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Auxin Response Factor2 (*ARF2*) and Its Regulated Homeodomain Gene *HB33* Mediate Abscisic Acid Response in *Arabidopsis*

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Abstract

The phytohormone abscisic acid (ABA) is an important regulator of plant development and response to environmental stresses. In this study, we identified two ABA overly sensitive mutant alleles in a gene encoding Auxin Response Factor2 (ARF2). The expression of *ARF2* was induced by ABA treatment. The *arf2* mutants showed enhanced ABA sensitivity in seed germination and primary root growth. In contrast, the primary root growth and seed germination of transgenic plants over-expressing ARF2 are less inhibited by ABA than that of the wild type. ARF2 negatively regulates the expression of a homeodomain gene *HB33*, the expression of which is reduced by ABA. Transgenic plants over-expressing HB33 are more sensitive, while transgenic plants reducing HB33 by RNAi are more resistant to ABA in the seed germination and primary root growth than the wild type. ABA treatment altered auxin distribution in the primary root tips and made the relative, but not absolute, auxin accumulation or auxin signal around quiescent centre cells and their surrounding columella stem cells to other cells stronger in *arf2-101* than in the wild type. These results indicate that ARF2 and HB33 are novel regulators in the ABA signal pathway, which has crosstalk with auxin signal pathway in regulating plant growth.

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Introduction

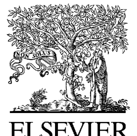
Abscisic acid regulates many important aspects including seed development, dormancy, germination, vegetative growth, and plant responses to environmental stresses [1]. ABA is required for normal plant growth as ABA-deficient mutants reduce cell vigor and are usually smaller [2]. Different developmental stages of *Arabidopsis* seedlings exhibit different response to ABA. In the early germination stage for establishing embryonic axis, the seed germination and post-germination growth are more sensitive to ABA (during 48 hr after seed imbibition) than other stages and more than 3 μM ABA will block the germination and post-germination growth [3,4]. Genetic screening during this stage has been performed and identified some specific ABA responsive factors such as ABA INSENSITIVE3 (ABI3) and ABI5, which play critical roles in regulating seedling growth mainly during seed germination and post-germination growth period [5,6]. However, after more than 48 hr of seed imbibition, higher concentrations of ABA are needed to inhibit seedling growth [1]. Recent studies have identified four core components in the ABA signaling pathway, which include soluble PYR1/PYL/RCAR ABA receptors, PP2C phosphatases, SnRK2 kinases and ABA-responsive transcriptional factors for gene regulation or SLAC1 and other channels for regulating guard cell movement, indicating a relative simple and short regulating pathway [7–12]. The SnRK2 (sucrose non-fermenting 1-related protein kinase) triple mutants and the dominant *abi1-1* and *abi2-1* mutants show insensitive to ABA in all ABA responses including seed germination, seedling growth and guard cell movement [4,13–15]. Low concentrations of ABA promote root growth through the promotion of the quiescent centre quiescence and the suppression of stem cell differentiation [16]. However, high concentrations of ABA can inhibit root growth through inhibiting cell division [17,18]. Some DNA replication related mutants are hypersensitive to ABA in seed germination and seedling growth, suggesting that ABA signal might inhibit cell division through regulating the DNA replication related proteins [18].

In order to find the new genes in ABA response, we performed a genetic screen by using ABA inhibiting root growth phenotype [18–20]. Here we identified two *ARF2* mutant alleles that were hypersensitive to ABA in both seed germination and primary root growth. ARF2 directly regulates the expression of a homeodomain gene *HB33*. Our data indicate that ARF2 is a negative, and HB33 is a positive regulator in ABA mediating seed germination and primary root growth.

Results

arf2 mutants are more sensitive to ABA than the wild type in both seed germination and root growth

The sensitivity of seed germination on ABA has been used to identify some classic ABA sensitive and ABA insensitive mutants [4]. In order to find more new ABA responsive mutants, we take



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The coupling of epigenome replication with DNA replication

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In multicellular organisms, each cell contains the same DNA sequence, but with different epigenetic information that determines the cell specificity. Semi-conservative DNA replication faithfully copies the parental nucleotide sequence into two DNA daughter strands during each cell cycle. At the same time, epigenetic marks such as DNA methylation and histone modifications are either precisely transmitted to the daughter cells or dynamically changed during S-phase. Recent studies indicate that in each cell cycle, many DNA replication related proteins are involved in not only genomic but also epigenomic replication. Histone modification proteins, chromatin remodeling proteins, histone variants, and RNAs participate in the epigenomic replication during S-phase. As a consequence, epigenome replication is closely linked with DNA replication during S-phase.

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Introduction

Although each cell of multicellular organisms maintains identical DNA sequences after each cell division during development, different types of cells exhibit different gene expression patterns that are determined by epigenetical marks. These epigenetical marks include DNA methylation, histone modifications, histone variants, chromatin structures, and noncoding RNAs. Each cell reads different epigenetic marks precisely during different developmental stages or in response to different environmental stresses. Establishment of epigenetic marks is partially mediated by noncoding RNAs, which are conserved in animals, plants, and yeasts [1]. Once these epigenetic marks are established, epigenetic inheritance will guarantee the transmission of these marks in each cell cycle and even from generation to generation.

Genomic regions with epigenetic marks can be distinguished by highly condensed heterochromatin that is localized in transposons, repetitive sequences, centromeric regions, and telomeres. Heterochromatin in plants is usually characterized by histone marks such as histone 3 lysine 9 mono/dimethylation, 27 mono/dimethylation, and histone 4 lysine 20 monomethylation [2]. Decondensed euchromatin that is localized in the gene-rich regions is usually modified by histone marks such as H3K4me3 and hyperacetylated histone [2]. Unlike DNA replication, however, epigenomic replication does not always precisely copy all the parental epigenetic marks in every cell cycle, which results in epigenomic changes that lead to various cell types (called cell differentiation during development) or to different cell responses to environmental stresses. In this review, we will summarize researches concerning the coupling of epigenetic inheritance with DNA replication.

Disruption of chromatin structures in preparation for DNA replication

DNA replication can be divided into four stages including pre-replication, initial replication, replication elongation, and maturation [3]. DNA replication machinery is highly conserved among eukaryotes. Euchromatic regions replicate early while heterochromatin regions replicate late in the S-phase, suggesting that loose chromatin structures are more easily opened than condensed chromatin structures for DNA replication [4]. The origin recognition complex (ORC) binds onto the origin sites of replication and sequentially recruits Cdc6 and Cdt1, which leads to the assembly of the mini-chromosome maintenance complex 2-7 (MCM2-7, a ring-shaped, heterohexameric AAA + ATPase) onto chromatin [5]. Disruption of parental nucleosomes is required for the formation of replication forks and for the synthesis of daughter strands because chromatin renders the DNA template structurally inaccessible. It is clear that ATP-dependent chromatin remodeling enzymes and chaperone proteins are required during this process. In mammalian cells, the Williams syndrome transcription factor interacts with the DNA clamp proliferating cell nuclear antigen (PCNA, a ring shaped homotrimer) and recruits ISWI chromatin remodeling ATPase protein SNF2H to the replication sites [6]. Another homolog, ACF1-ISWI, is required for DNA replication through heterochromatin [7]. The Swi2/Snf2 chromatin remodeling protein INO80 is recruited to the replication origins, and is responsible for replication fork stability and restarting replication under replication

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Active DNA demethylation by oxidation and repair

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DNA methylation and demethylation are increasingly recognized as important epigenetic factors in both plants and animals. DNA methylation, which is catalyzed by DNA methyltransferases (DNMTs), is a relatively stable and heritable modification that controls gene expression, cellular differentiation, genomic imprinting, paramutation, transposon movement, X-inactivation, and embryogenesis [1]. The methylation of cytosine to 5-methylcytosine (5mC) is an important example of DNA modification in animals and plants. This highlight concerns DNA demethylation mechanisms in mammals and whether they are similar to that in plants.

