A ROP GTPase Signaling Pathway Controls Cortical Microtubule Ordering and Cell Expansion in *Arabidopsis*

Ying Fu,1,2,∗ Tongda Xu,2 Lei Zhu,1 Mingzhang Wen,2,3 and Zhenbiao Yang2,∗

1State Key Laboratory of Plant Physiology and Biochemistry, Department of Plant Sciences, College of Biological Sciences, China Agricultural University, Beijing 100193, China
2Center for Plant Cell Biology, Department of Botany and Plant Sciences, University of California, Riverside, Riverside, CA 92521, USA

Summary
Cortical microtubules (MTs) participate in the spatial control of cell expansion and division that is required for plant growth and morphogenesis. Well-ordered transverse cortical MTs promote cell elongation and restrict radial cell expansion [1, 2]. The molecular mechanism controlling their ordering is poorly understood. We report the first known signaling pathway that promotes the organization of cortical MTs into parallel arrays oriented perpendicular to the axis of cell elongation in plants. Well-ordered MTs locally restrict cell expansion to promote indentation formation in the cell elongation of plants. Well-ordered MTs locally restrict cell expansion to promote indentation formation in the jigsaw-puzzle-shaped pavement cells of *Arabidopsis* leaves [3, 4]. Deleting ROP6, a Rho-family GTPase, randomized cortical MTs and released the localized restriction of cell expansion, whereas ROP6 overexpression enhanced MT ordering, turning the jigsaw-puzzle appearance of cells into a cylindrical shape. ROP6 directly binds and activates MT-associated RIC1 to achieve the MT ordering. The ROP6–RIC1 pathway also affects MT ordering of hypocotyl cells, showing a broad role for this pathway in the spatial regulation of cell expansion.

Results and Discussion
Well-ordered cortical microtubules (MTs) are believed to restrict cell expansion to the direction perpendicular to their dominant orientation. Accordingly, transversely arranged MTs are important for cell elongation and plant growth along the apical-basal axis, whereas cells grow isotropically with cortical MTs arranged randomly. Several MT dynamic behaviors, including angle-dependent modification of sustained treadmilling, rotary movement, and polar coalignment caused by selective stabilization, have been suggested to regulate cortical MT ordering [5–8]. Furthermore, a number of MT-associated proteins (MAPs), e.g., RIC1, SPR1/SKU6, and SPR2, are known to affect the orientation of cortical MTs [3, 9, 10]. However, these MT behaviors and MAPs are linked to developmental and environmental signals that control the ordering of cortical MTs remains mysterious.

We use the *Arabidopsis* leaf epidermal pavement cells (PCs) with their jigsaw-puzzle appearance as a model system to investigate signaling mechanisms regulating the organization of the cytoskeleton in plants [2–4, 11–13]. In PCs, ordered cortical MTs are associated with indenting regions of PCs and are excluded from the protruding region that contains fine cortical actin microfilaments (MFs). We recently showed that ROP2 and ROP4, *Arabidopsis* members of the conserved Rho GTPase family [14–16], promote the protrusion by activating the localized actin accumulation and inhibiting MT organization by inactivating the microtubule-associated RIC1 protein in *Arabidopsis* PCs [3, 4]. In the indenting region, the ordering of cortical MTs is activated by RIC1, which belongs to the RIC (ROP-interactive CRIB motif-containing proteins) family of ROP effector proteins that interact with the active form of ROPs through the CRIB motif [3, 17]. In this report, we demonstrate that RIC1’s function in promoting MT ordering is activated by another Rho family GTPase, ROP6.

We speculated that, as a ROP effector protein, RIC1 must be activated to promote MT organization by one of the 11 ROP GTPases in *Arabidopsis*. We anticipated that a loss-of-function mutant for a RIC1 activating ROP would mimic ric1 knockout mutants, and we analyzed available *Arabidopsis* rop knockout mutants for PC-shape phenotype. Indeed, a ROP6 null mutant, rop6-1, resulting from a T-DNA insertion into the sixth exon, exhibited a phenotype similar to that of the ric1-1 knockout mutant. Lateral cell expansion in the indentation regions of PCs was significantly increased in rop6-1, which resulted in wider PC necks (Figures 1A, 1B, and 1D; see Figure S1A available online). To confirm that the wider neck region was due to the absence of ROP6, we transformed GFP-ROP6 driven by the ROP6 promoter into rop6-1. Several lines with a GFP-ROP6 transcript level similar to that of wild-type ROP6 indeed showed the same PC shape as the wild-type (Figure S2). Furthermore, ROP6 overexpression strongly inhibited lateral expansion of both lobes and indentations, as does RIC1 overexpression [3], which causes PCs to lose the intercalating jigsaw-puzzle appearance (Figure 1C; Figure S3). Thus, ROP6 plays an important role in restricting lateral cell expansion, as does RIC1 [3]. PCs of the rop6-1ric1-1 double mutant showed a phenotype identical to that of the ric1-1 or rop6-1 single mutant (Figures 1A, 1B, and 1D; Figure S1A), which provides genetic evidence that ROP6 and RIC1 act in the same pathway.

We next visualized MTs by using GFP-tagged tubulin to investigate whether ROP6, like RIC1, affects the organization of cortical MTs (Figure 2A) [3, 4, 18]. Similar to young ric1-1 cells, young rop6-1 cells (stage I) contained fewer cortical MTs than the wild-type (Figure 2A) [3]. Both rop6-1 and ric1-1 cells at stage II had more randomly oriented cortical MTs and fewer MT bundles than wild-type cells. Immunostaining with anti-tubulin antibody revealed a similar MT organization pattern in fixed rop6-1 PCs (Figure 2B). As in RIC1-overexpressing cells, ROP6-overexpressing PCs (ROP6S3) exhibited nearly all cortical MTs highly ordered in the orientation transverse to the axis of cell elongation (Figure 2B). These results also support the hypothesis that ROP6 and RIC1 function in the same pathway to promote the ordering of cortical MTs and to spatially control cell expansion.

To further test this hypothesis, we analyzed the organization of fine cortical MFs in PCs altered in ROP6 expression, given...
Epigenetic Regulation, Somatic Homologous Recombination, and Abscisic Acid Signaling Are Influenced by DNA Polymerase ε Mutation in Arabidopsis

Haibo Yin,a Xia Zhang,a,b Jun Liu,a Youqun Wang,a Junna He,a Tao Yang,a Xuhui Hong,a Qing Yang,a and Zhizhong Gonga,c,d,1

a State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing, 100193, China
b Biotecnology Center of Shandong Academy of Science, Jinan, 250014, China
c China Agricultural University-University of California-Riverside Center for Biological Sciences and Biotechnology, Beijing, 100193, China
d National Center for Plant Gene Research, Beijing, 100193, China

Based on abscisic acid (ABA) inhibition of seed germination and seedling growth assays, we isolated an ABA overly sensitive mutant (abo4-1) caused by a mutation in the Arabidopsis thaliana POL2a/TILTED1(TIL1) gene encoding a catalytic subunit of DNA polymerase ε. The dominant, ABA-insensitive abil-1 or abil2-1 mutations suppressed the ABA hypersensitivity of the abo4-1 mutant. The abo4/til1 mutation reactivated the expression of the silenced Athila retrotransposon transcriptional silent information (TSI) and the silenced 35S-NPTII in the ros1 mutant and increased the frequency of somatic homologous recombination (HR) ~60-fold. ABA upregulated the expression of TSI and increased HR in both the wild type and abo4-1. MEIOTIC RECOMBINATION11 and GAMMA RESPONSE1, both of which are required for HR and double-strand DNA break repair, are expressed at higher levels in abo4-1 and are enhanced by ABA, while KU70 was suppressed by ABA. abo4-1 mutant plants are sensitive to UV-B and methyl methanesulfonate and show constitutive expression of the G2/M-specific cyclin CycB1;1 in meristems. The abo4-1 plants were early flowering with lower expression of FLOWER LOCUS C and higher expression of FLOWERING LOCUS T and changed histone modifications in the two loci. Our results suggest that ABO4/POL2a/TIL1 is involved in maintaining epigenetic states, HR, and ABA signaling in Arabidopsis.

INTRODUCTION

Plants are sessile and unable to escape acute environmental stresses, but they have developed sophisticated responses to cope with and survive stress conditions. Plants are able to quickly counteract environmental stresses through actions, such as up- or downregulating gene expression and accumulating adaptive metabolic compounds. Abiotic stresses, such as drought, high salinity, and low temperature, induce the accumulation of the phytohormone abscisic acid (ABA) (Finkelstein et al., 2002; Koornneef et al., 2002). ABA modulates embryogenesis, maturation, and dormancy during seed development and plays vital roles in a wide range of biological processes throughout plant growth, development, and stress responses (Finkelstein et al., 2002; Koornneef et al., 2002). Through forward and reverse genetic studies, many genes involved in ABA responses have been isolated and characterized (Finkelstein et al., 2002; Koornneef et al., 2002; Xiong et al., 2002; Shinozaki and Yamaguchi-Shinozaki, 2007). Increased ABA in plant cells inhibits DNA replication and cell division, which results in retarded plant growth (Finkelstein et al., 2002; Swiatek et al., 2002; Chandrasekharan et al., 2003). However, the molecular mechanism for ABA inhibition of plant growth is largely unknown. One hint is that ABA induces expression of the cyclin-dependent kinase inhibitor KRP1/ICK1 (Wang et al., 1998). Cyclin-dependent kinases regulate the progression of the cell cycle in eukaryotes. Studies thus far have focused on ABA-mediated rapid physiological responses that are important for plant stress resistance in the short term. Whether ABA or an ABA-regulated signal can affect epigenetic states and genome stability, which might be important for the long-term adaptation of plants to environmental stresses and possibly contribute to plant evolution, is not known.

During the cell cycle, both DNA methylation patterns and specific chromatin structures must be duplicated with high fidelity throughout DNA replication. The DNA replication process involves DNA biosynthesis, transient disruption of parental nucleosomes, and nucleosome reassembly (Groth et al., 2007), and many different proteins participate in this complex process. During S-phase, the leading and lagging strand are replicated by different mechanisms in a continuous and discontinuous fashion, respectively. In the lagging strand synthesis, DNA polymerase ε uses RNA primers initiated by the primase to synthesize a short
Our results demonstrate that WRKY6 and WRKY42 are involved in development, serves various basic biological functions in the INTRODUCTION PHO1 expression. MG132, suggesting that low Pi–induced release of blot analysis showed that the decrease in WRKY6 protein induced by low Pi treatment was inhibited by a 26S proteosome proteolysis. In addition, WRKY42 also showed binding to W-boxes of the PHOSPHATE1 (PHO1) expression.

