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Structural insight into the role of VAL1 B3 domain for targeting to FLC locus in *Arabidopsis thaliana*

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ABSTRACT

Vernalization is a pivotal stage for some plants involving many epigenetic changes during cold exposure. In *Arabidopsis*, an essential step in vernalization for further flowering is successful silence the potent floral repressor Flowering Locus C (FLC) by repressing histone mark. *At*Val1 is a multi-function protein containing five domains that participate into many recognition processes and is validated to recruit the repress histone modifier PHD-PRC2 complex and interact with components of the ASAP complex target to the FLC nucleation region through recognizing a *cis* element known as CME (cold memory element) by its plant-specific B3 domain. Here, we determine the crystal structure of the B3 domain in complex with Sph/RY motif in CME. Our structural analysis reveals the specific DNA recognition by B3 domain, combined with our *in vitro* experiments, we provide the structural insight into the important implication of *At*VAL1-B3 domain in flowering process.

1. Introduction

Vernalization is the main method used by some plants to cope with the environmental stimuli during cold exposure [1,2]. In *Arabidopsis*, a key regulating mechanism of vernalization is through silencing the potent floral repressor Flowering Locus C (FLC) [2–5]. Sets of histone modifiers and a long noncoding RNA known as COOLAIR were indicated as regulators that dynamically modulated the expression of FLC [1,2,5]. The N-terminal tails of histone proteins carry various reversible post-translational modifications. One of these modifications, H3K27me3, is validated to be a repress mark to FLC that dynamically installed and uninstalled by a series of modifiers including Polycomb group (PcG) and Trithorax group [6]. Polycomb-mediated silencing typically involves PcG complex recruitment and spreading of repression mark at target loci [4,7]. An interacting partner of PcG known as Polycomb repressive complex 2 (PRC2), is pointed to catalyze the repressive H3K27me3 in animals and in plants [8]. Cold exposure will induce the formation of a PHD-PRC2 complex to localize to FLC chromatin involving the expression of two plant homeodomain (PHD) protein called VERNALIZATION INSENSITIVE 3 (VIN3) and VIN3-like protein 1(VRN5) [9,10]. This PHD-PRC2 complex accumulates specifically in a region, termed the "nucleation region" that covers the first exon and part of the first intron of FLC, to catalyze the H3K27me3 during vernalization [11]. After winter, when the temperature rises, the H3K27me3 modification spreads to cover the entire FLC locus, resulting in stable FLC silencing to render the vernalized plants competent to flower [11]. Thus, the PHD-PRC2 complex targeting to the "nucleation region" of *FLC* is a key step for downstream functions.

A *cis* DNA element in FLC locus containing two Sph/RY motifs (5′-TGCATG-3'; referred to hereafter as RY motifs) were recently discovered crucial for FLC repression during vernalization [12,13]. The two RY motifs containing region that essential for the maintenance of FLC silencing upon return to warm temperatures were then named cold memory element (CME) as its capability to remember the past cold [14]. Furthermore, H3K27me3 levels were

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not found to increase when mutated the RY motif during vernalization, manifesting that the RY motifs enable PHD-PRC2 nucleation. Meanwhile, two B3 domain family protein *At*VAL1 and *At*VAL2 but not *At*VAL3 were found as the transacting protein binding to the RY motifs and confirmed that the binding was mediated by its B3 domain [12,13], which was previously shown to recognize the RY motif [15]. These two homologs belong to the ABSCISIC ACID INSENSITIVE 3 (ABI3), FUSCA3 (FUS3) and LEAFY COTYLEDON 2 (LEC2) family of transcriptional repressor proteins [16]. *At*VAL1/2 were shown to function with redundancy, e.g. *At*VAL1 and *At*VAL2 were previously shown to function together during seedling development with sugar signaling to repress ectopic expression of seed maturation genes [17]; simultaneous disruption of *val1* and *val2* resulted in seedlings that developed embryonic traits and accumulated seed storage proteins and oils, whereas individual *val1* and *val2* mutants had no apparent morphological phenotypes [17,18]. Moreover, *At*VAL1/2 were also shown to function with redundancy and physically interact with the Polycomb component LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) [13], and interact with components of the ASAP complex and promote histone deacetylation during vernalization [12].