DNA demethylation can be passive or active. In both plants and animals, passive DNA demethylation occurs when cells fail to maintain the methylation during DNA replication [1]. In plants, active DNA demethylation is mainly carried out by a small group of bifunctional DNA glycosylases, including ROS1, DME, DML2 and DML3; after these DNA glycosylases remove the methylated cytosine base and create an abasic site, the gap is refilled with an unmethylated cytosine through a base-excision-repair (BER) pathway [1]. Animal genomes, however, do not contain ROS1 homologues, and active DNA demethylation in animals is proposed to result from several other mechanisms [2]. An oxidative mechanism in mammals has recently been revealed by studies on a small family of 5-methylcytosine hydroxylases (TET1, TET2, TET3; "TET" refers to Ten-Eleven-Translocation); these studies suggest that active DNA demethylation in mammals also goes through BER, but unlike that in plants, it must be preceded by oxidation and/or deamination [3-7].

5mC can be hydroxylated by TETs to become 5-hydroxymethylcytosine (5hmC). 5hmC was first reported in bacteriophage nucleic acids [8] and later in animal cells [9]. The occurrence of 5hmC in animal cells was debated until 2009, when two groups independently confirmed the presence of 5hmC in the animal genome [7, 10]. Tahiliani *et al*. found that TET1 in humans is responsible for the conversion of 5mC to 5mhC in a 2-oxoglutarate- and Fe(II)-dependent manner [7]. Later, Zhang and colleagues at the University of North Carolina found that all three mouse TET proteins can catalyze this conversion [6]. Recently, Zhang and colleagues reported that 5mC can also be converted into 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [11]. Their data showed that TET proteins oxidize the 5mC to generate 5hmC, 5fC, and 5caC *in vitro*. Their analysis of the genomic DNA indicated that all four cytosine modifications exist in different quantities. They further showed that through increasing or decreasing the expression of TET proteins, the contents of 5hmC, 5fC, and 5caC are increased or decreased, respectively [11].

Recent studies indicate that active DNA demethylation in mammals may follow a oxidation-deamination-BER pathway [3-5]. AID (activation-induced deaminase)-dependent DNA demethylation was found during a study of nuclear reprogramming when mouse embryonic stem cells (mESCs) were fused with human fibroblasts [12]. AID is required for promoter demethylation of *OCT4* and *NANOG* genes [12]. Guo *et al*. provided further evidence that the AID/APOBEC (apolipoprotein B mRNA-editing catalytic polypeptides) family of cytidine deaminases deaminates 5hmC, but not 5mC, into 5hmU [4]. In another study, Cortellino *et al*. identified the thymine DNA glycosylase (TDG) as a key enzyme that exhibits high glycosylase activity on 5hmU:G mismatches in double-stranded DNA but not on 5hmC [5]. After TDG removes 5hmU, the DNA is repaired and an unmethylated cytosine is inserted through the BER pathway [4, 5]. Mouse primordial germ cells with knockout of AID exhibit higher genome-wide DNA methylation than the wild type, indicat-

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The Plant-Specific Actin Binding Protein SCAB1 Stabilizes Actin Filaments and Regulates Stomatal Movement in *Arabidopsis*

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Microfilament dynamics play a critical role in regulating stomatal movement; however, the molecular mechanism underlying this process is not well understood. We report here the identification and characterization of STOMATAL CLOSURE-RELATED ACTIN BINDING PROTEIN1 (SCAB1), an *Arabidopsis thaliana* actin binding protein. Plants lacking *SCAB1* were hypersensitive to drought stress and exhibited reduced abscisic acid-, H_2O_2-, and $CaCl_2$-regulated stomatal movement. In vitro and in vivo analyses revealed that SCAB1 binds, stabilizes, and bundles actin filaments. SCAB1 shares sequence similarity only with plant proteins and contains a previously undiscovered actin binding domain. During stomatal closure, actin filaments switched from a radial orientation in open stomata to a longitudinal orientation in closed stomata. This switch took longer in *scab1* plants than in wild-type plants and was correlated with the delay in stomatal closure seen in *scab1* mutants in response to drought stress. Our results suggest that SCAB1 is required for the precise regulation of actin filament reorganization during stomatal closure.

INTRODUCTION

Plants must cope with various environmental changes during their life cycle. For example, during periods of drought, plants close their stomata to prevent excessive water vapor loss from their leaves. Many cellular signaling molecules and ions, including abscisic acid, H_2O_2, Ca^{2+}, and NO, regulate stomatal closure by controlling K^+ and anion channel activities, leading to a reduction in guard cell turgor (Pei et al., 2000; Hosy et al., 2003; Desikan et al., 2004; Hirayama and Shinozaki, 2007). Guard cell movement involves rearrangement of the actin cytoskeletal network (Kim et al., 1995; Eun and Lee, 1997; Liu and Luan, 1998; Hwang and Lee, 2001; Lemichez et al., 2001; MacRobbie and Kurup, 2007; Choi et al., 2008; Gao et al., 2008; Higaki et al., 2010). During stomatal closure and opening, microfilaments (MFs) undergo dynamic changes that are associated with changes in the activity of osmosensitive and stretch-activated Ca^{2+}-permeable channels (Zhang et al., 2007) or inward K^+ channels (Hwang et al., 1997). Consistent with this, the inhibition of MF reorganization via pharmacological means interrupts stomatal closure and opening (Kim et al., 1995; MacRobbie and Kurup, 2007).

Actin is a highly conserved globular protein in eukaryotic cells. Less than 10% of the actin in plant cells is in the filamentous form, whereas the majority of the actin in yeast or animal cells is filamentous, suggesting that the MFs of plant cells are remarkably dynamic, likely enabling plants to efficiently cope with environmental changes (Karpova et al., 1995; Gibbon et al., 1999; Snowman et al., 2002; Wang et al., 2005). Besides their function in regulating stomatal movement, MF dynamics are involved in many other cellular processes, including cell division, expansion, motility, organelle trafficking, endocytosis, exocytosis, and signal transduction (Thomas et al., 2009). To perform these functions, MF dynamics must be precisely regulated, and this requires many specific actin-associated factors, including actin binding proteins.

Actin binding proteins are essential for normal functioning of the actin cytoskeleton during plant growth and development (Barrero et al., 2002; Jedd and Chua, 2002; Oikawa et al., 2003; Ketelaar et al., 2004b; Ingouff et al., 2005; Szymanski, 2005; Djakovic et al., 2006; Xiang et al., 2007; Holweg and Nick, 2008; Peremyslov et al., 2008; Prokhnevsky et al., 2008; Sparkes et al., 2008; Tian et al., 2009; Li et al., 2010; Peremyslov et al., 2010; Suetsugu et al., 2010; Treitschke et al., 2010; Ueda et al., 2010; Vidali et al., 2010). Although pharmacological data suggest that

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Glutathione-Indole-3-Acetonitrile Is Required for Camalexin Biosynthesis in *Arabidopsis thaliana* W OA

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Camalexin, a major phytoalexin in *Arabidopsis thaliana*, consists of an indole ring and a thiazole ring. The indole ring is produced from Trp, which is converted to indole-3-acetonitrile (IAN) by CYP79B2/CYP79B3 and CYP71A13. Conversion of Cys(IAN) to dihydrocamalexic acid and subsequently to camalexin is catalyzed by CYP71B15. Recent studies proposed that Cys derivative, not Cys itself, is the precursor of the thiazole ring that conjugates with IAN. The nature of the Cys derivative and how it conjugates to IAN and subsequently forms Cys(IAN) remain obscure. We found that protein accumulation of multiple glutathione *S*-transferases (GSTs), elevation of GST activity, and consumption of glutathione (GSH) coincided with camalexin production. *GSTF6* overexpression increased and *GSTF6*-knockout reduced camalexin production. *Arabidopsis* GSTF6 expressed in yeast cells catalyzed GSH(IAN) formation. GSH(IAN), (IAN)CysGly, and γGluCys(IAN) were determined to be intermediates within the camalexin biosynthetic pathway. Inhibitor treatments and mutant analyses revealed the involvement of γ-glutamyl transpeptidases (GGTs) and phytochelatin synthase (PCS) in the catabolism of GSH(IAN). The expression of *GSTF6*, *GGT1*, *GGT2*, and *PCS1* was coordinately upregulated during camalexin biosynthesis. These results suggest that GSH is the Cys derivative used during camalexin biosynthesis, that the conjugation of GSH with IAN is catalyzed by GSTF6, and that GGTs and PCS are involved in camalexin biosynthesis.