Arabidopsis thaliana WRKY family comprises 74 members and some of them are involved in plant responses to biotic and abiotic stresses. This study demonstrated that WRKY6 is involved in Arabidopsis responses to low-Pi stress through regulating PHOSPHATE1 (PHO1) expression. WRKY6 overexpression lines, similar to the pho1 mutant, were more sensitive to low Pi stress and had lower Pi contents in shoots compared with wild-type seedlings and the wrky6-1 mutant. Immunoprecipitation assays demonstrated that WRKY6 can bind to two W-boxes of the PHO1 promoter. RNA gel blot and β-glucuronidase activity assays showed that PHO1 expression was repressed in WRKY6-overexpressing lines and enhanced in the wrky6-1 mutant. Low Pi treatment reduced WRKY6 binding to the PHO1 promoter, which indicates that PHO1 regulation by WRKY6 is Pi dependent and that low Pi treatment may release inhibition of PHO1 expression. Protein gel blot analysis showed that the decrease in WRKY6 protein induced by low Pi treatment was inhibited by a 26S proteosome inhibitor, MG132, suggesting that low Pi-induced release of PHO1 repression may result from 26S proteosome–mediated proteolysis. In addition, WRKY42 also showed binding to W-boxes of the PHO1 promoter and repressed PHO1 expression. Our results demonstrate that WRKY6 and WRKY42 are involved in Arabidopsis responses to low Pi stress by regulation of PHO1 expression.

INTRODUCTION

Phosphorus (P), as a major essential nutrient for plant growth and development, serves various basic biological functions in the plant life cycle (Raghothama, 1999). Phosphate (H₂PO₄⁻, or in short, Pi) is the major form that is most readily taken up and transported in the plant cell (Ulrich-Eberius et al., 1981; Tu et al., 1990). The Pi concentration in the soil, typically 10 μM or less, results in Pi starvation for plant growth and survival, which is one of major limiting factors for crop production in the cultivated soils. A number of studies have shown that plants have evolved different strategies to overcome limited Pi availability. In response to low Pi stress or Pi starvation, plants may increase the Pi uptake from the soil by alteration of root architecture and function (López-Bucio et al., 2003; Ticconi and Abel, 2004; Osmond et al., 2007). Under Pi-limiting conditions, plants may also increase their Pi acquisition by changing their metabolic and developmental processes (Raghothama and Karthikeyan, 2005), such as increasing phosphatase activity (Lipton et al., 1987) and secretion of organic acids (Marschner, 1995).

PHOSPHATE1 (PHO1) has been shown to play roles in Pi translocation from root to shoot (Hamburger et al., 2002), which is also important for plant adaptation to a low Pi environment. A single nuclear recessive mutation in PHO1 led to its inability to load Pi into xylem (Poirier et al., 1991; Hamburger et al., 2002).

PHO1 is predominantly expressed in the stellar cells of the root and the lower part of the hypocotyls and is believed have a role in Pi efflux out of root stellar cells for xylem loading (Hamburger et al., 2002). However, PHO1 shares no homology with any previously described Pi transporter proteins in plants and fungi (Hamburger et al., 2002). It is interesting that PHO1 contains a SPX domain, which can be found in several proteins that are involved in phosphate transport and/or Pi signaling pathways in plants and yeast. For example, an SPX protein in yeast named PHO81 is a key regulator in transporting and sensing phosphate, as well as in sorting proteins to endomembranes (Lenburg and O’Shea, 1996; Wykoff and O’Shea, 2001). In Arabidopsis, the SPX proteins SPX1-SPX3 are involved in Pi signaling pathways and regulate the expression of the Pi transporter genes Pht1;4 and Pht1;5 (Duan et al., 2008). Thus, the possibility cannot be excluded that PHO1 may not be a direct Pi transporter but rather may regulate Pi loading of the xylem either by directly influencing the activity of transporter proteins or via signal transduction.

PHO1 gene expression can be induced by Pi starvation (Stefanovic et al., 2007; Ribot et al., 2008; also see Figure 5B in this study), but the transcription factors that regulate PHO1 expression remain unknown. Transcriptome analysis has demonstrated that expression of many genes is significantly changed in Oryza sativa (Wasaki et al., 2003) and Arabidopsis thaliana (Wu et al., 2003; Missen et al., 2005) under Pi-limiting conditions, indicating that transcriptional regulation may play important roles in plant responses to low Pi stress. More recently, a number of regulatory components that may be involved in plant responses to low Pi stress have been reported, such as microRNA miR339 (Bari et al., 2006; Chiou et al., 2006), Arabidopsis posttranscription regulators PHOSPHATE TRANSPORTER TRAFFIC FACILITA-

TOR1 (At PHF1) (González et al., 2005) and E3 SUMO Ligase (At SIZ1) (Miura et al., 2005), transcription factors PHOSPHATE
A mutation in THERMOSENSITIVE MALE STERILE 1, encoding a heat shock protein with DnaJ and PDI domains, leads to thermosensitive gametophytic male sterility in Arabidopsis

Ke-Zhen Yang1, Chuan Xia1, Xiao-Lei Liu1, Xiao-Ying Dou1, Wei Wang1, Li-Qun Chen1, Xue-Qin Zhang1, Li-Fen Xie2, Luyan He1, Xuan Ma1 and De Ye1,3,*
1State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100193, China,
2Temasek Life Science Laboratory, 1 Research Link, National University of Singapore 117604, Singapore, and
3National Center for Plant Gene Research (Beijing), Institute of Genetics and Developmental Biology, 917 Datun Road, Beijing 100101, China

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*For correspondence (fax +86 10 62734839; e-mail yede@cau.edu.cn).

Summary
In most flowering plant species, pollination and fertilization occur during the hot summer, so plants must have evolved a mechanism that ensures normal growth of their pollen tubes at high temperatures. Despite its importance to plant reproduction, little is known about the molecular basis of thermotolerance in pollen tubes. Here we report the identification and characterization of a novel Arabidopsis gene, THERMOSENSITIVE MALE STERILE 1 (TMS1), which plays an important role in thermotolerance of pollen tubes. TMS1 encodes a Hsp40-homologous protein with a DnaJ domain and an a_ERdj5_C domain found in protein disulfide isomerases (PDI). Purified TMS1 expressed in Escherichia coli (BL21 DE3) had the reductive activity of PDI. TMS1 was expressed in pollen grains, pollen tubes and other vegetative tissues, including leaves, stems and roots. Heat shock treatment at 37°C increased its expression levels in growing pollen tubes as well as in vegetative tissues. A knockout mutation in TMS1 grown at 30°C had greatly retarded pollen tube growth in the transmitting tract, resulting in a significant reduction in male fertility. Our study suggests that TMS1 is required for thermotolerance of pollen tubes in Arabidopsis, possibly by functioning as a co-molecular chaperone.

Keywords: THERMOSENSITIVE MALE STERILE 1, heat shock protein, male sterility, gametophyte, pollen, Arabidopsis.

Introduction
In flowering plants, male and female gametes are separated in male and female organs. The male gametes are enclosed in male gametophytes (pollen grains) in anthers. Fertilization requires the delivery of male gametes to female gametophytes (embryo sacs) in ovules that are embedded deep inside an ovary. The process begins with the deposition of pollen grains onto stigmatic tissues. A compatible interaction between a pollen grain and a stigmatic cell triggers hydration and germination of the pollen grain. The resulting pollen tube invades the stigmatic tissue, directionally extends through the transmitting tract, and is finally directed into an embryo sac, so that the male gametes (sperm) in the pollen tube are transported into the embryo sac. Therefore, growth of pollen tubes is important for fertilization in flowering plants.

In most flowering plants, pollination and fertilization occur during hot summers. Pollen tubes are sensitive to high-temperature stress (Ahmed et al., 1992; Dupuis and Dumas, 1990; Herrero and Johnson, 1980; Mascarenhas and Crone, 1996; Monterroso and Wien, 1990; Schoper et al., 1987), so plants must have evolved a mechanism of thermotolerance to maintain normal growth of their pollen tubes at high temperatures. Little is known about the molecular basis of thermotolerance in pollen tubes. Studies...
Involvement of OsSPX1 in phosphate homeostasis in rice

Chuang Wang†, Shan Ying†, Hongjie Huang, Kuan Li, Ping Wu and Huixia Shou*
State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou 310058, China

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*For correspondence (fax +86 571 88206133; e-mail huixia@zju.edu.cn).
†These authors contributed equally to this work.

Summary

Arabidopsis thaliana SPX (SYG/PHO81/XPR1) domain genes have recently been shown to be involved in the phosphate (Pi) signaling pathway. We show here that a rice (Oryza sativa) SPX gene, OsSPX1, is specifically induced by Pi starvation in roots. Suppression of OsSPX1 by RNA interference resulted in severe signs of toxicity caused by the over-accumulation of Pi, similar to that found in OsPHR2 (phosphate starvation response transcription factor 2) overexpressors and pho2 (phosphate-responsive mutant 2). Quantitative RT-PCR showed that expression of OsSPX1 was strongly induced in OsPHR2 overexpression and pho2 mutant plants, indicating that OsSPX1 occurs downstream of OsPHR2 and PHO2. The expression of 10 genes associated with the phosphate-starvation signal pathways was analyzed. Expression of OsPT2 (phosphate transporter 2) and OsPT8 was significantly induced in OsSPX1-RNAi (OsSPX1-Ri) plants, suggesting that over-accumulation of Pi in OsSPX1-Ri plants results from an increase in Pi transport. In contrast, overexpression of OsSPX1 suppressed the induction of expression by Pi starvation of all 10 phosphate starvation-induced genes tested: IPS1, IPS2, OsPAP10 (purple acid phosphatase 10), OsSQD2 (sulfoquinovosyldiacylglycerol 2), miR399d and miR399j (microRNA 399), OsPT2, OsPT3, OsPT6 and OsPT8. This suggests that OsSPX1 acts via a negative feedback loop to optimize growth under phosphate-limited conditions.

Keywords: rice, SPX domain, phosphate, homeostasis, suppressor.

