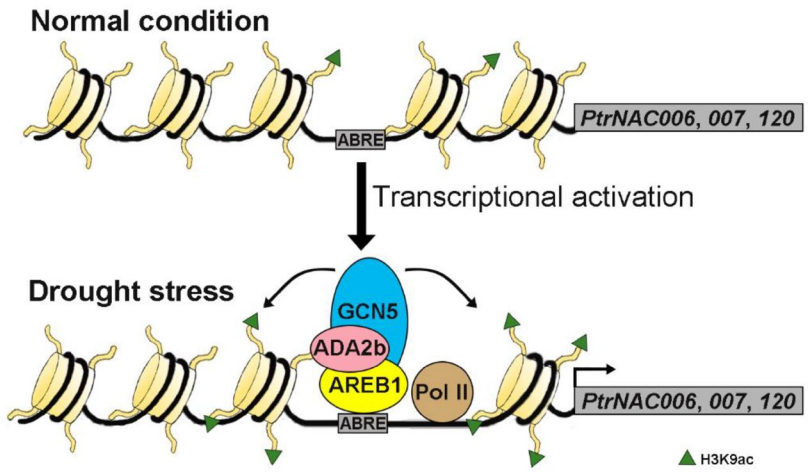
Because ABA-associated genes encoding transcription factors (TFs) have been shown to be effective for drought tolerance, the genes of interest were narrowed down to TF genes that met three important criteria: (1) differentially expressed during drought stress; (2) contained the ABRE motif; and (3) possessed changes in promoter H3K9ac levels. From this set of TF genes, Li et al. (2018) systematically narrowed them further to those with orthologs in other plant species that can enhance drought tolerance. Ultimately, three were selected as representative *Populus* TF genes for detailed functional characterization - *PtrNAC006*, *-007* and *-120*.

When these *PtrNAC* genes were overexpressed, the transgenic plants showed drought-tolerance or -avoidance phenotypes. The authors concluded that the transgenic plants had enhanced drought tolerance. However, many of the transgenic plants were also smaller than the wild-type controls and thus could have avoided the severe drought experienced by the larger control plants, since by transpiring less they would reduce soil moisture less (see the reader comment by Verslues, 2019). For example, Li et al. (2018) noted that the soil in which the transgenic plants "remained sufficiently moist, whereas the soil of wild-type control plants was dried out", supporting the notion that these plants avoided drought by reduced transpiration in these experiments. More definitive experiments are needed to show if the transgenic plants overexpressing *PtrNAC* genes are tolerant of reduced soil moisture, or if this is a drought avoidance effect due to reduced transpiration.

Probing deeper into the molecular mechanisms that might link ABA-responsive gene expression and the drought response, a protoplast system (Lin et al., 2014) was used to show that the *Populus* ortholog of AREB1 protein (PtrAREB1-2) can activate the transcription of *PtrNAC006*, *-007* and *-120* genes. ChIP and electrophoretic mobility shift assay (EMSA) confirmed that PtrAREB1-2 binds to the ABRE promoter motifs of these *PtrNAC* genes.

Because these *PtrNAC* genes also had differential H3K9ac levels in their promoter regions, it was important to test if the PtrAREB1-2 transcriptional activator can associate with a histone acetyltransferase (HAT) complex. Remarkably, using in vitro and in vivo protein interaction assays, PtrAREB1-2 was found to interact with the HAT complex catalytic subunit PtrGCN5 and the adaptor protein PtrADA2b. The *P. trichocarpa* AREB1-ADA2b-GCN5 regulatory complex dramatically induced transcription of the three *PtrNAC* genes by increasing H3K9ac levels and RNA polymerase II recruitment. Functional characterization of the ternary complex proteins by RNAi silencing and CRISPR editing provided evidence that PtrAREB1, PtrADA2b, and PtrGCN5 are required for drought tolerance.

This study by Li et al. (2018) provides new insight into the molecular mechanisms underlying plant responses to drought stress, with the characterization of genome-wide H3K9ac patterns and discovery of a regulatory protein complex controlling histone acetylation



Model for drought tolerance enhancement through the ARBE1-ADA2b-GCN5 regulatory complex in *Populus trichocarpa*.
The drought-induced AREB1 transcription factor binds to the ABRE promoter motif of *PtrNAC* genes associated with the water deprivation response. AREB1 recruits HAT complex proteins ADA2b and GCN5 leading to increased H3K9 acetylation, enriched Pol II recruitment and induced *PtrNAC* gene expression levels. *[Adapted from Li et al., (2018), Figure 9.]*

during drought stress being particularly interesting observations. In the future, it would be interesting to characterize the downstream target genes of the PtrNAC transcription factors as well as the upstream cellular signals leading to the formation of the AREB1-ADA2b-GCN5 ternary complex.

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