INTRODUCTION

Phytoalexins are defined as low molecular weight antimicrobial compounds that are produced by plants after infection or stress (Hammerschmidt, 1999). Camalexin (3-thiazol-2'-yl-indole) is the major phytoalexin that accumulates in *Arabidopsis thaliana* plants after infection with microorganisms (Tsuji et al., 1992; Thomma et al., 1999; Glawischnig, 2007) and following treatment with abiotic factors (Tsuji et al., 1993; Zhao et al., 1998; Mert-Turk et al., 2003; Bouizgarne et al., 2006; Kishimoto et al., 2006). Several intermediates and key enzymes for camalexin biosynthesis in *Arabidopsis* have been identified through analysis of camalexin-deficient mutants and incorporation of radiolabeled compounds. Many phytoalexins produced by cruciferous plants are indoles with sulfur-containing moieties. The sulfur-containing moiety for camalexin is a thiazole ring at the 3-position of the indole (Devys et al., 1990; Monde et al., 1990; Tsuji et al., 1992). The indole ring of camalexin originates from Trp (Zook, 1998). Trp is converted to indole-3-acetaldoxime (IAOx) by the cytochrome P450 enzymes CYP79B2/CYP79B3 (Hull et al., 2000; Mikkelsen et al., 2000; Glawischnig et al., 2004). IAOx is a key metabolic intermediate for camalexin, indole glucosinolates, and auxin biosynthesis (Glawischnig et al., 2004). The dehydration of IAOx to indole-3-acetonitrile (IAN), catalyzed by CYP71A13, is required for IAOx flux through camalexin biosynthesis (Nafisi et al., 2007). The final two steps of camalexin biosynthesis, the catalysis of Cys(IAN) into dihydrocamalexic acid (DHCA) and DHCA into camalexin, are catalyzed by CYP71B15 (PAD3) (Schuhegger et al., 2006; Böttcher et al., 2009).

Early reports showed that the thiazole ring of camalexin originates from Cys (Zook and Hammerschmidt, 1997). It is unknown whether Cys itself or a Cys derivative conjugates to IAN to subsequently form Cys(IAN). However, recent studies indicate that a Cys derivative, but not Cys itself, conjugates to IAN. The first evidence of this is from the analysis of *phytoalexin-deficient2* (*pad2*) mutant plants. Camalexin accumulation in the *pad2* mutant was reduced by ~90% compared with wild-type plants following infection with *Pseudomonas syringae* pv *maculicola* strain ES4326 (*psm ES4326*) (Glazebrook and Ausubel, 1994). Gene cloning indicated that *PAD2* encodes glutamylcysteine synthetase 1 (GSH1) (Parisy et al., 2007). However, the *pad2* mutant contained about five times more Cys and 22% of glutathione (GSH) compared with wild-type plants. An exogenous supply of GSH restored pathogen-induced camalexin accumulation. Therefore, GSH or GSH derivatives, but not Cys itself, may function as the Cys donors in camalexin biosynthesis. The second point of evidence that a Cys derivative conjugates to IAN comes from IAN feeding and intermediates identification experiments. In plants fed with IAN and challenged with silver nitrate to induce camalexin biosynthesis, Böttcher et al. (2009) identified GSH(IAN), γGluCys(IAN), and Cys(IAN). These results suggest that GSH or GSH catabolites, such as γGluCys, may directly

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Arabidopsis Kinesin KP1 Specifically Interacts with VDAC3, a Mitochondrial Protein, and Regulates Respiration during Seed Germination at Low Temperature [W][OA]

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The involvement of cytoskeleton-related proteins in regulating mitochondrial respiration has been revealed in mammalian cells. However, it is unclear if there is a relationship between the microtubule-based motor protein kinesin and mitochondrial respiration. In this research, we demonstrate that a plant-specific kinesin, Kinesin-like protein 1 (KP1; At KIN14 h), is involved in respiratory regulation during seed germination at a low temperature. Using in vitro biochemical methods and in vivo transgenic cell observations, we demonstrate that KP1 is able to localize to mitochondria via its tail domain (C terminus) and specifically interacts with a mitochondrial outer membrane protein, voltage-dependent anion channel 3 (VDAC3). Targeting of the KP1-tail to mitochondria is dependent on the presence of VDAC3. When grown at 4°C, *KP1* dominant-negative mutants (TAILOEs) and *vdac3* mutants exhibited a higher seed germination frequency. All germinating seeds of the *kp1* and *vdac3* mutants had increased oxygen consumption; the respiration balance between the cytochrome pathway and the alternative oxidase pathway was disrupted, and the ATP level was reduced. We conclude that the plant-specific kinesin, KP1, specifically interacts with VDAC3 on the mitochondrial outer membrane and that both KP1 and VDAC3 regulate aerobic respiration during seed germination at low temperature.

INTRODUCTION

Much of the aerobic oxidation in eukaryotic cells takes place in mitochondria. A number of studies have shown that microfilaments and microtubules function in mitochondrial movement and positioning in eukaryotic cells (Hirokawa, 1998). Cytoskeletal proteins are also involved in regulating the permeability of the mitochondrial outer membrane to ADP in animal cells (Rappaport et al., 1998; Saks et al., 1995). It is well known that the membrane permeability of mitochondria is mainly dependent on the voltage-dependent anion channel (VDAC) (also named as a porin), the most abundant integral membrane protein in the mitochondrial outer membrane (Benz, 1994; Colombini, 1979; Liu and Colombini, 1992). Recently, both tubulin and actin from human and yeast (*Saccharomyces cerevisiae*) cells were found to interact with VDACs (Carré et al., 2002; Roman et al., 2006). In vitro reconstitution studies demonstrated that fungal VDACs have two main conductance states: an open state that allows the diffusion of large metabolites, including nucleotides, and a closed state that regulates ATP flux through the membrane (Rostovtseva and Colombini, 1996). Based on patch-clamp and planar lipid bilayer techniques, Rostovtseva et al. (2008) demonstrated that tubulin could induce reversible blockage of VDACs and decrease ATP/ADP permeability through the mitochondrial outer membrane and that the addition of tubulin in the reaction system could reduce the respiration rate of mitochondria (Rostovtseva et al., 2008). Genomic sequence analysis revealed that there are five VDAC isoforms in *Arabidopsis thaliana*, four of which have been cloned and identified (Clausen et al., 2004). However, it is still not known whether plant VDACs interact with cytoskeletal proteins.