Introduction

Phosphorus (P) is a macronutrient that is essential for plant growth and development. Although abundant in the soil, it is often limited for plants because of its low bioavailability (Raghothama, 2000; Vance et al., 2003). To cope with an inadequate supply of phosphate (Pi), plants have evolved adaptive responses that include expansion of the root system to accelerate soil exploration, the adjustment of metabolism to protect intracellular Pi homeostasis, and the induction and secretion of phosphatases and organic acids to mobilize Pi from organic matter (Raghothama, 2000; Rausch and Bucher, 2002; Ticconi and Abel, 2004). Genes encoding proteins involved in responses to Pi starvation, and the mechanism of these proteins, have been intensively studied (Schachtman and Shin, 2007). Mutation in a MYB transcription factor, phosphate starvation response 1 (PHR1), reduces the expression of several phosphate starvation-induced (PSI) genes and results in evident signs of Pi deficiency (Rubio et al., 2001). Arabidopsis PHR1 was found to bind to an imperfect palindromic sequence (GNATATNC) that has been found in the promoter regions of many PSI genes (Franco-Zorrilla et al., 2004; Rubio et al., 2001). A microRNA gene, miR399, the expression of which is induced by Pi starvation, is under the control of PHR1. miR399 targets the mRNA of a ubiquitin-conjugating E2 enzyme, UBC24 (PHO2), and reciprocally regulates the expression of PHO2 at the transcriptional level (Aung et al., 2006; Chiou et al., 2006; Fujii et al., 2005), and consequently regulates several phosphate transporters in Arabidopsis (Bari et al., 2006). Over-expression of AtPHR1 and miR399 and mutation in AtPHO2 led to over-accumulation of Pi in Arabidopsis (Bari et al., 2006; Chiou et al., 2006; Nilsson et al., 2007). A member of the PSI cDNAs of the Mt4/TPS1 family, IPS1 (induced by phosphate starvation 1), IPS2, OsPAP10 (purple acid phosphatase 10), OsSQD2 (sulfoquinovosyldiacylglycerol 2), miR399d and miR399j (microRNA 399), OsPT2, OsPT3, OsPT6 and OsPT8. This suggests that OsSPX1 acts via a negative feedback loop to optimize growth under phosphate-limited conditions.
Elongator mediates ABA responses, oxidative stress resistance and anthocyanin biosynthesis in Arabidopsis

Xiaofeng Zhou1, Deping Hua1, Zhizhong Chen1, Zhengjun Zhou1 and Zhizhong Gong1,2,3,*

1State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100193, China,
2China Agricultural University – University of California – Riverside Center for Biological Sciences and Biotechnology, Beijing 100193, China,
3National Center for Plant Gene Research, Beijing 100193, China

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*For correspondence (fax +86 10 62733733; e-mail gongzz@cau.edu.cn).

SUMMARY

Elongator is a histone acetyl-transferase complex consisting of six subunits, and is highly conserved in eukaryotic organisms. Here, we isolated two novel mutants, elp2 and elp6, during a genetic screening for ABA-hypersensitive Arabidopsis mutants. Map-based cloning identified ELP2 and ELP6, which encode the orthologs of the yeast Elongator subunits, ELP2 and ELP6, respectively. Another Elongator subunit mutant, elp4/elo1, was obtained from the SALK T-DNA collection. The elp1/abo1/elo2 mutant was isolated in a previous study. All four of the Elongator mutants had narrow leaves, reduced root growth, ABA hypersensitivity and an increased accumulation of anthocyanins. Mutations in the core subcomplex subunits ELP1/ABO1 and ELP2, but not in the accessory subcomplex subunits ELP4/ELO1 and ELP6, caused stomatal closing to be supersensitive to ABA. In addition, the four mutants were all more resistant than the wild type to oxidative stress produced by methyl viologen, and to CsCl. Gene expression analysis indicated that the four mutants had increased transcript levels of CAT3 under normal conditions, increased transcript levels of ZAT10 when treated with ABA and reduced transcript levels of MYBL2, which encodes a single-repeat MYB protein, acting as a negative regulator of anthocyanin biosynthesis. Our results suggest that Elongator plays crucial roles in regulating plant responses to ABA, oxidative stress resistance and anthocyanin biosynthesis in Arabidopsis.

Keywords: Elongator, ABA, oxidative stress, anthocyanin biosynthesis.

INTRODUCTION

The plant hormone ABA regulates many processes during plant growth and development, including seed maturation, seed dormancy, seed germination and seedling growth, and plays vital roles in plant responses to environmental stresses such as drought, salt, cold and oxidative stress (Leung and Giraudat, 1998; Shinozaki and Yamaguchi-Shinozaki, 2000; Finkelstein et al., 2002; Zhu, 2002). Early studies mainly focused on ABA biosynthesis and ABA-mediated stress responses (Finkelstein et al., 2002; Xiong and Zhu, 2003). Recent studies showed that the ABA signal pathway is affected by RNA metabolism, including RNA splicing, RNA export from the nucleus to the cytoplasm, RNA stability and RNA elongation (Hugouvieux et al., 2001; Xiong et al., 2001, 2002a,b; Koiva et al., 2002; Gong et al., 2005; Chen et al., 2006; Reyes and Chua, 2007; Chinnusamy et al., 2008).

ABA signaling is associated with Elongator, a histone acetyl-transferase complex (Chen et al., 2006). Elongator was first identified based on its direct association with the RNA polymerase-II (RNAPII) holoenzyme, in transcriptional elongation in yeast (Otero et al., 1999). Elongator consists of two subcomplexes: the core subcomplex with subunits ELP1, 2 and 3, and the accessory subcomplex with subunits ELP4, 5 and 6 (Krogan and Greenblatt, 2001). The known biological roles for Elongator are in RNAPII-mediated transcription through the acetylation of histone H3 and H4 in chromatin, the modification of certain tRNAs and the acetylation of α-tubulin (Otero et al., 1999; Fellows et al., 2000; Jablonowski et al., 2001; Huang et al., 2005; Esberg et al., 2006; Svejstrup, 2007; Creppe et al., 2009). In humans, a splicing mutation in the IKBKAP gene (encoding IKAP, human ELP1) causes familial dysautonomia disease (Anderson et al., 2001). Recent reports indicated that mutations of Elongator subunits (ELO1/ELP4, ABO1/ELP2 and ELO3/ELP3) in Arabidopsis have pleiotropic effects on plant
LEW3, encoding a putative α-1,2-mannosyltransferase (ALG11) in N-linked glycoprotein, plays vital roles in cell-wall biosynthesis and the abiotic stress response in Arabidopsis thaliana

Min Zhang¹, Maurice Henquet², Zhizhong Chen¹, Hairong Zhang¹, Yi Zhang¹, Xiaozhi Ren¹, Sander van der Krol³, Martine Gonneau⁴, Dirk Bosch²,⁵ and Zhizhong Gong¹,⁶,⁷, *

¹State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing, 100193, China,
²Business Unit Bioscience, Plant Research International, Wageningen University and Research Centre, 6708 PB Wageningen, The Netherlands,
³Laboratory of Plant Physiology, Wageningen University, The Netherlands,
⁴Laboratoire de Biologie Cellulaire, Unité de Recherche 501, Institut Jean-Pierre Bourgín–Institut National de la Recherche Agronomique, Route de St Cyr, 78026 Versailles Cedex, France,
⁵Membrane Enzymology, Department of Chemistry, Utrecht University, 3584 CH Utrecht, The Netherlands,
⁶China Agricultural University/University of California Riverside Center for Biological Sciences and Biotechnology, and
⁷National Center for Plant Gene Research, Beijing 100193, China

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*For correspondence (fax 86 10 6273 3733; e-mail gongzz@cau.edu.cn).

SUMMARY

N-linked glycosylation is an essential protein modification that helps protein folding, trafficking and translocation in eukaryotic systems. The initial process for N-linked glycosylation shares a common pathway with assembly of a dolichol-linked core oligosaccharide. Here we characterize a new Arabidopsis thaliana mutant lew3 (leaf wilting 3), which has a defect in an α-1,2-mannosyltransferase, a homolog of ALG11 in yeast, that transfers mannose to the dolichol-linked core oligosaccharide in the last two steps on the cytosolic face of the ER in N-glycan precursor synthesis. LEW3 is localized to the ER membrane and expressed throughout the plant. Mutation of LEW3 caused low-level accumulation of Man3GlcNAc2 and Man4GlcNAc2 glycans, structures that are seldom detected in wild-type plants. In addition, the lew3 mutant has low levels of normal high-mannose-type glycans, but increased levels of complex-type glycans. The lew3 mutant showed abnormal developmental phenotypes, reduced fertility, impaired cellulose synthesis, abnormal primary cell walls, and xylem collapse due to disturbance of the secondary cell walls. lew3 mutants were more sensitive to osmotic stress and abscisic acid (ABA) treatment. Protein N-glycosylation was reduced and the unfolded protein response was more activated by osmotic stress and ABA treatment in the lew3 mutant than in the wild-type. These results demonstrate that protein N-glycosylation plays crucial roles in plant development and the response to abiotic stresses.

Keywords: protein N-glycosylation, α-1,2-mannosyltransferase, unfolded protein response, abiotic stresses.

INTRODUCTION

In eukaryotic cells, N-linked glycosylation is an essential protein modification and shares a common pathway with the synthesis of core dolichol-linked oligosaccharides in the endoplasmic reticulum (Figure 1) (Herscovics and Orlean, 1993; Burda and Aebi, 1999; Gemmill and Trimble, 1999; Cipollo et al., 2001; Helenius and Aebi, 2004; O’Reilly et al., 2006; Weerapana and Imperiali, 2006). Most genes involved in this pathway have been identified in yeast. First, on the cytosolic surface of the ER, a series of glycotransferases add two N-acetylglucosamines (GlcNAc) and five mannoses stepwise to a lipid carrier, dolichylphosphate (Herscovics and Orlean, 1993; Burda and Aebi, 1999; Gemmill and
Transcriptional Gene Silencing Mediated by a Plastid Inner Envelope Phosphoenolpyruvate/Phosphate Translocator CUE1 in Arabidopsis\[OA\]

Jie Shen, Xiaozhi Ren, Rui Cao, Jun Liu, and Zhizhong Gong*

State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100193, China (J.S., X.R., R.C., J.L., Z.G.); China Agricultural University-University of California Riverside Center for Biological Sciences and Biotechnology, Beijing 100193, China (Z.G.); and National Center for Plant Gene Research, Beijing 100193, China (Z.G.)

Mutations in REPRESSOR OF SILENCING1 (ROSI) lead to the transcriptional gene silencing (TGS) of Pro\textsubscript{35S}\textsubscript{LUC} (LUCIFERASE) and Pro\textsubscript{ros1}NPTII (Neomycin Phosphotransferase II) reporter genes. We performed a genetic screen to find suppressors of ros1 that identified two mutant alleles in the Arabidopsis (Arabidopsis thaliana) CHLOROPHYLL A/B BINDING PROTEIN UNDERTEXPRESSED1 (CUE1) gene, which encodes a plastid inner envelope phosphoenolpyruvate/phosphate translocator. The cue1 mutations released the TGS of Pro\textsubscript{ros1}NPTII and the transcriptionally silent endogenous locus TRANSCRIPTIONAL SILENCING INFORMATION in a manner that was independent of DNA methylation but dependent on chromatin modification. The cue1 mutations did not affect the TGS of Pro\textsubscript{35S}\textsubscript{LUC} in ros1, which was dependent on RNA-directed DNA methylation. It is possible that signals from chloroplasts help to regulate the epigenetic status of a subset of genomic loci in the nucleus.