*At*VAL1/2 proteins seem to be sequence-specific epigenome readers that, through multivalent recognitions and interactions, can be tethered to a particular allele or locus [13]. Whereas, the structural basis of these domains of *At*VAL1/2 in the regulation of gene expression is yet to be elucidated. Here, we report the crystal structure of B3 domain of *At*VAL1. Our *At*VAL1 B3 domain in complex with RY motif and biochemical studies unveil the specific DNA recognition by *At*VAL1/2 and give a structural evidence that why *At*VAL1/2 but not *At*VAL3 can recognize the RY motif. We also illustrate the structural basis for different sequence selectivity among B3 domain-containing proteins in plants.

2. Materials and methods

2.1. Protein expression and purification

The PCR-amplified cDNA fragments encoding the *At*VAL1 B3 domain (273–400) were cloned into a modified pSUMO vector encoding 6xHis tag at N-terminus following a SUMO fusion tag and Ulp1 protease site. The plasmid containing the DNA insert of interest was transformed into *Escherichia coli* strain BL21 (DE3) grown in LB medium supplemented with 50 mg/ml kanamycin. The recombinant protein expression was induced by 0.2 mM IPTG at 37 °C, followed by 16–18 h incubation at 18 °C. The cell pellets were resuspended in buffer containing 20 mM Tris-HCl pH 8.0, 500 mM NaCl, 25 mM imidazole pH 8.0 and lysed using the high press and further clarified by centrifugation at 18,000 rpm. Supernatants were purified with nickel-chelating affinity column HisTrap (GE Healthcare), the target protein was washed with lysis buffer and then eluted with a buffer containing 20 mM Tris-HCl, pH 8.0, 500 mM NaCl and 500 mM imidazole pH 8.0. Ulp1 protease was added to remove the N-terminal tag and the fusion protein of the recombinant protein and dialyzed with lysis buffer containing 20 mM Tris pH 8.0, 500 mM NaCl 3 h. The mixture was applied to another Ni-NTA resin to remove the protease and uncleaved proteins with the buffer containing 20 mM HEPES pH7.5, 500 mM NaCl, 25 mM Imidazole pH 8.0. Eluted proteins were concentrated by centrifugal ultrafiltration, loaded onto a pre-equilibrated HiLoad 16/60 Superdex 75-pg column in an Äkta-purifier (GE Healthcare), eluted at a flow rate of 1 ml/min with the buffer containing 10 mM HEPES pH 7.5, 200 mM NaCl. Peak fractions were analyzed by SDS-PAGE (15%, w/v) and stained with Coomassie Brilliant Blue R-250. Purified fractions were pooled together and concentrated by centrifugal ultrafiltration. The concentration was determined by A_{280}.

2.2. ITC measurements

For B3 domain and DNA interactions, ITC assays were carried out on a MicroCal iTC200 calorimeter (GE Healthcare) at 25 °C. The buffer used for testing the binding affinity between proteins and DNA was 10 mM HEPES pH 7.5, 100 mM NaCl. The concentrations of proteins were determined spectrophotometrically. The DNA substrates were diluted in the reaction buffer. The ITC experiments involved 25 injections of protein into DNA. The sample cell was loaded with 250 μl of DNA at 50 μM and the syringe with 80 μL of

Table 1
Data collection and refinement statistics.

	B3 (273–400) and 13bp-DNA (Hg-II) (5YZY)	B3 (273–400) and 13bp-DNA (5YZZ)
Data collection		
Space group	$I4_122$	$I4_122$
Cell dimensions		
a, *b*, *c* (Å)	87.780, 87.780, 148.735	86.896, 86.896, 148.237
α, β γ, (°)	90, 90, 90	90, 90, 90
Wavelength(Å)	0.97853	0.97853
Resolution (Å)	75.60–2.608 (2.69–2.60)	74.97–2.585 (2.67–2.58)
R_{merge}	0.096	0.077
$I/\sigma I$	37.3 (2.25)	29.4 (2.33)
Completeness (%)	100.0(100.0)	99.5(95.7)
Redundancy	16.3	13.9
Refinement		
Resolution (Å)	30.00–2.61	30.00–2.58
No. reflections	7578	7414
R_{work}/R_{free}	0.2103/0.2567	0.2260/0.2552
No. atoms	1394	1393
Protein	866	866
DNA	527	527
Water/Ligands	1	0
Average *B*-factor (Å)	37.787	44.338
R.m.s. deviations		
Bond lengths (Å)	0.013	0.013
Bond angles (°)	1.70	1.89
Ramachandran plot		
Favored/allowed (%)	93.58/6.42	92.66/6.42

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