We identified a plant-specific kinesin member in *Arabidopsis*, Kinesin-like protein 1 (KP1) (standardized nomenclature: At KIN14h; see Malcos and Cyr, 2009), and found that it binds to mitochondria based on immunoblot analysis (Ni et al., 2005). The motor domain of KP1 has nucleotide-dependent microtubule binding ability and microtubule-stimulated ATPase activity (Li et al., 2007). Kinesins constitute a superfamily of microtubule motor proteins and play critical roles in the transport of vesicles and organelles, cytokinesis, morphogenesis, and signal transduction (Reddy, 2001; Verhey et al., 2001; Lee and Liu, 2004; Hirokawa et al., 2009). Several animal kinesins, such as KIF1B and KIF5B in mouse cells (Nangaku et al., 1994; Tanaka et al., 1998) and KLP67A in early *Drosophila melanogaster* embryos (Pereira et al., 1997), have been implicated in the movement of mitochondria. Green fluorescent protein (GFP) fusion and transient expression assays showed that two *Arabidopsis* kinesins, MKRP1 and MKRP2, were expressed in mitochondria via their N-terminal mitochondrial targeting signals (Itoh et al., 2001). It is

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MDP25, A Novel Calcium Regulatory Protein, Mediates Hypocotyl Cell Elongation by Destabilizing Cortical Microtubules in *Arabidopsis* [C][W][OA]

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The regulation of hypocotyl elongation is important for plant growth. Microtubules play a crucial role during hypocotyl cell elongation. However, the molecular mechanism underlying this process is not well understood. In this study, we describe a novel *Arabidopsis thaliana* microtubule-destabilizing protein 25 (MDP25) as a negative regulator of hypocotyl cell elongation. We found that MDP25 directly bound to and destabilized microtubules to enhance microtubule depolymerization in vitro. The seedlings of *mdp25* mutant *Arabidopsis* lines had longer etiolated hypocotyls. In addition, MDP25 overexpression resulted in significant overall shortening of hypocotyl cells, which exhibited destabilized cortical microtubules and abnormal cortical microtubule orientation, suggesting that MDP25 plays a crucial role in the negative regulation of hypocotyl cell elongation. Although MDP25 localized to the plasma membrane under normal conditions, increased calcium levels in cells caused MDP25 to partially dissociate from the plasma membrane and move into the cytosol. Cellular MDP25 bound to and destabilized cortical microtubules, resulting in their reorientation, and subsequently inhibited hypocotyl cell elongation. Our results suggest that MDP25 exerts its function on cortical microtubules by responding to cytoplasmic calcium levels to mediate hypocotyl cell elongation.

INTRODUCTION

Etiolation occurs as buried seedlings fully elongate their hypocotyls upward in search of the soil surface. Light perception signifies soil emergence and significantly inhibits hypocotyl elongation. The cotyledons unfold and the photosynthetic growth process begins. Thus, the regulation of hypocotyl elongation is crucial for plant growth and development. Hypocotyl cells elongate quickly without division during postgermination and are widely used as a model to study the regulation of cell elongation (Gendreau et al., 1997; Wang et al., 2002; Tsuchida-Mayama et al., 2010). Hypocotyl cells begin their elongation in the basal region. They proceed in the acropetal direction and reach their maximum length when grown in the dark. The elongation of hypocotyl cells is strongly influenced by both external and internal cues. Numerous studies have detailed the mechanisms involved in hypocotyl cell elongation regulated by light, phytohormones, transcription factors, and the cytoskeleton (Wang et al., 2002; Ehrhardt, 2008; Niwa et al., 2009).

Microtubules are involved in cell elongation, expansion, division, and plant morphogenesis (Thitamadee et al., 2002; Smith and Oppenheimer, 2005; Ehrhardt and Shaw, 2006; Buschmann and Lloyd, 2008). Cortical microtubules regulate cell elongation by orientating cellulose fibrils and cellulose fibril arrays, thereby influencing the mechanical properties of the cell wall (Baskin, 2005; Paredez et al., 2006; Somerville, 2006; Lloyd and Chan, 2008). Long-term time-lapse imaging has revealed that clockwise and counterclockwise rotations are important dynamic features of cortical microtubules in growing hypocotyl cells (Chan et al., 2007). The parallel array of cortical microtubules is transversely oriented to the hypocotyl longitudinal growth axis in elongating hypocotyl cells and longitudinally oriented when elongation has stopped (Le et al., 2005). Thus, the regulation of the organization and dynamics of cortical microtubules is crucial for hypocotyl cell growth.

Microtubule regulatory proteins regulate the organization and dynamics of microtubules (Hamada, 2007; Kaloriti et al., 2007; Buschmann and Lloyd, 2008; Sedbrook and Kaloriti, 2008). Mutations in these regulatory proteins usually result in abnormal plant growth and cell morphogenesis by altering microtubule organization and dynamics. For example, a point mutation in *Arabidopsis thaliana MICROTUBULE ORGANIZATION1* (*MOR1*)/*GEMINI POLLEN1* induces organ twisting and isotropic cell expansion in the roots by altering the dynamic instability of microtubules (Whittington et al., 2001; Twell et al., 2002). In addition, *Arabidopsis* SPIRAL1 (SPR1) is involved in hypocotyl cell elongation. The expression pattern of *SPR1* in etiolated hypocotyls correlates with hypocotyl cell growth status, and SPR1 overexpression increases hypocotyl length, suggesting a

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LARGE-SCALE BIOLOGY ARTICLE

The Predicted *Arabidopsis* Interactome Resource and Network Topology-Based Systems Biology Analyses[W][OA]

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Predicted interactions are a valuable complement to experimentally reported interactions in molecular mechanism studies, particularly for higher organisms, for which reported experimental interactions represent only a small fraction of their total interactomes. With careful engineering consideration of the lessons from previous efforts, the Predicted Arabidopsis Interactome Resource (PAIR; http://www.cls.zju.edu.cn/pair/) presents 149,900 potential molecular interactions, which are expected to cover ~24% of the entire interactome with ~40% precision. This study demonstrates that, although PAIR still has limited coverage, it is rich enough to capture many significant functional linkages within and between higher-order biological systems, such as pathways and biological processes. These inferred interactions can nicely power several network topology-based systems biology analyses, such as gene set linkage analysis, protein function prediction, and identification of regulatory genes demonstrating insignificant expression changes. The drastically expanded molecular network in PAIR has considerably improved the capability of these analyses to integrate existing knowledge and suggest novel insights into the function and coordination of genes and gene networks.

INTRODUCTION

Protein–protein interactions are essential for almost all cellular processes. Deciphering the protein interaction network not only provides insights into protein functions but also advances our understanding of higher-level phenotypes and their regulation. In *Saccharomyces cerevisiae* (Ito et al., 2000; Uetz et al., 2000; Gavin et al., 2002; Ho et al., 2002; Yu et al., 2008), *Homo sapiens* (Rual et al., 2005; Stelzl et al., 2005), *Drosophila melanogaster* (Formstecher et al., 2005), and *Caenorhabditis elegans* (Li et al., 2004), genome-wide yeast two-hybrid screens and large-scale affinity purification/mass spectrometry studies have been reported. A number of databases, such as IntAct (Aranda et al., 2010), BioGRID (Stark et al., 2006), BIND (Alfarano et al., 2005), and TAIR (Swarbreck et al., 2008), have been established as repositories for interaction data. However, no experiment aiming to chart an entire plant interactome has been attempted. Even for the best-studied plant model, *Arabidopsis thaliana*, <6000 interactions currently can be found in the major interaction repositories.

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Although current high-throughput interaction detection experiments are still prone to false-positives or false-negatives (Huang et al., 2007), the connection topology of many well-charted interactomes has been demonstrated to be robust enough to reflect significant functional linkages within and between higher-order biological systems, such as pathways and biological processes. These interactomes have been successfully explored to suggest potential coordination between pathways (Li et al., 2008; Dotan-Cohen et al., 2009), to predict novel gene functions (Vazquez et al., 2003; Sharan et al., 2007), and to identify robust expression signatures in response to perturbations at the interaction module level, including genes without detectable differential expression (Chuang et al., 2007). Unfortunately, the scarcity of the available *Arabidopsis* interactome limits the power of these approaches to produce novel hypotheses. Recognizing the need for a more comprehensive plant interactome, several efforts have been made to infer interactions using a variety of computational strategies. For instance, Geisler-Lee et al. (2007) predicted ~20,000 *Arabidopsis* interactions (interologs) based on homologous interactions in other species, De Bodt et al. (2009) filtered the interologs with functional association data to improve prediction reliability, Cui et al. (2008) predicted ~23,000 interactions from multiple types of indirect evidence using a relatively simple statistical learning tool, and Brandão et al. (2009) established a database integrating both experimentally reported and predicted interactions. However, none of these works rigorously assessed the coverage and reliability of the predicted interactions with externally reported experimental interactions.