Heterochromatin is characterized by DNA methylation and/or histone modifications, including histone H3K9, H3K27 methylation, and H3 as well as H4 hypoacetylation. Heterochromatin can lead to transcriptional gene silencing (TGS), which plays a central role in repressing transposon movement and mediating gene repression during developmental and cellular differentiation in plants (Martienssen and Colot, 2001; Henderson and Jacobsen, 2007; Matzke et al., 2007, 2009). TGS relies on both DNA methylation-dependent and independent pathways in plants (Martienssen and Colot, 2001; Henderson and Jacobsen, 2007; Matzke et al., 2007). Various components in the RNA-directed DNA methylation pathway have been identified (Henderson and Jacobsen, 2007; Matzke et al., 2007, 2009). The reversible removal of DNA methylation is catalyzed by REPRESSOR OF SILENCING1 (ROSI), DEMETER, and two DEMETER-like proteins, DML2 and DML3, through a base excision repair mechanism (Gong et al., 2002; Agius et al., 2006; Morales-Ruiz et al., 2006). In the DNA methylation-independent pathway, several proteins involved in DNA replication and repair regulate the epigenetic status of the transcriptionally silent endogenous locus TRANSCRIPTIONAL SILENCING INFORMATION (TSI) in Arabidopsis (Arabidopsis thaliana). These proteins include BRUSHY1 (BRU1), a DNA repair-related protein (Takeda et al., 2004), REPRESSOR OF ROS1/REPLICATION PROTEIN A2 (ROR1/RPA2A; Xia et al., 2006), FASCIATA1 (FAS1) and FAS2, two subunits of CHROMATIN ASSEMBLY FACTOR1 (Kaya et al., 2001; Takeda et al., 2004), MSI1 (Hennig et al., 2003), TOUSLED (TSL) protein kinase (Wang et al., 2007), NUCLEOSOME ASSEMBLY PROTEIN1-RELATED proteins (histone chaperones; Zhu et al., 2006), TSO2-1 and RNR2a-1 (two subunits of ribonucleotide reductase; Wang and Liu, 2006), DNA polymerase \gamma (Yin et al., 2009), and others. These proteins participate in chromatin assembly, DNA recombination, DNA replication, and/or DNA repair, indicating crucial roles of DNA replication and repair in maintaining chromatin structures. Previous studies also indicate that TGS of some developmental genes is regulated by other chromatin-remodeling proteins such as Polycomb group (PcG) proteins. A well-studied example is the regulation of FLOWER LOCUS C expression by the PcG protein VERNALIZATION2 in Arabidopsis (Dennis and Peacock, 2007) during extended cold treatment. The extended cold treatment suppresses FLOWER LOCUS C expression by increasing H3K27 methylation, which is mediated by the VERNALIZATION2 repressor complex (Bastow et al., 2004).
Cortical microtubule as a sensor and target of nitric oxide signal during the defence responses to *Verticillium dahliae* toxins in *Arabidopsis*

FU-MEI SHI1,2*, LIN-LIN YAO1*, BAO-LEI PEI1, QUN ZHOU1, XIU-LI LI1, YUN LI1 & YING-ZHANG LI1

1State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100193, China and 2School of Life Science, Liaocheng University, Liaocheng 252059, China

ABSTRACT

The molecular mechanisms of signal transduction of plants in response to *Verticillium dahliae* (VD) are not known. Here, we show that *Arabidopsis* reacts to VD-toxins with a rapid burst of nitric oxide (NO) and cortical microtubule destabilization. VD-toxins treatment triggered a disruption of cortical microtubules network. This disruption can be influenced by NO production. However, cortical microtubule disruptions were not involved in regulating the NO production. The results indicated that NO may act as an upstream signalling molecule to trigger the depolymerization of cortical microtubule. Cortical microtubules may act as a target of NO signal and as a sensor to mediate the activation of PR-1 gene expression. These results suggested that NO production and cortical microtubule dynamics appeared to be parts of the important signalling system and are involved in the defence mechanisms to VD-toxins in *Arabidopsis*.

Key-words: cortical microtubules dynamics; defence mechanisms; NO signal.

INTRODUCTION

Plant cytoskeletons play important roles in plant cell growth, development and other cellular processes (Wasteneys & Galway 2003). The cytoskeleton is a highly conserved, dynamic subcellular complex that is adaptively rearranged in response to environmental stimuli (Cahill et al. 2002; Hashimoto & Kato 2006). It has been demonstrated that microfilaments and microtubules are necessary for plants to block fungal penetration (Kobayashi, Kobayashi & Hardham 1994; Škalamera & Heath 1998; Genre & Bonfante 2002; Kobayashi & Hakuno 2003). The cytoskeletons may assist the formation of physical and chemical barriers by recruiting defence-related products to the site of invasion (Thordal-Christensen 2003). Changes of the plant cytoskeleton during plant interactions with microbes are complex and varied, with some aspects remaining to be elucidated (Takemoto & Hardham 2004). The cytoskeleton has been studied in several plant–fungal systems, and the cytoskeletal rearrangements in these systems are not always the same. In barley and flax, this rearrangement occurs before the fungus penetrates the cell (Kobayashi et al. 1994, 1997). In parsley, the change occurs immediately after penetration is initiated (Schmelzer et al. 1995). It was also observed that rearrangements and fragmentation of microtubular cytoskeleton were induced in nematode feeding sites (de Almeida Engler et al. 2004), and the abundance of cortical microtubules increased around the penetration site in lettuce (Sedláková, Binarová & Lebeda 2001). In contrast, it was suggested that microtubules did not become focused on the penetration site in *Arabidopsis* epidermal cells after penetration by oomycete pathogens (Takemoto, Jones & Hardham 2003, 2006). These results indicated that microtubule may play an important part in the mobilization of the plant defence response. However, details of the contribution of microtubules have not yet been studied, especially in terms of the molecules that signal and bring about the dramatic reorganizations that are often observed.

*Verticillium dahliae* (VD) is a soilborne pathogen that causes *Verticillium* wilt in a variety of important plant species worldwide (Bhat & Subbarao 1999). Although the physiology of plant defence against *Verticillium* infection is well established, comprising the production of the pathogenesis-related (PR) proteins, phytoalexins and phenolic compounds (Williams et al. 2002; Zhen & Li 2004), and active expression of some disease response genes (MaFadden et al. 2001; Fradin & Thomma 2006; Hou, Shi & Li 2008), the regulatory signal and pathways that control genes expression and the cytoskeletons involved in plant defence responses to *Verticillium* remain largely unknown. Recently, we demonstrated that VD-toxins induced alteration of cytoskeletons and nucleoli in *Arabidopsis* suspension cells (Yuan et al. 2006). However, the precise role of cytoskeletons and its relation with other signalling molecules and mediators still need clarification.

Nitric oxide (NO) has been identified as an essential molecule that mediates defence gene activation in plants (Delledonne et al. 1998; Huang et al. 2002; Neill, Desikan &
Adventitious root formation in rice requires OsGNOM1 and is mediated by the OsPINs family

Shiping Liu¹,², Jirong Wang¹, Lu Wang¹, Xiaofei Wang¹, Yanhong Xue¹, Ping Wu¹, Huixia Shou¹

¹State Key Laboratory of Plant Physiology and Biochemistry, College of Life Science, Zhejiang University, Hangzhou 310058, China; ²College of Chemistry and Life Science, China Three Gorges University, Yichang 443002, China

Correspondence: Ping Wu¹, Huixia Shou²
Fax: +86 571 88206146; E-mail: clspwu@zju.edu.cn
E-mail: huixia@zju.edu.cn

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The fibrous root system in cereals comprises primarily adventitious roots (ARs), which play important roles in nutrient and water uptake. Current knowledge regarding the molecular mechanism underlying AR development is still limited. We report here the isolation of four rice (Oryza sativa L.) mutants, from different genetic backgrounds, all of which were defective in AR formation. These mutants exhibited reduced numbers of lateral roots (LRs) and partial loss of gravitropism. The mutants also displayed enhanced sensitivity to N-1-naphthylphthalamic acid, an inhibitor of polar auxin transport (PAT), indicating that the mutations affected auxin transport. Positional cloning using one of the four mutants revealed that it was caused by loss-of-function of a guanine nucleotide exchange factor for ADP-ribosylation factor (OsGNOM1). RT-PCR and analysis of promoter::GUS transgenic plants showed that OsGNOM1 is expressed in AR primordia, vascular tissues, LRs, root tips, leaves, anthers and lemma veins, with a distribution pattern similar to that of auxin. In addition, the expressions of OsPIN2, OsPIN5b and OsPIN9 were altered in the mutants. Taken together, these findings indicate that OsGNOM1 affects the formation of ARs through regulating PAT.

Keywords: Oryza sativa L., adventitious root, OsGNOM1, polar auxin transport

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Introduction

In contrast to the tap root system of Arabidopsis thaliana, monocot cereals consist almost entirely of a complex fibrous root system, comprising a mass of adventitious roots (ARs) that develop from the stem base postembryonically. Rice (Oryza sativa L.) is a model cereal crop with seminal roots that die during the growing period [1]. Thus, lateral roots (LRs) and AR are the key determinants of nutrient and water use efficiency in rice.

A number of Arabidopsis mutants with defects in root development have been characterized [2, 3]. These research efforts have comprehensively documented the molecular control and hormonal regulation of root initiation and development in dicotyledonous plants [3-5]. In recent years, an increasing number of cereal mutants with impaired root formation have been discovered [6-9]. However, due to the complex structure and intricate regulation of their root systems, the mechanism of AR development is still far from clear.

Phytohormone is required for the establishment of appropriate root architecture. It affects the number and length of LR and AR [2]. Many genes required in root development have connections with the auxin-signaling pathway [4, 10], including the Aux/IAA family [11], the auxin response factor family [12] and certain hormone-related transporters [13]. An LOB domain-containing transcription factor controlled by auxin responsive factor was known to play a critical role in the formation of AR primordia in rice and maize (Zea mays L.) [8-9, 14].

GNOM is a large guanine nucleotide exchange factor (GEF) for ADP-ribosylation factor (ARF), a small GTPase. GNOM affects polar auxin transport (PAT) and development in Arabidopsis [15-18]. Over 20 allelic mutants of gnom have been identified [19-21]. Most of these mutants exhibited severe defects in both early embryonic structure and subsequent post-embryonic development such as LR formation and gravitropism, resulting in lethality [19, 22, 23]. GNOM is found in...
A novel chloroplast-localized protein EMB1303 is required for chloroplast development in *Arabidopsis*

Xiaozhen Huang¹, Xiaoyan Zhang¹, Shuhua Yang¹, ²

¹State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100193, China; ²National Plant Gene Research Center, Beijing 100193, China

To understand the molecular mechanisms underlying chloroplast development, we isolated and characterized the albino mutant *emb1303-1* in *Arabidopsis*. The mutant displayed a severe dwarf phenotype with small albino rosette leaves and short roots on a synthetic medium containing sucrose. It is pigment-deficient and seedling lethal when grown in soil. Embryo development was delayed in the mutant, although seed germination was not significantly impaired. The plastids of *emb1303-1* were arrested in early developmental stages without the classical stack of thylakoid membrane. Genetic and molecular analyses uncovered that the EMB1303 gene encodes a novel chloroplast-localized protein. Microarray and RT-PCR analyses revealed that a number of nuclear- and plastid-encoded genes involved in photosynthesis and chloroplast biogenesis were substantially downregulated in the mutant. Moreover, the accumulation of several major chloroplast proteins was severely compromised in *emb1303-1*. These results suggest that EMB1303 is essential for chloroplast development.

