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Structural Basis of Specificity in DNA Recognition by Plant WRKY Transcription Factors

Plants frequently encounter various types of stress factors such as abiotic stressors (e.g. extreme temperature, salinity, drought, UV radiation, wounding or presence of heavy metals) or biotic stressors, mainly caused by pathogen infections. To withstand these stressors, plants evolved many defense mechanisms that require precise reprogramming of a large number of genes. Regulation of gene expression is attained by the action of transcription factors (TFs) that are proteins deciding which genes get "turned on" (expressed) or "turned off" at any given time in a cell. TFs are activated after receiving stress signals, acting as molecular switches to regulate the expression of their target genes by interacting with gene promoters.

WRKY proteins are one of the largest families of transcription factors in plants and play a pivotal role in various signaling cascades and regulatory networks, particularly in defensive responses against environmental stresses and pathogen invasions. The distinctive feature of WRKY proteins lies in their DNA binding domain (DBD), known as the WRKY domain, a ~60-residue region that houses the highly conserved WRKYGQK sequence motif. While sequences within the DBD domain show high identity, WRKY TFs exhibit low overall similarity outside this domain, which is consistent with their functional diversity. Despite the essential role of WRKY proteins in plant defense, the molecular basis of DNA recognition by full-length WRKY TFs remains unexplored. Structures deposited so far in Protein Data Bank (PDB) offer only a glimpse into the DBDs of a few WRKY representatives, while indirect evidence suggests that regions outside the WRKY domain strongly contribute to the DNA binding selectivity.

To address this scientific gap, our project focuses on the structural and functional characterization of full-length *Arabidopsis thaliana* WRKY TFs. By employing cutting-edge techniques such as cryogenic electron microscopy (cryo-EM) and crystallography, as well as various DNA binding assays, we aim to unveil the intricate interactions of WRKY TFs with DNA and other protein binding partners. The application of several biophysical methods will provide clues about how tightly the proteins attach to DNA and how they influence gene expression.

The exploration of the structural and functional nuances of WRKY proteins holds significance not only for the fundamental understanding of plant physiology but also for practical applications e.g. in agriculture. The insights gained from this research can be harnessed to develop plant strains resistant to diverse environmental challenges, from pathogens to adverse conditions like cold, drought, or high salinity. Furthermore, our findings may contribute to refining breeding processes and guiding genetic engineering strategies. The identification of specific markers associated with stress resistance could revolutionize plant breeding, offering sustainable solutions to enhance crop resilience. Finally, structures of full-length WRKY TFs will advance algorithms for protein structure predictions, allowing the software to build more reliable models of protein-DNA complexes in the future. Therefore, our project that focuses on WRKY TFs will discover their structural and functional properties to advance both basic and applied science, with the potential to revolutionize plant defense strategies and contribute to the ever-expanding field of structural biology.