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BON1 interacts with the protein kinases BIR1 and BAK1 in modulation of temperature-dependent plant growth and cell death in Arabidopsis

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SUMMARY

The Arabidopsis copine gene *BON1* encodes a calcium-dependent phospholipid-binding protein involved in plant growth homeostasis and disease resistance. However, the biochemical and molecular mechanisms by which BON1 modulates plant growth and defense responses are not well understood. Here, we show that BON1 interacts physically with the leucine-rich-repeat receptor-like kinases BIR1 (BAK1-interacting receptor-like kinase 1) and pathogen-associated molecular pattern (PAMP) receptor regulator BAK1 *in vitro* and *in vivo*. Additionally, *bon1* and *bir1* mutants exhibit synergistic interaction. While a *bir1* null mutant has similar growth and cell-death defects compared with *bon1*, a *bir1 bon1* double mutant displays more severe phenotypes than does the single mutants. The *bon1-1* and *bir1-1* phenotypes are partially suppressed by overexpression of *BIR1* and *BON1*, respectively. Furthermore, the *bir1* phenotype is attenuated by a loss-of-function mutation in the *resistance* (*R*) gene *SNC1* (*Suppressor of npr1-1, constitutive* 1), which mediates defense responses in *bon1*. Intriguingly, BON1 and BIR1 can be phosphorylated by BAK1 *in vitro*. Our findings suggest that BIR1 functions as a negative regulator of plant resistance and that BON1 and BIR1 might modulate both PAMP- and R protein-triggered immune responses.

Keywords: BON1, receptor-like kinases BIR1 and BAK1, growth, cell death, Arabidopsis.

INTRODUCTION

The copines are a family of highly evolutionarily conserved proteins that are found in protozoa, nematodes, mammals, and plants (Creutz *et al*., 1998). Copine proteins are characterized by two C2 domains at the N-terminus and a von Willebrand A domain at the C terminus. The C2 domains are calcium-dependent phospholipid-binding domains found mostly in signal transduction and membrane trafficking molecules (Rizo and Sudhof, 1998). The A domain of human copines associates with various proteins, such as mitogen-activated protein kinase kinase 1, protein phosphatase 5, and the NEDD8-conjugating enzyme UBC12 (Tomsig *et al*., 2003, 2004). The A domain of human copine III has also been shown to possess intrinsic kinase activity (Caudell *et al*., 2000). Copine structures and activities suggest that they may play crucial roles in membrane trafficking and signal transduction.

Genetic studies have revealed that the Arabidopsis copine gene *BON1* affects temperature-dependent plant growth homeostasis and defense responses (Hua *et al*., 2001; Jambunathan *et al*., 2001). *BON1* is a repressor of the disease resistance (*R*) gene *SNC1* (*suppressor of npr1-1, constitutive* 1) (Yang and Hua, 2004). *SNC1* encodes a TIR-NB-LRR protein, which is a member of the RPP4/RPP5 protein family (Zhang *et al*., 2003). The loss of *BON1* function results in activation of *SNC1* and subsequent temperature-dependent growth defects (Yang and Hua, 2004). *BON1* has two homologs in Arabidopsis, *BON2* and *BON3*, that have redundant functions essential for plant viability (Yang *et al*., 2006b). The double mutants of *bon1 bon2* and *bon1 bon3* exhibit extensive cell death and show seedling lethality, both of which can be suppressed either by *pad4* and *eds1* or by a higher temperature of 28°C (Yang

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MALE GAMETOPHYTE DEFECTIVE 4 encodes a rhamnogalacturonan II xylosyltransferase and is important for growth of pollen tubes and roots in Arabidopsis

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SUMMARY

In flowering plants, the growth of pollen tubes is essential for the delivery of sperm to the egg cells. Although many factors (including cell-wall properties) are involved in this process, little is known about the underlying molecular mechanisms that regulate the growth of pollen tubes. We report here the characterization of an Arabidopsis mutant *male gametophyte defective 4* (*mgp4*) that is severely defective in pollen tube growth. The *mgp4* mutation also impairs root growth of pollen-rescued *mgp4* mutant plants generated by expressing *MGP4* cDNA under the control of a pollen grain/tube-specific promoter. The *MGP4* gene encodes a putative xylosyltransferase and is expressed in many organs/tissues, including pollen tubes and roots. MGP4 protein expressed in *Pichia pastoris* exhibited xylosyltransferase activity and transferred D-xylose onto L-fucose. The pectic polysaccharide rhamnogalacturonan II (RG-II), isolated from 7-day-old pollen-rescued mutant seedlings, exhibited a 30% reduction in 2-*O*-methyl D-xylose residues. Furthermore, an exogenous supply of boric acid enhanced RG-II dimer formation and partially restored the root growth of the pollen-rescued mutant seedlings. Taken together, these results suggest that MGP4 plays important roles in pollen tube and root growth by acting as a xylosyltransferase involved in the biosynthesis of pectic RG-II.

Keywords: MGP4, xylosyltransferase, rhamnogalacturonan II, pollen, gametophyte, Arabidopsis.

INTRODUCTION

In flowering plants, the male gametes are enclosed in male gametophytes (pollen grains) in anthers. During anthesis, mature pollen grains are released from the anthers and delivered to the female stigmatic tissue. The interaction between the pollen grain and stigmatic cells triggers hydration and germination of the pollen grain. The resulting pollen tube invades the stigmatic cell, directionally elongates in the transmitting tract, and finally enters an embryo sac. The two sperm in the pollen tube are then released into the embryo sac for double fertilization. Growth of the pollen tube is therefore indispensable for fertilization in flowering plants (Taylor and Hepler, 1997; Malhó *et al.*, 2006; Krichevsky *et al.*, 2007).

Pollen tube growth is a complex developmental process that requires the involvement of a plethora of factors, including biosynthesis and modification of cell walls (Krichevsky *et al.*, 2007; Zonia and Munnik, 2008, 2009). The pollen tube wall has a tensile and plastic polysaccharide structure, generally consisting of two layers. The inner sheath contains callose, and the outer layer is primarily composed of pectin and a small amount of cellulose and hemicellulose (Taylor and Hepler, 1997). The wall properties,

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Root hair-specific expansins modulate root hair elongation in rice

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SUMMARY

Root hair growth requires intensive cell-wall modification. This study demonstrates that root hair-specific expansin As, a sub-clade of the cell wall-loosening expansin proteins, are required for root hair elongation in rice (*Oryza sativa* L.). We identified a gene encoding *EXPA17* (*OsEXPA17*) from a rice mutant with short root hairs. Promoter::reporter transgenic lines exhibited exclusive *OsEXPA17* expression in root hair cells. The OsEXPA17 mutant protein (OsexpA17) contained a point mutation, causing a change in the amino acid sequence (Gly104 → Arg). This amino acid alteration is predicted to disrupt a highly conserved disulfide bond in the mutant. Suppression of *OsEXPA17* by RNA interference further confirmed requirement for the gene in root hair elongation. Complementation of the *OsEXPA17* mutant with other root hair EXPAs (OsEXPA30 and Arabidopsis EXPA7) can restore root hair elongation, indicating functional conservation of these root hair EXPAs in monocots and dicots. These results demonstrate that members of the root hair EXPA sub-clade play a crucial role in root hair cell elongation in Graminaceae.

Keywords: *Oryza sativa* L., expansin, root hair elongation, EXPA17, root hair-specific gene, disulfide bond.