**Keywords**: EMB1303, albino, chloroplast development, embryogenesis, *Arabidopsis*

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**Introduction**

Chloroplast is a special and important organelle of plant cells carrying out many essential processes such as photosynthesis and biosynthesis of fatty acids, pigments, and amino acids from inorganic nitrogen [1].

In addition to the outer and inner membrane, mature chloroplasts have an internal membrane network of thylakoids, where the light reactions take place, converting the light energy into chemical energy stored in ATP and NADPH. The formation of the chloroplast complex and elaborate thylakoid membrane initiates from proplastids and is dependent on the coordinated expression of nuclear- and plastid-encoded genes [2, 3]. These genes encode proteins involved in many processes of chloroplast development such as RNA processing, protein translation and folding, and protein transport. Such proteins include PAC, a nuclear-encoded protein functioning in plastid mRNA maturation and accumulation [4]; HCF136, a chaperone-like assembly factor for the stability of photosystem II (PSII) [5]; SLP, chaperonin-60α for protein folding [6]; APG2, a major component of ΔpH-dependent thylakoid protein transporter [7]; and ALB3, a subunit of the thylakoid Sec protein transport system [8, 9]. Loss of these proteins usually impairs chloroplast development resulting in abnormal chloroplast morphology. Chloroplast is also involved in the biosynthesis of plastid isoprenoids such as chlorophyll and carotenoid. Mutations in the isoprenoid biosynthesis pathways, such as *cla1*, *ispD*, *ispE*, *ispG*, *ispH*, *pds3*, and *zds*, block chloroplast development and result in albino phenotype [10-14].

Chloroplast development is tightly linked to embryogenesis. Mutants interfering with or blocking chloroplast development usually exhibit embryogenesis defects [6, 15-19]. For example, mutations in some genes participating in chloroplast membrane lipid biosynthesis result in embryonic lethality [20, 21]. However, a deficiency in the photosynthetic capacity of embryo plastids does not always cause an embryonic lethal phenotype. Some albino and pigment-deficient mutants produce morphologically normal seeds that are able to germinate and grow to
A transcription factor with a bHLH domain regulates root hair development in rice

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Dear Editor,

In plants, root hairs are important organs for the uptake of nutrients and water from the rhizosphere and serve as sites of interaction with soil microorganisms [1]. A root hair grows as an extension of a single epidermal cell and is a simple system, making it an elegant model for studying higher plant cell differentiation and cell fate determination. Three types of root hair pattern have been suggested [2, 3]. Arabidopsis has the striped pattern (Type 3) of root hairs, which has been extensively reported [4, 5]. Type 2 patterns depend on asymmetrical cell division, which is found in rice (Oryza sativa L.), Barley (Hordeum vulgare) and wheat (Triticum aestivum). Hair-forming cells (trichoblasts) in Type 2 roots derive from a late, unequal transverse cell division in the epidermal stem cell [2]. These different patterns of root hair development in different plant species imply the existence of different genetic and molecular mechanisms controlling epidermal cell behaviors, while the knowledge of the molecular mechanisms of Type 2 root hair pattern formation in monocot crops is still limited.

Here, we report the identification and characterization of a novel basic helix-loop-helix (bHLH) transcription factor that regulates root hair development in rice (O. sativa L.). To isolate mutants with defects in root hair growth, an ethyl methanesulfonate-generated rice (O. indica, Kasalath) mutant library was screened in solution culture. Two mutants defective in root hair elongation were isolated by examining root hair using stereomicroscope, and designated as Osrhl1-1 and Osrhl1-2. The mutant plants showed no significant differences in root length, number of lateral roots and adventitious roots (Figure 1A), except for very short root hair (Figure 1B and 1C). To examine the morphology of the epidermal cells of the mutants in more detail, seedlings were grown on Murashige and Skoog media for 3 days after germination. Roots from wild-type (WT) and Osrhl1-1 mutant plants were compared using cryo-scanning electron microscopy (Cryo-SEM) (see Supplementary information, Data S1).

Root hairs located 2-3 mm from the apex were examined, and their length and diameter were determined (Figure 1D and 1E). The length of root hairs of Osrhl1-1 was reduced remarkably relative to WT, while no difference in root hair diameter was found (Figure 1J). The image of Cryo-SEM also showed that the WT epidermal cell pattern as Type 2 formed by unequal transverse cell division in the epidermal stem cell [2] may be remediated. In the mutant, no clear shorter and longer cells were observed as in the WT (Figure 1D and 1E), suggesting that RHL1 may control the root hair elongation as well as epidermal cell patterning in rice.

Genetic analysis of 700 F2 progenies derived from a cross between a homozygous rhl1-1 line and the japonica cultivar Nipponbare revealed that rhl1-1 possessed a recessive mutation at a single nuclear locus. The OsRHL1 locus was mapped to chromosome 6 between STS1 and STS2 in a PAC clone P0554A06 (Supplementary Information, Figure S1A). Within the mapped region, one gene with a bHLH domain (GenBank accession No. BAD72512) was found. Sequencing analysis indicated that the point mutation in Osrhl1-1 disrupted the splicing site between exon 2 and intron 2, resulting in the addition of intron 2 to the mRNA of OsRHL1 and a shift in the reading frame. In Osrhl1-2, two base pairs (GC) at position 179 were deleted (Supplementary information, Figure S1B and S1C).

To determine the function of RHL1, complementation analysis was performed in the Osrhl1-1 mutant line using Agrobacterium tumefaciens-mediated transformation. The 1 263-bp coding region of OsRHL1 was cloned into the pCAMBIA 1301 vector and expression was driven by the 35S promoter. Ten independent transgenic lines were obtained, with 10-20 sibling plants in each line. Insertion and expression of the transgene were confirmed by RT-PCR and Southern analysis (Figure 1H and 1I). Cryo-SEM images of primary roots of WT and two independent Osrhl1-1 mutant transgenic lines with over-expression of OsRHL1 showed that the transgenic lines had longer root hairs on primary roots than WT plants.
A cotton kinesin GhKCH2 interacts with both microtubules and microfilaments

Tao Xu1, Zhe Qu1, Xueyong Yang, Xinghua Qin, Jiyuan Xiong, Youqun Wang, Dongtao Ren2 and Guoqin Liu2

State key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100193, China

INTRODUCTION

Many biological processes require the co-operative involvement of both microtubules and microfilaments; however, only a few proteins mediating the interaction between microtubules and microfilaments have been identified from plants. In the present study, a cotton kinesin GhKCH2, which contains a CH (calponin homology) domain at the N-terminus, was analysed in vitro and in vivo in order to understand its interaction with the two cytoskeletal elements. A specific antibody against GhKCH2 was prepared and used for immunolabelling experiments. Some GhKCH2 spots appeared along a few microtubules and microfilaments in developing cotton fibres. The His-tagged N-terminus of GhKCH2 (termed GhKCH2-N) could co-precipitate with microfilaments and strongly bind to actin filaments at a ratio of monomeric actin/GhKCH2-N of 1:0.6. The full-length GhKCH2 recombinant protein was shown to bind to and cross-link microtubules and microfilaments in vitro. A GFP-fusion protein GFP–GhKCH2 transiently overexpressed in Arabidopsis protoplasts decorated both microtubules and microfilaments, confirming the binding ability and specificities of GhKCH2 on microtubules and microfilaments in living plant cells. The results of the present study demonstrate that GhKCH2, a plant-specific microtubule-dependent motor protein, not only interacts with microtubules, but also strongly binds to microfilaments. The cytoskeletal dual-binding and cross-linking ability of GhKCH2 may be involved in the interaction between microtubules and microfilaments and the biological processes they co-ordinate together in cotton cells.

Key words: calponin homology domain, cotton fibre, cross-link, kinesin, microfilament, microtubule.

1 Both of these authors contributed equally to the present study.
2 Correspondence may be addressed to either of these authors (email Liu@cau.edu.cn or Ren@cau.edu.cn).
AtKinesin-13A is located on Golgi-associated vesicle and involved in vesicle formation/budding in Arabidopsis root-cap peripheral cells
Liqin Wei†1,2, Wei Zhang†1, Zhaohui Liu1 and Yan Li*1

Address: 1State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100193, PR China and 2Research Center of Molecular and Developmental Biology, Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, PR China

Email: Liqin Wei - weiliqin@ibcas.ac.cn; Wei Zhang - wei_zhang6423@126.com; Zhaohui Liu - zhaoh@cau.edu.cn; Yan Li* - liyan@cau.edu.cn
* Corresponding author †Equal contributors

Abstract

Background: AtKinesin-13A is an internal-motor kinesin from Arabidopsis (Arabidopsis thaliana). Previous immunofluorescent results showed that AtKinesin-13A localized to Golgi stacks in plant cells. However, its precise localization and biological function in Golgi apparatus is unclear.

Results: In this paper, immunofluorescent labeling and confocal microscopic observation revealed that AtKinesin-13A was co-localized with Golgi stacks in Arabidopsis root tip cells. Immunoelectron microscopic observations indicated that AtKinesin-13A is primarily localized on Golgi-associated vesicles in Arabidopsis root-cap cells. By T-DNA insertion, the inactivation of the AtKinesin-13A gene (NM-112536) resulted in a sharp decrease of size and number of Golgi vesicles in root-cap peripheral cells. At the same time, these cells were vacuolated in comparison to the corresponding cells of the wild type.

Conclusion: These results suggest that AtKinesin-13A decorates Golgi-associated vesicles and may be involved in regulating the formation of Golgi vesicles in the root-cap peripheral cells in Arabidopsis.

