

New aspects of DNA recognition by plant WRKY transcription factors revealed by structural and functional study

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WRKY proteins are one of the largest families of transcription factors (TFs) in plants [1]. This family has expanded enormously in higher plants but not in bacteria, fungi, or animal lineages [2]. Biosynthesis of specific WRKYs is induced during certain stages of plant development, as a response to abiotic stress, or upon pathogen infection. WRKY TFs are essential players in several signalling cascades and regulatory networks that have crucial implications for defence responses in plants. They recognize the so-called W-box motif [(T)TGAC(C/T)] that usually occurs as tandem repeats. Despite the biological importance, structural knowledge of full-length WRKY proteins remains limited, with only a few DNA-binding domain (DBD) structures currently available in the Protein Data Bank (PDB). Based on domain and motifs architecture, WRKY TFs are categorised into three main Groups (I-III) and Group II further in five subgroups (a-e). The WRKY subgroup IIa includes three representatives in *A. thaliana*, AtWRKY18, AtWRKY40, and AtWRKY60, that are involved in biotic and abiotic stress responses. Subgroup IIa WRKY TFs are known to form homo and heterodimers. Here we determined the crystal structures of the AtWRKY18 DNA binding domain (DBD) alone and in the complex with a W-box containing DNA duplex [3]. Our data suggest that the dimerization interface of the full-length AtWRKY18 involves direct contacts between the DBD subunits. Structural and DNA binding analyses point out novel aspects of WRKY DNA recognition. In particular, AtWRKY18-DBD preferentially binds an overlapping tandem of W-boxes accompanied by a quasi-W-box motif [3]. The binding of DNA induces deformation of the B-type double helix, suggesting a requirement for intrinsic DNA flexibility. This may help explain why despite the short W-box consensus, WRKY TFs can precisely control gene expression. Additionally, our work provides the first experimental structure of a Group II WRKY transcription factor, enabling comparisons across Groups I to III.

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