INTRODUCTION

Root hairs are important for nutrient and water uptake from the rhizosphere, and serve as a site of interaction with soil micro-organisms. Root hair development can be divided into three phases: cell specification, initiation and elongation. Numerous experimental observations in Arabidopsis indicate that these three phases involve various cellular and genetic processes (for reviews see Schiefelbein, 2000; Foreman and Dolan, 2001). The root hair distribution pattern in the root epidermis varies among vascular plants (Clowes, 2000; Dolan and Costa, 2001; Kim *et al.*, 2006). Several genes for root hair elongation or initiation in monocot crops have been reported. Two EXPANSIN B (EXPB) genes in barley (*Hordeum vulgare*) (*HvEXPB1*) and rice (*OsEXPB5*) were shown to be root hair-specific (Won *et al.*, 2010), and *HvEXPB1* was shown to be intimately involved in root hair initiation (Kwasniewski and Szarejko, 2006). Hochholdinger *et al.* (2008) described a *roothairless3* gene (*RTH3*) encoding a monocot-specific COBRA-like protein in maize. The genes *cellulose synthase-like D1* (*OsCSLD1*) and *OsAPY* for root hair elongation in rice have also been isolated (Kim *et al.*, 2007; Yuo *et al.*, 2009). A root hair-specific basic helix-loop-helix (bHLH) transcription factor gene in rice, designated *RHL1* (*root hairless 1*), was reported to be involved in root hair elongation (Ding *et al.*, 2009). More recently, Kwasniewski *et al.* (2010) identified 10 new genes that were thought to be involved in root hair formation in barley. Compared with Arabidopsis, however, root hair formation in monocots is still poorly characterized at the molecular level.

The root hair is a growing extension of a single epidermal cell. The initiation and growth of root hairs are thought to require loosening of cell-wall components. This loosening is potentially mediated by cell wall-loosening expansin proteins (EXPs) (Cho and Cosgrove, 2002). There are two major EXP sub-groups: EXPA and EXPB (Cosgrove, 2000; Kende *et al.*, 2004; Choi *et al.*, 2006). Multiple members of the EXPA and EXPB sub-groups have been shown to possess *in vitro* expansin activity, namely the ability to induce rapid extension or stress relaxation of cell walls placed under uniaxial tension (Cosgrove, 2000). The promoters of two Arabidopsis EXPA genes, *AtEXPA7* and

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OsIAA23-mediated auxin signaling defines postembryonic maintenance of QC in rice

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SUMMARY

Although the quiescent center (QC) is crucial to root development, the molecular mechanisms that regulate its postembryonic maintenance remain obscure. In this study, a semi-dominant mutant that exhibits pleiotropic defects in root tissues, which includes the root cap, lateral and crown roots, was isolated. The mutant is characterized by a loss of QC identity during postembryonic development, and the displayed defects result from a stabilizing mutation in domain II of OsIAA23 (Os06g39590). Expression of *OsIAA23* is specific to the QC of the root tip during the development of primary, lateral and crown roots. Consistent with *OsIAA23* expression in the QC, the auxin signaling marked by *DR5p::GUS* (ß-glucuronidase) was absent in the QC region of *Osiaa23*. Transgenic rice plants harboring *Osiaa23* under the control of the *QHB* promoter mimic partially the defects of *Osiaa23*. These results indicate that the maintenance of the QC is dependent on OsIAA23-mediated auxin signaling in the QC. These findings provide insight into Aux/IAA-based auxin signaling during postembryonic maintenance of the QC in plants.

Keywords: *Oryza sativa L.*, root development, quiescent center, OsIAA23, auxin signaling.

INTRODUCTION

The importance of the quiescent center (QC) to root development has been well documented in Arabidopsis. In Arabidopsis, nearly all of the cells in the root are derived from four types of stem cells. These stem cells are controlled by the QC, which is composed of a small number of mitotically inactive central cells (Benfey and Scheres, 2000). The QC acts to maintain the surrounding stem cells (SC) in an undifferentiated state, and laser ablation of the QC has been shown to cause differentiation of stem cells (van den Berg *et al.*, 1997).

Differences in the root structure of rice and Arabidopsis have been noted. In contrast with the single layer epidermis–endodermis structure in Arabidopsis, rice roots undergo eight successive asymmetrical periclinal cell divisions following the first anticlinal division. This generates the epidermis–endodermis, sclerenchyma layer, exodermis and five layers of cortex (Rebouillat *et al.*, 2009). In Arabidopsis, the epidermis and lateral root cap cell files are derived from the same stem cell (Dolan *et al.*, 1993). In rice, the epidermis and lateral root cap cell files derive from independent initials (Coudert *et al.*, 2010). In spite of these differences, the basic developmental model of cell types in rice roots is similar to Arabidopsis (Kamiya *et al.*, 2003a,b; Cui *et al.*, 2007). The molecular mechanism of QC/SC maintenance and the development of different cell files in Arabidopsis have been well studied (reviewed by Iyer-Pascuzzi and Benfey, 2009), while knowledge of these processes in rice, the monocot model plant, remains limited.

Auxin is crucial to many aspects of plant development, including lateral and crown root formation, embryonic development, and stem cell maintenance (Inukai *et al.*, 2005; Liu *et al.*, 2005; Woodward and Bartel, 2005; Galinha *et al.*, 2007). Inhibition of auxin transport by *N*-1-naphthylphthalamic acid (NPA) causes abnormal auxin accumulation, resulting in changes in root tip cell identities (Sabatini *et al.*, 1999). Accordingly, the disruption of auxin transport around the QC in one of the Arabidopsis PIN (PIN-FORMED) mutants, *Atpin4*, causes a defect in the maintenance of endogenous auxin gradients and leads to abnormal cell division (Friml *et al.*, 2002). PLETHORA (PLT) protein expression patterns are thought to be determined by auxin gradients. The dosage can be translated into distinct cellular responses, and high levels of PLT activity promote stem cell identity and maintenance (Galinha *et al.*, 2007). High-resolution measurements of endogenous indole-3-acetic acid (IAA) concentrations revealed a distinct maximum in the QC

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OsPHF1 Regulates the Plasma Membrane Localization of Low- and High-Affinity Inorganic Phosphate Transporters and Determines Inorganic Phosphate Uptake and Translocation in Rice[1][W][OA]

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PHOSPHATE TRANSPORTER TRAFFIC FACILITATOR1 (PHF1) is known to regulate the plasma membrane localization of PHT1;1, a high-affinity inorganic phosphate (Pi) transporter in Arabidopsis (*Arabidopsis thaliana*). *OsPHF1*, a rice (*Oryza sativa*) gene homologous to *AtPHF1*, was isolated and found to regulate the localization of both low- and high-affinity Pi transporters to the plasma membrane. Three *OsPHF1* allelic mutants carrying one-point mutations at the fifth WD-repeat motif and two at the transmembrane helix, respectively, showed arsenate resistance and severely reduced Pi accumulation. The data indicate that mutation of *OsPHF1* results in the endoplasmic reticulum retention of the low-affinity Pi transporter OsPT2 and high-affinity Pi transporter OsPT8. Mutation of *OsPHF1* also reduced Pi accumulation in plants exhibiting excessive shoot Pi accumulation due to the overexpression of *OsPHR2*. However, the transcript level of *OsPHF1* itself is not controlled by OsPHR2. Overexpression of *OsPHF1* increased Pi accumulation in both roots and shoots in a solution culture with Pi-supplied condition. These results indicate that the role of OsPHF1 is unique in the localization of both low- and high-affinity Pi transporters on the plasma membrane in rice and determines Pi uptake and translocation in rice. The similar function of PHF1 required to facilitate PHT1 transit through the endoplasmic reticulum between Arabidopsis and rice provides an example of expectations from what one would deduce from sequence comparisons to extend knowledge from Arabidopsis to crops.

Phosphorus is an essential macronutrient for plant growth and development. Plants acquire inorganic phosphate (Pi) directly from their environment by active absorption into the epidermal and cortical cells of the root via Pi transporters. After entry into the root cortical cells, Pi must eventually be loaded into the apoplastic space of the xylem, transported to the shoot, and then redistributed within the plant via Pi transporters (Schachtman et al., 1998).