Background
Kinesins are a large super-family of microtubule motor proteins that can use the energy of ATP hydrolysis to produce force and move along microtubules [1,2]. Based on their motor domain location within the primary sequence of the proteins, different kinesins may have their motor domains affixed at C-terminal, N-terminal or internal positions [3]. The C-terminal and N-terminal motor kinesins transport various vesicles and organelles toward the microtubules minus-terminal or plus-terminal, respectively. The internal motor kinesins found in animal cells are not able to move along the microtubules in the conventional form, but instead depolymerize microtubules from both ends [4]. The completed Arabidopsis genome contains at least 61 genes encoding polypeptides with the kinesin catalytic core. Among these kinesins, AtKinesin-13A and AtKinesin-13B are two internal-motor kinesins [5,6]. However, the similarity of AtKinesin-13A and AtKinesin-13B to kinesins of the same subfamily from other kingdoms is only limited to the catalytic core, and they lacks a Lys-rich neck motif commonly found in animal Kinesin-13s. Plant Kinesin-13A and animal Kinesin-13s also have different localization patterns [7,8]. Lu et al. reported that AtKinesin-13A was co-localized with Golgi stacks in various Arabidopsis cells, indicating that AtKinesin-13A is a special plant internal-motor
Database

plantsUPS: a database of plants' Ubiquitin Proteasome System
Zhou Du, Xin Zhou, Li Li and Zhen Su*

Address: State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing, 100193, PR China

Email: Zhou Du - adugduzhou@gmail.com; Xin Zhou - xzhou82@gmail.com; Li Li - walkcoolboyli@gmail.com; Zhen Su* - zhensu@cau.edu.cn

* Corresponding author

Abstract

Background: The ubiquitin 26S/proteasome system (UPS), a serial cascade process of protein ubiquitination and degradation, is the last step for most cellular proteins. There are many genes involved in this system, but are not identified in many species. The accumulating availability of genomic sequence data is generating more demands in data management and analysis. Genomics data of plants such as Populus trichocarpa, Medicago truncatula, Glycine max and others are now publicly accessible. It is time to integrate information on classes of genes for complex protein systems such as UPS.

Results: We developed a database of higher plants' UPS, named plantsUPS. Both automated search and manual curation were performed in identifying candidate genes. Extensive annotations referring to each gene were generated, including basic gene characterization, protein features, GO (gene ontology) assignment, microarray probe set annotation and expression data, as well as cross-links among different organisms. A chromosome distribution map, multi-sequence alignment, and phylogenetic trees for each species or gene family were also created. A user-friendly web interface and regular updates make plantsUPS valuable to researchers in related fields.

Conclusion: The plantsUPS enables the exploration and comparative analysis of UPS in higher plants. It now archives > 8000 genes from seven plant species distributed in 11 UPS-involved gene families. The plantsUPS is freely available now to all users at http://bioinformatics.cau.edu.cn/plantsUPS.

Background

The ubiquitin/26S proteasome system (UPS) is the major pathway of protein degradation. UPS can affect all aspects of cellular function, and plays an important role in physiological processes like hormonal responses, biotic stress and photomorphogenesis. In UPS, substrate proteins destined for degradation are tagged with 76-residue ubiquitin proteins through a serial cascade process of so-called ubiquitination, and finally hydrolysed by 26S proteasome. There are three steps in ubiquitination, catalyzed by three different enzymes or enzyme complexes: ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2), and ubiquitin protein ligase (E3). There are approximately 1300 E3s in the Arabidopsis genome, and similarly large numbers in other plants. However, in most plant species, the genome-wide classification and annotation of UPS genes, especially E3 families, are not yet available. The rapidly accumulating genome sequences has
Arabidopsis microtubule-associated protein AtMAP65-2 acts as a microtubule stabilizer

Hua Li · Xian Zeng · Zi-Qiang Liu · Qiu-Tao Meng · Ming Yuan · Tong-Lin Mao

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Abstract Nine genes that encode proteins of the MAP65 family have been identified in the Arabidopsis thaliana genome. In this study, we reported that AtMAP65-2, a member of the AtMAP65 family, could strongly stabilize microtubules (MTs). Bacterially-expressed AtMAP65-2 fusion proteins induced the formation of large MT bundles in vitro. Although AtMAP65-2 showed little effect on MT assembly or nucleation, AtMAP65-2 greatly stabilized MTs that were subjected to low-temperature treatment in vitro. Analyses of truncated versions of AtMAP65-2 indicated that the region that encompassed amino acids 495–578, which formed a flexible extended loop, played a crucial role in the stabilization of MTs. Analysis of suspension-cultured Arabidopsis cells that expressed the AtMAP65-2-GFP fusion protein showed that AtMAP65-2 co-localized with MTs throughout the cell cycle. Cortical MTs that were decorated with AtMAP65-2-GFP were more resistant to the MT-disrupting drug propyzamide and to ice treatment in vivo. The results of this study demonstrate that AtMAP65-2 strongly stabilizes MTs and is involved in the regulation of MT organization and dynamics.

Keywords Microtubules · AtMAP65-2 · Stabilization · Arabidopsis

Abbreviations EGTA Ethylene glycol-bis-(β-aminoethyl ether)-N,N,N,N-tetraacetic acid
MAPs Microtubule-associated proteins
MT Microtubule
PIPES Piperazine-N,N-bis (2-ethanesulfonic acid; 1,4-piperazinediethanesulfonic acid)

Introduction

Plant cells are remarkable for their ability to organize microtubules (MTs) into distinct arrays, both in dividing cells and in differentiated cells (Cyr and Palevitz 1995; Wasteneys and Galway 2003). The microtubule-associated proteins (MAPs) play crucial roles in regulating MT organization and dynamics (Lloyd and Hussey 2001). The regulation of MT dynamics is commonly carried out by two groups of proteins, namely, MT stabilizers and destabilizers (Heald and Nogales 2002). MAPs that act as destabilizers, such as katanin (Burk et al. 2001; Bouquin et al. 2003) and MAP18 (Wang et al. 2007b), have been identified in plant cells. A number of MT stabilizers, in particular those that act on cortical MTs, have also been identified in plant cells. For example, MOR1 from Arabidopsis thaliana stabilizes cortical MTs (Whittington et al. 2001), and MAP60 from carrot stabilizes MTs against cold or dilution treatment in vitro (Rutten et al. 1997). A number of evolutionarily conserved MAPs of ~65 kD were first identified in tobacco and suspension-cultured carrot cells, and these proteins comprise the...
Recruitment of AtWHY1 and AtWHY3 by a distal element upstream of the kinesin gene AtKP1 to mediate transcriptional repression

Ji-Yuan Xiong · Cheng-Xia Lai · Zhe Qu · Xue-Yong Yang · Xing-Hua Qin · Guo-Qin Liu

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Abstract A 43-bp distal element, the AtKP1-related element (KPRE), was previously shown to repress the promoter activity of the kinesin gene AtKP1 in Arabidopsis thaliana. In order to identify KPRE-binding factor 1 (KBF1), a combination of ion-exchange chromatography, gel-filtration chromatography and DNA-affinity chromatography was used to purify KBF1 from whole cell extracts of Arabidopsis seedlings. Mass spectrometric identification showed that KBF1 contains two members of the whirly family of transcription factors, AtWHY1 and AtWHY3. KBF1 is a single and double-stranded DNA-binding factor. A ChIP assay showed that AtWHY1 and AtWHY3 bind to the upstream region of AtKP1 gene in vivo. Over-expression of AtWHY1 and AtWHY3 led to an obvious decrease of AtKP1 transcripts, based on quantitative real-time PCR analysis. Interestingly, salicylic acid treatment resulted in an increase of AtWHY1 and AtWHY3 transcripts, and a decrease of AtKP1 transcripts. Thus, AtWHY1 and AtWHY3, as two components of KBF1, can be recruited at the KPRE site to mediate the transcriptional repression of AtKP1. Our results prove that AtKP1 is a new downstream target of the whirly family of transcription factors.

Keywords KPRE-binding factor 1 · Whirly family of transcription factors · Transcriptional repression · Kinesin · AtKP1-related element

Introduction

Transcription factors play important roles in the activation and repression of gene transcription by binding to the regulatory regions of genes (Jones et al. 1988). The interactions between transcription factors and promoters at the DNA level are a decisive step in transcriptional regulation, in which a multitude of DNA-protein and protein-protein interactions activate or repress gene transcription by forming functional and regulatory complexes (Suzuki et al. 1998). Transcription is a cumulative result of multiple reactions and components, and the elucidation of critical reactions such as DNA-protein interactions will increase understanding of the whole process, and facilitate further investigation of gene function.

Transcription factors are members of a superfamily that performs different functions in the process of cell development (Hori et al. 2003; Perez-Rodriguez et al. 2005), differentiation (Reimold et al. 2001; Igarashi et al. 2007), and signal transduction (Uno et al. 2000; Middleton et al. 2007), etc. The whirly family of transcription factors are plant-specific proteins, and are predicted to have the ability to bind to single-stranded DNA (Desveaux et al. 2005). The first identified member of the whirly proteins is PBF-2, which activates potato PR-10a gene expression in an ERE element-dependent manner. The cDNA for PBF-2 was cloned and termed SquWHY1, and demonstrated to play roles in defense gene regulation (Desveaux et al. 2000, 2004). Analysis of its crystal structure showed that four SquWHY1 molecules associate with cyclic C4 symmetry, and the
Isolation and characterization of conserved non-coding sequences among rice (*Oryza sativa* L.) paralogous regions

Xianran Li · Lubin Tan · Liguo Wang · Songnian Hu · Chuanqing Sun

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Abstract Segmental duplication is particularly frequent within plant genomes and the ability of the original single-copy gene to gain a new function for the change of regulatory elements is one of the prominent consequences of duplication. Thus, it is important to study the pattern of conserved non-coding sequence (CNS) between paralogous genes. We report the result of a survey of CNSs among paralogous regions in rice (*Oryza sativa* L.), as well as the comparison of CNS dataset between rice and *Arabidopsis* *thaliana*. Some common properties, such as the change of A + T content near the CNS boundaries and CNS are enriched in regulatory genes, were observed. However, the content of CNSs differs between rice and *Arabidopsis*, and it is interesting that the rice metabolic network includes both CNS-poor and CNS-rich genes, which indicated a fine-tuned metabolic network presents in rice.

Keywords *Oryza sativa* L. · Conserved non-coding sequence · Metabolic network

Introduction

The increasing availability of genomic sequences has accelerated the identification of conserved non-coding sequences (CNSs) in animals (Levy et al. 2001; McEwen et al. 2006; Prabhakar et al. 2006; Venkatesh et al. 2006; Vavouri et al. 2007) and in plants (Kaplinsky et al. 2002; Guo and Moose 2003; Inada et al. 2003). It has been proposed that some CNSs function as regulatory elements via in vivo transgenic analysis (Woolfe et al. 2005; Bejerano et al. 2006; Pennacchio et al. 2006). Recently, a conserved non-coding sequence (*vgt1*) among maize (*Zea mays* L.), rice (*Oryza sativa* L.), and sorghum (*Sorghum bicolor*) was shown to function as a key cis-element associated with maize flowering time (Salvi et al. 2007). Also, one non-coding sequence (k-box), which locates in the 5′ upstream region of *SHOOT MERISTEMLESS* (*STM*) and is conserved among monocots and dicots, is involved in the regulation of *STM* expression in the developing leaves (Uchida et al. 2007).

