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A Direct Link between Abscisic Acid Sensing and the Chromatin-Remodeling ATPase BRAHMA via Core ABA Signaling Pathway Components

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ABSTRACT

Optimal response to drought is critical for plant survival and will affect biodiversity and crop performance during climate change. Mitotically heritable epigenetic or dynamic chromatin state changes have been implicated in the plant response to the drought stress hormone abscisic acid (ABA). The *Arabidopsis* SWI/SNF chromatin-remodeling ATPase BRAHMA (BRM) modulates response to ABA by preventing premature activation of stress response pathways during germination. We show that core ABA signaling pathway components physically interact with BRM and post-translationally modify BRM by phosphorylation/dephosphorylation. Genetic evidence suggests that BRM acts downstream of SnRK2.2/2.3 kinases, and biochemical studies identified phosphorylation sites in the C-terminal region of BRM at SnRK2 target sites that are evolutionarily conserved. Finally, the phosphomimetic $BRM^{S1760D\ S1762D}$ mutant displays ABA hypersensitivity. Prior studies showed that BRM resides at target loci in the ABA pathway in the presence and absence of the stimulus, but is only active in the absence of ABA. Our data suggest that SnRK2-dependent phosphorylation of BRM leads to its inhibition, and PP2CA-mediated dephosphorylation of BRM restores the ability of BRM to repress ABA response. These findings point to the presence of a rapid phosphorylation-based switch to control BRM activity; this property could be potentially harnessed to improve drought tolerance in plants.

Key words: abscisic acid, hormone signaling, chromatin remodeling

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INTRODUCTION

The stress hormone abscisic acid (ABA) elicits plant responses through binding to soluble PYRABACTIN RESISTANCE1 (PYR1)/PYR1-LIKE (PYL)/REGULATORY COMPONENTS OF ABA RECEPTORS (RCAR) receptors, which constitute a 14-member family in *Arabidopsis thaliana*. PYR/PYL/RCAR receptors perceive ABA in different subcellular locations (Rodriguez et al., 2014) and, as a result, form ternary complexes with clade A protein phosphatases type 2C (PP2Cs), thereby inactivating these negative regulators of ABA signaling (Ma et al., 2009; Park et al., 2009; Santiago et al., 2009; Nishimura et al., 2010). Therefore, ABA sensing prevents the PP2C-mediated dephosphorylation of ABA-activated sucrose non-fermenting 1-related protein kinases (SnRKs) subfamily 2 (SnRK2s), i.e. SnRK2.2/D, 2.3/I, and 2.6/E/OST1, and ABA receptors indirectly control the activity of these SnRK2s by allowing *cis*- and *trans*-autophosphorylation of the SnRK2 activation loop (Cutler et al., 2010; Soon et al., 2012; Ng et al., 2011; Minkoff et al., 2015). This results in the activation of an SnRK2-dependent phosphorylation cascade affecting a high number of targets in the plant cell (Umezawa et al., 2013; Wang et al., 2013). As a result, ABA-activated SnRK2s regulate different cellular processes, among them ion transport, cytosolic pH, and transcriptional response to ABA (Planes et al., 2015; Yoshida et al., 2015). Conversely, in the absence of ABA

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SnRK2 kinases are kept in an inactive state by clade A PP2Cs (Umezawa et al., 2009; Vlad et al., 2009).

ABA signaling regulates plant growth and development as well as stress responses (Cutler et al., 2010; Finkelstein, 2013). Plant developmental processes regulated by ABA are embryo maturation, seed development, dormancy and germination, seedling establishment, primary and lateral root growth, and transition from vegetative to reproductive stage (Cutler et al., 2010; Finkelstein, 2013). In addition, ABA mediates the response to both biotic and abiotic stresses (Cutler et al., 2010; Finkelstein, 2013). ABA signaling, in addition to key effects on ion transporters at the plasma membrane, leads to coordinated transcriptional reprogramming of gene expression in a ligand-dependent manner (Cutler et al., 2010; Finkelstein, 2013). Inducible alteration of gene expression requires changes in the chromatin state (Weake and Workman, 2010). Chromatin-mediated control of gene expression involves enzymes that covalently modify histones (e.g. by acetylation, methylation, phosphorylation, and ubiquitylation) or the DNA (methylation) as well as non-covalent change nucleosome occupancy or positioning through chromatin-remodeling complexes, such as SWI/SNF subgroup complexes that form around BRAHMA (BRM) (Han et al., 2015). Indeed, the ABA response has been linked to mitotically heritable and dynamic chromatin state changes (Chinnusamy and Zhu, 2009; Yaish et al., 2011; Han and Wagner, 2014). For example, chromatin remodeling (Han et al., 2012), histone deacetylation (Zhu et al., 2008; Luo et al., 2012; Ryu et al., 2014), and histone demethylation (Zhao et al., 2015) have been reported to regulate ABA response.

