BMB Graduate Group M.S. Defense

Wednesday, April 1 at 1:30 p.m. in Ackert 324A

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The role of the GIR1-AMTL1 interaction in sepal cell fate and glucosinolate accumulation

HD-Zip IV transcription factors are the key regulators of epidermal development in *Arabidopsis*. As the epidermis serves as the first line of defense against the environment, its proper development is vital for plant survival. One HD-Zip IV member, *Arabidopsis thaliana* MERISTEM LAYER1 (ATML1), is a known regulator of giant cell formation in sepals, a modified leaf structure protecting the developing reproductive tissues within the flower bud. GLABRA2 INTERACTING REPRESSOR1 (GIR1) is a small protein that was previously reported to play a role as a transcriptional coregulator. In this work, it is shown that GIR1 physically interacts with ATML1. Since the function of GIR1 in transcription is poorly understood, we sought to elucidate the mechanism and downstream developmental impacts of this interaction.

Protein-protein interaction analyses revealed that the GIR1-ATML1 interaction occurs through the predicted zinc finger of GIR1 and START adjacent domain (STAD) of ATML1. Additionally, GIR1 displayed interactions with TOPLESS (TPL) and TOPLESS-RELATED (TPR) proteins, which are corepressors known to recruit histone deacetylase chromatin remodelers. The complex between ATML1, GIR1, and TPL/TPR proteins was detected using co-immunoprecipitation, indicating that GIR1 acts as an adaptor protein to bridge the interactions. The *gir1* loss-of-function mutant was observed to have more giant cells in sepals, a phenotype similar to *ATML1* overexpressing lines, suggesting that GIR1 functions as a repressor to ATML1. RNA sequencing revealed several genes upregulated in *gir1* sepals compared to wild type that are involved in the glucosinolate (GSL) biosynthesis pathway. GSLs are sulfur- and nitrogen-containing secondary metabolites that serve as biotic defense compounds in the Brassicales. Chemical analysis along with mass spectrometry imaging confirmed GSL accumulation in gir1 sepals. Thus, GIR1 acts as a repressor to ATML1 that functions to negatively regulate giant cell formation and GSL biosynthesis in sepals.

The interaction between GIR1 and ATML1 was shown in both plant and yeast systems but less is known about the minimal requirements or biological nature of the interaction. Identification of an acidic patch in ATML1 STAD and yeast bait autoactivation assays suggest STAD's role as a transcriptional activation domain. Mutational analysis revealed residues in the acidic patch critical for the transactivation activity. In order to detect the direct interaction between STAD and GIR1, we employed a biophysical method known as surface plasmon resonance (SPR). It was confirmed that STAD can directly interact with GIR1 with a low micromolar affinity. Moreover, no effective K_D could be calculated when the interaction occurred with a gir1 zinc finger mutant protein. Interaction of wild-type GIR1 with a STAD acidic patch mutant resulted in a two-fold decrease in affinity.

Together, these studies define a novel transcriptional repression complex involved in regulating giant cell formation and GSL accumulation in sepals. Given the agricultural importance of this compound, this work can be leveraged for engineering crops with tailored cell-specific GSL profiles.