As a constituent of nucleic acids, phospholipids, and cellular metabolites, living cells require millimolar amounts of Pi. However, most soil Pi is immobile and the Pi concentration available to roots is in micromolar quantities (Raghothama, 1999). To coordinate plant growth with the limited Pi availability, high-affinity Pi transporters have evolved to enable increased Pi acquisition from soils (Raghothama, 1999; Paszkowski et al., 2002; Rausch and Bucher, 2002; Ticconi and Abel, 2004). High-affinity plant Pi transporters were originally identified by sequence similarity with the high-affinity transporter of yeast (*Saccharomyces cerevisiae*), PHO84. Genes encoding some of these transporters are able to complement *pho84* yeast mutants (Rausch and Bucher, 2002). These proteins belong to the PHOSPHATE TRANSPORTER1 (PHT1) family of Pi/H^+ symporters (Rausch and Bucher, 2002). Nine PHT1 genes have been identified in Arabidopsis (*Arabidopsis thaliana*), and 13 PHT1 genes have been identified in rice (*Oryza sativa*; Goff et al., 2002; Rausch and Bucher, 2002).

Yeast cells starved for Pi activate feedback loops that regulate low- and high-affinity phosphate transport. In this manner, the interplay of positive and negative feedback loops leads to the bistability of phosphate transporter usage. Cells express predominantly either low- or high-affinity transporters, both of which have similar phosphate uptake capacities (Wykoff et al., 2007). Previous reports demonstrated that at least one member of the OsPHT1 family, OsPT2, is a low-affinity Pi transporter and may play a primary role during the Pi translocation process (Ai et al., 2009). OsPT2 is under the transcriptional control of OsPHR2, the functional ortholog of *AtPHR1* in rice (Zhou et al., 2008). Recently, the function of OsPT8 as a high-

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Investigating the Contribution of the Phosphate Transport Pathway to Arsenic Accumulation in Rice[1][W]

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Arsenic (As) accumulation in rice (*Oryza sativa*) may pose a significant health risk to consumers. Plants take up different As species using various pathways. Here, we investigated the contribution of the phosphate (Pi) transport pathway to As accumulation in rice grown hydroponically or under flooded soil conditions. In hydroponic experiments, a rice mutant defective in OsPHF1 (for phosphate transporter traffic facilitator1) lost much of the ability to take up Pi and arsenate and to transport them from roots to shoots, whereas transgenic rice overexpressing either the Pi transporter OsPht1;8 (OsPT8) or the transcription factor OsPHR2 (for phosphate starvation response2) had enhanced abilities of Pi and arsenate uptake and translocation. OsPT8 was found to have a high affinity for both Pi and arsenate, and its overexpression increased the maximum influx by 3- to 5-fold. In arsenate-treated plants, both arsenate and arsenite were detected in the xylem sap, with the proportion of the latter increasing with the exposure time. Under the flooded soil conditions, the *phf1* mutant took up less Pi whereas the overexpression lines took up more Pi. But there were no similar effects on As accumulation and distribution. Rice grain contained predominantly dimethylarsinic acid and arsenite, with arsenate being a minor species. These results suggest that the Pi transport pathway contributed little to As uptake and transport to grain in rice plants grown in flooded soil. Transgenic approaches to enhance Pi acquisition from paddy soil through the overexpression of Pi transporters may not increase As accumulation in rice grain.

Inorganic arsenic (As) is a human carcinogen, with exposure coming mainly from drinking water and food (Tsuji et al., 2007; European Food Safety Authority, 2009). Recent studies have identified rice (*Oryza sativa*) as a major dietary source of inorganic As, which may pose a significant health risk (Kile et al., 2007; Mondal and Polya, 2008; Meharg et al., 2009). This is because paddy rice is rather efficient at As accumulation due to a combination of the anaerobic conditions prevailing in paddy soil, which leads to arsenite mobilization (Takahashi et al., 2004; Williams et al., 2007b; Xu et al., 2008), and the inadvertent uptake of arsenite through the rice silicic acid uptake pathway (Ma et al., 2008). This problem is further exacerbated by the widespread contamination of As in paddy fields as a result of irrigation with As-laden groundwater in south Asia (Meharg and Rahman, 2003; Dittmar et al., 2010), mining, and the past use of arsenical agrochemicals (Williams et al., 2007a; Zhu et al., 2008). As contamination of paddy soils not only compromises food safety but also can cause substantial losses in rice production (Panaullah et al., 2009). It is important, therefore, to understand the mechanisms of As uptake by rice.

As is a redox-sensitive metalloid, with arsenate [As(V)] and arsenite [As(III)] being commonly found in soil. The two inorganic As species are readily interconvertible depending on the environmental conditions (especially the redox potential and pH). Arsenate predominates under aerobic conditions, whereas anaerobic conditions in flooded paddy soil favor arsenite. Arsenite can also be methylated by soil microorganisms, producing various forms of methylated As (e.g. monomethylarsonous acid [MMA] and dimethylarsinic acid [DMA]; Cullen and Reimer, 1989). Plants are able to take up various As species through different mechanisms (for review, see Zhao et al., 2010b). Arsenate is a chemical analog of phosphate (Pi) and is taken up by Pi transporters. Evidence for this comes from physiological studies showing competitive inhibition of arsenate uptake by Pi (Abedin et al., 2002b) and isolation of arsenate-resistant mutants of Arabidopsis (*Arabidopsis thaliana*) defective in Pi transporters (Shin et al., 2004; González et al., 2005). In the rice genome, there are 13 sequences belonging to the *Pht1* family encoding putative high-affinity Pi transporters (Paszkowski et al., 2002). Some of them have been characterized with regard to Pi uptake and transport (Ai et al., 2009). However, their roles in arsenate transport remain unknown. Furthermore, Pi uptake is highly regulated in

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Phosphorylation of SOS3-Like Calcium-Binding Proteins by Their Interacting SOS2-Like Protein Kinases Is a Common Regulatory Mechanism in Arabidopsis[1[W][OA]]

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The Arabidopsis (*Arabidopsis thaliana*) genome encodes nine Salt Overly Sensitive3 (SOS3)-like calcium-binding proteins (SCaBPs; also named calcineurin B-like protein [CBL]) and 24 SOS2-like protein kinases (PKSs; also named as CBL-interacting protein kinases [CIPKs]). A general regulatory mechanism between these two families is that SCaBP calcium sensors activate PKS kinases by interacting with their FISL motif. In this study, we demonstrated that phosphorylation of SCaBPs by their functional interacting PKSs is another common regulatory mechanism. The phosphorylation site serine-216 at the C terminus of SCaBP1 by PKS24 was identified by liquid chromatography-quadrupole mass spectrometry analysis. This serine residue is conserved within the PFPF motif at the C terminus of SCaBP proteins. Phosphorylation of this site of SCaBP8 by SOS2 has been determined previously. We further showed that CIPK23/PKS17 phosphorylated CBL1/SCaBP5 and CBL9/SCaBP7 and PKS5 phosphorylated SCaBP1 at the same site in vitro and in vivo. Furthermore, the phosphorylation stabilized the interaction between SCaBP and PKS proteins. This tight interaction neutralized the inhibitory effect of PKS5 on plasma membrane H^+-ATPase activity. These data indicate that SCaBP phosphorylation by their interacting PKS kinases is a critical component of the SCaBP-PKS regulatory pathway in Arabidopsis.