Specifically, with respect to chromatin remodeling, loss- or reduction-of-function of the SWI/SNF ATPase *BRM* or associated complex component *SWI3C* causes ABA hypersensitivity during post-germination growth due to de-repression of a positive regulator of ABA response, the bZIP transcription factor *ABA INSENSITIVE 5* (*ABI5*) (Han et al., 2012). While basal levels of *ABI5* were increased in *brm* mutants, fold induction of *ABI5* in ABA-treated versus mock-treated plants was similar in the *brm* mutant to that in the wild-type (wt) plants, suggesting that BRM is specifically required to prevent *ABI5* expression in the absence of the cue. Accordingly, BRM maintains a well-positioned nucleosome at the *ABI5* transcription start site in the absence of ABA, and this nucleosome is destabilized upon ABA sensing (Han et al., 2012). Intriguingly, BRM binds to the critical region at this locus in the absence and presence of the ABA signal (Han et al., 2012). These findings, combined with those from a prior study that revealed a link between the putative BRM complex component SWI3B and a core ABA signaling component, the clade A PP2C HAB1 (Saez et al., 2008), suggested the possibility that BRM activity might be controlled by ABA.

Further support for this idea came from *in vivo* phosphoproteomic studies. For example, a global analysis of the *Arabidopsis* phosphoproteome after ABA treatment in the wt and the *snrk2.2/2.3/2.6* triple mutant identified new putative substrates of the ABA-activated SnRK2s (Umezawa et al., 2013; Wang et al., 2013). BRM phosphopeptides were identified preferentially in ABA-treated wt that were not detected in the *snrk2.2/2.3/2.6* triple mutant. These results suggested that BRM might be substrate of the ABA-activated SnRK2s, either directly or indirectly, through activation of additional downstream kinases such as MPKs (Umezawa et al., 2013; Wang et al., 2013). Here, we provide evidence that BRM is a direct target of SnRK2s and of PP2Cs, identify conserved OST1 phosphorylation sites in the C-terminal region of BRM, which are dephosphorylated by PP2CA, and provide evidence that phosphomimetic *BRM* mutants are ABA hypersensitive. Our results reveal roles for the core ABA signaling pathway, including PYR/PYL ABA receptors, clade A PP2Cs, and SnRK2s, in directly controlling a chromatin regulatory protein. Moreover, our results suggest that phosphorylation of BRM by SnRK2s is a mechanism to release BRM-mediated repression of *ABI5* expression and, thus, ABA response, whereas PP2C-mediated dephosphorylation of BRM likely maintains the repressive function of BRM on ABA response.

RESULTS

Genetic Interaction between SnRK2.2/2.3 and BRM Suggests that BRM Is a Target of the Core ABA Signaling Pathway

To test for a functional link between the core ABA signaling pathway and BRM, we crossed the ABA-hypersensitive *brm-3* mutant to the ABA-insensitive *snrk2.2/2.3* mutant to generate a *brm-3/snrk2.2/2.3* triple mutant. ABA-mediated inhibition of seedling establishment was compared among the different genetic backgrounds (Figure 1A and 1B). We found that the ABA-insensitive phenotype of the *snrk2.2/2.3* double mutant was attenuated when *brm-3* was introduced in this genetic background. Likewise, the reduced sensitivity of *snrk2.2/2.3* to ABA-mediated inhibition of root growth was attenuated in the *brm-3/snrk2.2/2.3* triple mutant (Figure 1C). These results suggest that the ABA insensitivity of *snrk2.2/2.3* is in part dependent on BRM repressing the ABA response. To further test this idea, we took advantage of a double mutant previously generated that combines the *brm-101* null mutant and a *35S:HAB1* overexpressing (OE) line (Saez et al., 2004; Han et al., 2012). HAB1 OE leads to enhanced dephosphorylation of SnRK2s at Ser residues of the kinase-activating loop, which prevents SnRK2 activation and ABA signaling (Umezawa et al., 2009; Vlad et al., 2009; Antoni et al., 2013), and thus phenocopies higher-order *snrk2* mutants. HAB1 OE causes ABA insensitivity in the root. The ABA-insensitive phenotype of HAB1 OE lines was attenuated in *brm-101 HAB1* OE plants (Figure 1D), which likewise suggests that the HAB1 gain-of-function effect on ABA signaling is partially dependent on BRM activity.

BRM Physically Interacts with SnRK2s and Clade A PP2Cs

BRM is an SWI/SNF subgroup ATPase and has the canonical domains found in this family of proteins (Han et al., 2015; Figure 2A). BRM has an N-terminal region with a glutamine-rich domain and a helicase SANT-associated domain (HSA), which frequently serves as docking site for recruiting transcription factors such as LFY and TCP4 (Farrona et al., 2004; Szerlong et al., 2008; Wu et al., 2012; Efroni et al., 2013). This is followed by the catalytic helicase-like ATPase domain, the Snf2 ATP-coupling (SnAC) domain, and a C-terminal domain which contains an

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