Identification of CNSs involves the comparison of two homologous sequences (orthologues and paralogues). To date, most identified CNSs are isolated from orthologous. However, pairwise genes in paralogues typically diverged...
Purification and characterisation of polyphenol oxidase from red Swiss chard (Beta vulgaris subspecies cicla) leaves

Zhao-Jian Gao, Xiao-Hong Han, Xing-Guo Xiao * 
State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Haidian District, Beijing 100193, China

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ABSTRACT

We report purification and characterisation of a polyphenol oxidase from red Swiss chard (rcPPO). Our purification procedure resulted in a 39-fold enrichment in specific activity and 17% recovery of total enzyme activity. The purified rcPPO appeared as a monomeric protein of 41 kDa, with a specific conformation conserved in the Cu2+ binding region. It was optimally active at pH 7.5 and 45 °C. It had a diphenolase substrate preference for both L-DOPA and catechol and chlorogenic acid, but also exhibited weak monophenolase activity towards 4-methoxyphenol and tyrosine. We also found that the enzyme was activated by K+, Na+, SDS and lauryl sarcosine, but inhibited by divalent cations including Ca2+, Cu2+. Its activity was completely inhibited by ascorbic acid, cysteine, 1,4-dithiothreitol, β-mercaptoethanol, sodium diethyldithiocarbamate, sodium metabisulphite, sodium sulphite and thiourea. This first report on the purification and characterisation of red Swiss chard PPO provides a basis for understanding and use of this enzyme.

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1. Introduction

Polyphenol oxidase (PPO) is a copper-containing enzyme that is capable of catalyzing the conversion of monophenols to their corresponding o-diphenols (hydroxylation of monophenols), and the conversion of o-diphenols to their corresponding o-diphenols (hydroxylation of diphenols). The enzyme activity is related to the oxidation of tyrosine to L-DOPA (3,4-dihydroxyphenyl-L-alanine) and oxidation of 2,3-diphenols such as L-DOPA to dopaquinone (diphenolase activity E.C. 1.10.3.1) (Sánchez-Ferrer, Rodríguez-López, García-Cánovas, & García-Carmona, 1995). This enzyme is widely distributed in microorganisms, animals and higher plants. In plants, a significant body of literature on the catalytic properties of PPO has been reported. Research to date is mainly focused on the following three aspects: (1) its antiviral and antioxidant properties, as well as the protective effect against damage from ultraviolet radiation (Mayer, 2006), (2) its roles in darkening damaged tissue and in fruit and vegetable browning during storage and processing (Friedman, 1996), (3) its potential involvement in the betalain biosynthetic pathway (Steiner, Schiemann, Böhm, & Strack, 1999; Gandía-Herrero, Escribano, & García-Carmona, 2005), in which the PPO catalyzes two different reactions: hydroxylation of tyrosine to form L-DOPA and oxidation of the DOPA to produce dopaquinone.

Red Swiss chard (Beta vulgaris subspecies cicla) is a herbaceous biennial leafy vegetable cultivated in many parts of the world for its year-round availability, low cost and wide use in many traditional dishes. Different from its sister variety garden beet, red chard is rich in red–violet betacyanins in its leaf (Kugler, Stingl, & Carle, 2004), not the root. The betacyanins in garden beet have been investigated widely for their physiological functions, such as scavenging powerful radical (Pavlov, Kovatcheva, Georgiev, Koleva, & Ilieva, 2002), inhibiting the proliferation of tumour cells in vitro (Reddy, Alexander-Lindo, & Nair, 2005), antimicrobial properties (Abdou, Abou-Beid, & El-Sherbini, 2002), protective UV-filter and fungal resistance (Sepúlveda-Jiménez, Rueda-Benítez, Porta, & Rocha-Sosa, 2004). The same physiological functions were demonstrated for the betacyanins of red chard. In addition to betacyanins, red chard contains in the leaf also a substantial amount of phenolic acids with antioxidant activity (Pavlov, Kovatcheva, Georgiev, Koleva, & Ilieva, 2002), not the root. The betacyanins in garden beet have been investigated widely for its physiological functions, such as scavenging powerful radical (Pavlov, Kovatcheva, Georgiev, Koleva, & Ilieva, 2002), inhibiting the proliferation of tumour cells in vitro (Reddy, Alexander-Lindo, & Nair, 2005), antimicrobial properties (Abdou, Abou-Beid, & El-Sherbini, 2002), protective UV-filter and fungal resistance (Sepúlveda-Jiménez, Rueda-Benítez, Porta, & Rocha-Sosa, 2004). The same physiological functions were demonstrated for the betacyanins of red chard.
FREEZING/CHILLING STRESS

Coronatine Enhances Chilling Tolerance in Cucumber (Cucumis sativus L.) Seedlings by Improving the Antioxidative Defence System


State Key Laboratory of Plant Physiology and Biochemistry, College of Agronomy and Biotechnology, China Agricultural University, Beijing, China

Abstract

Coronatine (COR) is a new plant growth regulator that mimics the biological activities of methyl jasmonate. We determined whether COR enhanced chilling tolerance of cucumber (Cucumis sativus L. cv. Jinchun 4) seedlings and if such tolerance was correlated with changes in the activity of antioxidant enzymes. COR was applied to seedlings at two-leaf stage at 0 (Control), 0.1, 1, 10, and 100 nm. Seedlings were then subjected to chilling stress at 5 ± 1 °C for 4 days. Seedlings treated with COR showed significant higher tolerance to chilling stress and the optimal concentration was 1–10 nm. Compared with control, the chilling injury index (CII) of the seedlings treated with COR at 1 and 10 nm was decreased by 44.9 % and 24.5 %, respectively, while the membrane chilling stability (MCS) expressed as the change of relative conductance was increased by 37.2 % and 17.0 %, respectively. The malondialdehyde (MDA) content in leaves treated with COR at 1 nm was decreased by 39.7 %, and the O2 production rate and H2O2 content reduced by 28.6 % and 8.5 %, respectively. Treatment with COR at 1 nm increased the activities of superoxide dismutase (SOD) in leaves by 34.4 %, catalase (CAT) by 58.7 % and ascorbate peroxidase (APX) by 23.0 % under low temperature. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities were also significantly improved by 28.9 % and 21.3 % following treatment with COR at 1 and 10 nm, respectively. The overall results suggest that COR enhanced chilling tolerance in cucumber seedlings by improving the antioxidative defence system.
Role of OsHAL3 Protein, a Putative 4'-Phosphopantothenoylcysteine Decarboxylase in Rice

Ning Zhang1,2, Xuechen Wang1, and Jia Chen1*

1State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100094, China; fax: +86-10-6273-3491; E-mail: chenja@public.bta.net.cn; xcwang@cau.edu.cn
2Laboratory of Development Biology, Biological Science and Technology College, Shenyang Agricultural University, Shenyang 110161, China; E-mail: zhangning_66@163.com

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Abstract—In this study, we cloned the OsHAL3 gene from rice Oryza sativa. Alignment analysis revealed that OsHAL3 has a high sequence identity to Dfp protein in Escherichia coli and AtHAL3a protein in Arabidopsis thaliana, which have 4'-phosphopantothenoylcysteine decarboxylase (PPC-DC) activity. OsHAL3 can complement mutation in the E. coli dfp gene encoding PPC-DC, so that the mutant strains with OsHAL3 can grow on rich media at 42°C and on VB minimal media at 30°C. Complementation tests with point mutations of OsHAL3 suggested that the conserved Cys176 residue of OsHAL3 is a key active-site residue. The mutant OsHAL3 G180A has a partly reduced activity. Related mRNA-level analysis showed that the OsHAL3 gene is induced by calcium pantothenate in rice.

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Key words: OsHAL3, 4'-phosphopantothenoylcysteine decarboxylase, coenzyme A, rice

Dfp protein in Escherichia coli and AtHAL3a protein in Arabidopsis thaliana belong to a flavoprotein family that was named HFCD (homo-oligomeric flavin containing Cys decarboxylases) [1, 2]. They have 4'-phosphopantothenoylcysteine decarboxylase (PPC-DC) activity [3, 4]. PPC-DC catalyzes the decarboxylation of (R)-4'-phospho-N-pantothenoylcysteine (PPC) to 4'-phosphopantetheine (PP), a key step in coenzyme A (CoA) biosynthesis from pantothenate in bacteria and plants [1, 3-6]. PPC-DC is a good target for the development of antimicrobials to treat many diseases [7, 8].

Dfp was originally described as a flavoprotein involved in DNA and pantothenate metabolism. Further study revealed that Dfp protein is a bifunctional enzyme catalyzing the synthesis of PPC and its decarboxylation to PP with ATP [5-7] and CTP [9]. Escherichia coli dfp-707 mutant can grow on TY (tryptone–yeast) media but required β-alanine or pantothenate for growth on VB (Vogel–Bonner) minimal media at 30°C. The mutant was temperature-sensitive for growth on TY or VB minimal media at 42°C, and it did not grow at 42°C on rich media supplemented with 1 mM pantothenate [10, 11].

The A. thaliana flavoprotein AtHAL3a shows sequence homology to the NH2-terminal domain of Dfp. It is related to salt and osmotic tolerance and to plant growth. Overexpression of the AtHAL3a gene improves growth rates and salt and drought tolerance in transgenic A. thaliana plants [12]. The X-ray structure of AtHAL3a reveals that the biological activity unit is a trimer [13]. AtHAL3a catalyzes the decarboxylation of PPC to PP in vitro [4]. Like Dfp, the conserved Cys175 residue in the substrate recognition clamp of the AtHAL3a protein is essential for this enzymatic activity [1, 5, 14]. The mutant AtHAL3a proteins M145L and C175S showed no PPC-DC activity, whereas the AtHAL3a mutants D177N and G179A had a significantly reduced PPC-DC activity [5].

NtHAL3a, NtHAL3b, and NtHAL3c from Nicotiana tabacum complement the temperature-sensitive mutation of the E. coli dfp gene [15].

Rice is one of the major grain crops in the world, but the role of the Oryza sativa OsHAL3 gene is not clear, and rice PPC-DC is little known. In order to reveal whether the OsHAL3 protein has PPC-DC activity, we cloned the
Patterns of nucleotide diversity in wild and cultivated rice

Xianran Li · Lubin Tan · Zuofeng Zhu ·
Haiyan Huang · Ying Liu · Songnian Hu ·
Chuanqing Sun

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Abstract There are few reports of the patterns of polymorphism in the non-coding regions of plant genomes. In this study, we explored nucleotide diversity and linkage disequilibrium (LD) in 47 non-coding regions on chromosome 4 of wild and cultivated rice. The cultivated rice retained about 70% of the diversity of wild rice, which was verified by coalescent simulations with one population bottleneck for 198 combinations of duration and population sizes. Multi-locus likelihood analysis showed that the severity of the bottleneck ranged from 2.25 to 3.33, with an average value of 2.70; i.e., the diversity found in the cultivated rice could be explained by a founding population of 2,700 individuals if the initial domestication event occurred over a period of 1,000 years. LD decreased more rapidly in wild rice than in cultivated rice within 10 kb, and the LD observed in cultivated rice was increased at 100–140 kb by comparison with wild rice. The patterns of LD indicated the possibility of a haplotype block in cultivated rice but not in wild rice.