Calcium is a uniform second messenger involved in many plant responses to environmental stimuli. There are many different types of calcium-binding proteins identified in plants (Luan et al., 2002; Harper et al., 2004). One of them shares significant sequence similarity with the yeast (*Saccharomyces cerevisiae*) calcineurine B subunit (Luan et al., 2002; Gong et al., 2004). The first gene cloned from this family is SOS3 (for Salt Overly Sensitive3) by genetic screening of Arabidopsis (*Arabidopsis thaliana*) salt-sensitive mutants and map-based cloning (Liu and Zhu, 1998). SOS3 physically interacts with and activates SOS2, a protein kinase (Halfter et al., 2000), and this complex in turn activates SOS1, a plasma membrane Na^+/H^+ antiporter (Shi et al., 2000; Qiu et al., 2002). SOS3 interacts with the FISL motif of SOS2 at its C-terminal regulatory domain, a SOS2 kinase-inhibiting domain (Guo et al., 2001). Recently a SOS3 homolog, SCaBP8 (for SOS3-like calcium-binding protein8), was identified that interacts with and activates SOS2 to protect Arabidopsis shoots from salt stress, while SOS3 primarily protects roots (Quan et al., 2007). SOS3 has an N-terminal myristoylation signal peptide and it is required for SOS3 function in plant salt tolerance (Ishitani et al., 2000). SCaBP8 shares most of the biochemical features of SOS3 in regulating SOS2 kinase activity and recruiting SOS2 to plasma membrane, however it lacks such myristyolation signal sequence, suggesting that multiple regulatory processes exist in the SCaBP family (Quan et al., 2007). Although capable of performing similar functions in biochemical and cellular tests, SCaBP8 and SOS3 must fulfill distinct regulatory functions in the salt stress response, as they could not replace each other in genetic complementation experiments. SOS2 phosphorylates SCaBP8 at its C-terminal Ser-237 but does not phosphorylate SOS3. This phosphorylation is induced by salt stress, occurs at the plasma membrane, stabilizes the SCaBP8-SOS2 interaction, and enhances plasma membrane Na^+/H^+ exchange activity (Lin et al., 2009).

In Arabidopsis, there are nine SCaBPs/calcineurin B-like proteins (CBLs) and 24 SOS2-like protein kinases (PKSs)/CBL-interacting protein kinases (CIPKs; Luan et al., 2002; Gong et al., 2004). The regulation of PKS kinase activity by SCaBP calcium sensors is essential for their function in vivo (Gong et al., 2002;

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Cell Wall Hemicellulose Contributes Significantly to Aluminum Adsorption and Root Growth in Arabidopsis[1[OA]]

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The cell wall (CW) has been recognized as the major target of aluminum (Al) toxicity. However, the components responsible for Al accumulation and the mechanisms of Al-induced CW function disruption are still elusive. The contribution of different CW components (pectin, hemicellulose 1 [HC1], and HC2) to adsorb Al and the effect of Al on xyloglucan endotransglucosylase/hydrolyase activity were investigated in Arabidopsis (*Arabidopsis thaliana*) in this study. A fractionation procedure was optimized to effectively extract different CW components, especially to prevent the HC fraction from pectin contamination. When CW materials extracted from Al-treated roots (50 μM Al for 24 h) were fractionated, about 75% of CW Al accumulated in the HC1 fraction. A time-dependent kinetic study showed that only when the HC1 fraction was removed was the amount of Al adsorbed decreased sharply. In vivo localization of xyloglucan endotransglucosylase (XET) activity showed that Al greatly inhibited this enzyme activity within 30 min of exposure, which was concomitant with Al-induced callose deposition in roots. Results from real-time reverse transcription-polymerase chain reaction indicated that three genes may constitute the major contributors to XET activity and that the inhibition of XET activity by Al is caused by transcriptional regulation. These results, to our knowledge for the first time, demonstrate that HC is the major pool for Al accumulation. Furthermore, Al-induced reduction in XET activity could play an important role in Al-induced root growth inhibition.

Aluminum (Al) is the most abundant metal and the third most abundant chemical element in the earth's crust. When soil pH drops below 5, toxic forms of Al release into soil solution and become a limiting factor of crop growth and yield. It has been estimated that acid soils constitute approximately 50% of the world's potentially arable land (von Uexküll and Mutert, 1995). Therefore, Al toxicity is the most serious abiotic stress to crop production after drought (Kochian, 1995). The earliest and most dramatic visual symptom of Al toxicity is the inhibition of root elongation. Although it has been shown that Al can alter a series of physiological and biochemical processes, cytoskeleton dynamics disruption, and Ca^{2+}-dependent signal transduction pathway distortion, the primary cause of Al-induced root growth inhibition is still elusive (Kochian, 1995; Matsumoto, 2000; Rengel and Zhang, 2003; Zheng and Yang, 2005).

The cell wall (CW) plays principal roles not only in the regulation of growth and development of plants but also in the perception and expression of Al toxicity. When plant roots are exposed to Al, CW is the first site in contact with Al. Many reports have demonstrated consistently that CW is the major pool of Al accumulation. For example, Clarkson (1967) reported that 85% to 90% of the total Al accumulated by barley (*Hordeum vulgare*) roots was tightly bound to CWs. Up to 99.9% of the total cellular Al accumulated in the CW of giant algal cells of *Chara corallina* (Taylor et al., 2000). Furthermore, CW pectin has been implicated as the major Al-binding site (Horst, 1995; Chang et al., 1999). On the other hand, several studies have reported that CW hemicellulose (HC) metabolism is more susceptible to Al stress. For example, Tabuchi and Matsumoto (2001) found that exposure of Al-sensitive wheat (*Triticum aestivum* 'Scout 66') to 10 μM Al for 6 h resulted in the accumulation of HC but not pectin and cellulose. In rice (*Oryza sativa*), the most significant change of the

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Identification of a novel mitochondrial protein, short postembryonic roots 1 (SPR1), involved in root development and iron homeostasis in *Oryza sativa*

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Summary

- A rice mutant, *Oryza sativa short postembryonic roots 1* (*Osspr1*), has been characterized. It has short postembryonic roots, including adventitious and lateral roots, and a lower iron content in its leaves.
- *OsSPR1* was identified by map-based cloning. It encodes a novel mitochondrial protein with the Armadillo-like repeat domain.
- *Osspr1* mutants exhibited decreased root cell elongation. The iron content of the mutant shoots was significantly altered compared with that of wild-type shoots. A similar pattern of alteration of manganese and zinc concentrations in shoots was also observed. Complementation of the mutant confirmed that *OsSPR1* is involved in post-embryonic root elongation and iron homeostasis in rice. *OsSPR1* was found to be ubiquitously expressed in various tissues throughout the plant. The transcript abundance of various genes involved in iron uptake and signaling via both strategies I and II was similar in roots of wild-type and mutant plants, but was higher in the leaves of mutant plants.
- Thus, a novel mitochondrial protein that is involved in root elongation and plays a role in metal ion homeostasis has been identified.

Introduction

Iron (Fe) is an essential co-factor for several enzymes involved in crucial cellular processes ranging from oxygen and electron transport to hormone production and DNA synthesis (Briat & Lobreaux, 1997). In addition, plants require Fe for several processes that occur in plastids and an adequate Fe supply is essential to maintain photosynthetic function at optimal rates (Briat *et al.*, 2007). However, excess Fe generates hydroxyl radicals via the Fenton reaction, which can damage biological molecules (Grotz & Guerinot, 2006).

Although abundant in soils, Fe often forms insoluble ferric hydroxide precipitates that limit its uptake by plants. Therefore, higher plants have evolved two distinct strategies to solubilize and acquire Fe from the rhizoshpere (Jeong & Guerinot, 2009; Morrissey & Guerinot, 2009). Nongraminaceous plants use the strategy I system, which involves the induction of membrane-bound Fe(III)-chelate reductases, which reduce Fe(III) to the more soluble form of Fe(II), followed by uptake of Fe(II) via the Fe(II) transporter iron-regulated transporter 1 (IRT1) (Eide *et al.*, 1996). By contrast, graminaceous plants use strategy II, which is mediated by the synthesis and secretion of natural Fe chelators, the mugineic acid (MA) family of phytosiderophores. The secreted MAs solubilize Fe(III) in the rhizosphere, and the resulting Fe(III)–MA complexes are absorbed into the root cells by the yellow-stripe-like (YSL) transporters (Curie *et al.*, 2001). Rice (*Oryza sativa*) can use both strategies I and II for Fe uptake (Cheng *et al.*, 2007).

Several genes involved in Fe uptake have been identified in rice. Loss-of-function mutants of *OsNAAT* (*Nicotianamine Aminotransferase*) were found to have a low assimilation capacity for Fe(III), but a normal assimilation capacity for Fe(II) (Cheng *et al.*, 2007). OsYSL15 and OsIRT1 were reported to be Fe(III)–MA and Fe(II) transporters in rice,

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