Keywords SNP · Linkage disequilibrium · Haplotype block structures · Rice

Abbreviations
LD Linkage disequilibrium
SNP Single nucleotide polymorphism

Introduction

Genetic diversity among crop plants is typically reduced during domestication, for the new crop lineage is derived from partially wild progenitors through a population bottleneck. In general, about 60–80% of polymorphisms are descended from wild ancestors (Buckler et al. 2001), with the exception of strongly selected loci (Clark et al. 2004; Whitt et al. 2002). The structures of linkage disequilibrium (LD) are also changed by the bottleneck (Flint-Garcia et al. 2003; Gupta et al. 2005). Thus, comparison of the patterns of polymorphism and LD in wild and cultivated rice will provide new insights into the history of the population.

Knowledge of the nucleotide diversity in well-characterized plant systems is growing rapidly due to the development of genotyping technology (Brown et al. 2004; Heuertz et al. 2006; Nordborg et al. 2002, 2005; Roselius et al. 2005). Although some generalities (such as loss of diversity and bias of distribution of Tajima’s $D$) have
Coronatine-induced lateral-root formation in cotton (Gossypium hirsutum) seedlings under potassium-sufficient and -deficient conditions in relation to auxin

Zhiyong Zhang1,2, Fuqiang Yang1, Bo Li1, A. Egrinya Eneji1, Jianmin Li1, Liusheng Duan1, Baomin Wang1, Zhaohu Li1, and Xiaoli Tian1*

1 State Key Laboratory of Plant Physiology and Biochemistry, Key Laboratory of Crop Cultivation and Farming System, Center of Crop Chemical Control, China Agricultural University, Beijing 100193, China
2 School of Life Science & Technology, Henan Institute of Science and Technology, Xinxiang, Henan 453003, China

Abstract
A large root system plays a decisive role in potassium (K)-acquisition efficiency of cotton. Coronatine (COR), a non-host-specific phytotoxin, may affect the auxin level in plants and might therefore be useful in regulating lateral-root (LR) development. Our objectives were (1) to examine the effects of COR on root development, especially the LR formation in hydroponically grown cotton seedlings, and (2) to explore possible mechanisms involved. The results showed that K deficiency (0.05 mM) significantly reduced LR formation in cotton seedlings, possibly due to the decrease of endogenous indole acetic acid (IAA) in roots by more than half. Following the application of 10 nM COR, the LRs significantly increased by 26% in K-sufficient (0.5 mM) solution and by 95% in K-deficient solution. Although COR did not increase the free IAA level in the primary root, the polar auxin-transport inhibitor N-1-naphthylphthalamic acid (NPA) decreased its stimulating effects on LR formation by 25%–30%, suggesting that the COR-induced LR formation was independent of increased auxin level but likely associated with auxin transport. Treatment of plants with 1-naphthalene acetic acid (NAA) increased LR formation at NAA concentrations of 100 nM, but had no effect at 10 nM. In the presence of 1 nM COR, however, NAA increased LR formation at 10 nM concentrations. This indicates that LR formation due to COR possibly involves changes in auxin sensitivity. In addition, the shorter LRs of COR-treated seedlings were clearly restored when COR was removed from solutions for 12 d, and the total root length, total root surface area as well as K uptake increased significantly, suggesting that COR may be potentially useful for enhancing the K-acquisition efficiency of cotton seedlings.

Key words: auxin / auxin transport / nutrient acquisition / potassium acquisition / potassium availability

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1 Introduction
Potassium comprises up to 10% of plant dry weight (Leigh and Jones, 1984) and is of considerable agricultural importance (Marschner, 1995), especially for cotton production. With the recent increase in yield and popularization of transgenic insect-resistant cotton in China, varying degrees of K deficiency have frequently occurred in cotton fields in the Yangtze River, Yellow River, and Xinjiang areas. Potassium deficiency has thus become one of the major constraints to cotton production in China. It has been well established that a large and/or efficient root system plays a decisive role in plant resistance to abiotic stress, including nutrient deficiency (Zhang and Forde, 1998; Linkohr et al., 2002; Casimiro et al., 2003; Lopez-Bucio et al., 2003; Rengel and Damon, 2008). Brouder and Cassman (1990, 1994) reported that K deficiency in cotton during late-season growth was related to root development and that the K-efficient cultivar had an increased rate of root extension and more total root surface area. Therefore, a large root system will enhance the adaptation of cotton to K deficiency in the soil.

Among phytohormones, auxins have the best-known effects on root development (Casson and Lindsey, 2003; De Smet et al., 2006). Exogenous application of auxin (Evans et al., 1994; Himanen et al., 2002) and an increase in endogenous auxin (Sitbon et al., 1992) stimulated lateral-root (LR) formation. Auxin transport is also necessary for LR development (Reed et al., 1998; Casimiro et al., 2001; Bhlerao et al., 2002). Furthermore, LR formation is related to auxin sensitivity (Zobel, 1974; Mudy et al., 1995); the Arabidopsis mutant aberrant lateral root formation–1 (alf4–1) was unable to produce lateral roots and did not respond to exogenous auxins (Celenza et al., 1995).

Coronatine (COR) is a non-host-specific phytotoxin produced by several members of the Pseudomonas syringae group of pathovars (Mitchell, 1982; Bender et al., 1999). It is a new biotic plant-growth regulator, structurally and functionally similar to methyl jasmonate (Feys et al., 1994; Uppalapati et al., 2005). Coronatine can lead to leaf chlorosis (Gnanamanickam et al., 1982), anthocyanin production (Feys et al., 1994; Uppalapati et al., 2005), coiling of Bryonia dioica tendril...
Mapping quantitative trait loci influencing panicle-related traits from Chinese common wild rice (Oryza rufipogon) using introgression lines

X. Luo, F. Tian, Y. Fu, J. Yang and C. Sun

1State Key Laboratory of Plant Physiology and Biochemistry, National Evaluation Central for Agricultural Wild Plant (Rice), Laboratory of Crop Heterosis and Utilization of Ministry of Education, Department of Plant Genetics and Breeding, China Agricultural University, Beijing, 100094, China; 2State Key Laboratory of Genetic Engineering, Morgan-Tan International Center for Life Sciences, School of Life Sciences, Fudan University, Shanghai 200433, China; 3Corresponding author, E-mail: suncq@cau.edu.cn

Abstract

Panicle-related traits are important agronomic traits which directly associated with grain yield. In this study, we investigated quantitative trait loci (QTLs) associated with panicle-related traits using a set of 265 introgression lines (ILs) of common wild rice (Oryza rufipogon Griff.) in the background of Indica high-yielding cultivar Guichao 2 (O. sativa L.). A total of 39 QTLs associated with panicle-related traits including panicle length (PL), primary branch number (PNB), secondary branch number (SBN), spikelet number per panicle (SPP), and spikelet density (SD), were detected in the ILs with single-point analysis. The alleles of 20 QTLs derived from wild rice showed positive effects, and some QTLs, such as QPl1b for PL, QPn1d for PNB, QSan and QSd1b for SD and QSpp1 for SPP showed larger positive effects, providing good candidates and useful information for marker-aided improvement of yield potential of rice. Most of the QTLs controlling SPP, SBN and SD were located in cluster or closely linked on chromosomes, and the directions of their additive effects were consistent, which explained the genetic basis of significant correlations between their phenotypic characters.

Key words: common wild rice — introgression lines — quantitative trait locus — panicle-related traits

Grain yield is one of most important traits in rice breeding, and breeders always expected to develop high-yielding varieties. However, because grain yield is a complex trait and influenced by many processes and factors, direct analysis of grain yield did not give ideal results. It will be helpful to make proper dissection and focused studies on yield components, instead of yield itself as a whole. Panicle-related traits, as important yield components, had been studied by many researchers (Kato and Takeda 1996, Xu et al. 2004, Yamagishi et al. 2004, Mei et al. 2005b, Ando et al. 2008, Xie et al. 2008). Currently, rice breeding faces the problem of yield plateau, caused by narrow genetic basis of parental materials (Rangel et al. 1996, Tankesley and McCouch 1997). Thus, new breeding resource must be exploited from unused local varieties or exotic germplasm like wild rice to broaden the genetic diversity. Common wild rice (Oryza rufipogon Griff.), as the wild ancestor species of cultivated rice (O. sativa L.) (Second 1982, Oka 1988, Wang et al. 1992), constitute a major gene pool for rice improvement. During the course of domestica-tion from wild rice to cultivated rice, profound changes of agronomic traits and genetic diversity occurred, and the number of alleles of cultivated rice was only 60% that of wild rice, and many alleles were lost, leading to lower genetic diversity of the cultivated rice (Sun et al. 2001). In recent years, a number of trait-enhancing quantitative trait loci (QTL) alleles derived from wild rice have been reported (Xiao et al. 1996, Moncada et al. 2001, Li et al. 2002, Septiningsih et al. 2003, Thomson et al. 2003, He et al. 2006, Tian et al. 2006a). However, there were few reports about the identification of favourable QTL of panicle-related traits from common wild rice. Mapping QTL influencing panicle-related traits, was benefit to not only uncover the low yield genetic basis of wild rice, but also understand the evolutionary mechanism of panicle-related traits from wild rice to cultivated rice.

Introgression lines (ILs) are the results of using marker-assisted selection (MAS) to introgress small chromosomal segments from the donor into the recurrent parent by consecutive backcrossing and selfing (Eshed and Zamir 1994, 1995). Any phenotypic difference between such an IL and its recurrent parent should be due to the QTL located on the introgressed segments of the donor. It was demonstrated that ILs are a powerful tool for identification of new genes (Eshed and Zamir 1994, 1995, Chetelat and Meglic 2000, Kubo et al. 2002, Ashikari et al. 2005, Tian et al. 2006a,b, Ando et al. 2008, Xie et al. 2008, Xing et al. 2008), distinguishing pleiotropy vs. linkage as well as pseudo-overdominance vs. true-dominance (Yamamoto et al. 1998, Monforte and Tankesley 2000), and eliminating the linkage drag and map-based cloning of QTL (Alpert and Tanksley 1996, Grandillo et al. 1996, Yamamoto et al. 2000, Yano et al. 2000, Takahashi et al. 2001, Ashikari et al. 2005). In our laboratory, a set of 159 ILs derived from the cross between Guichao 2, a high-yielding commercial ‘Indica’ cultivar (O. sativa), as the recurrent parent and an accession of common wild rice collected from Dongxiang county, Jiangxi Province, China, as the donor, were constructed and QTL analysis for yield-related traits in this population based on phenotypic evaluations of multiple years and sites was conducted (Tian et al. 2006a).

In this study, a larger population consisting of 265 ILs including the 159 ILs described previously by Tian et al. (2006a) and 106 new selected ILs from BC3F4 of the same cross by genotyping of 160 polymorphic simple sequence repeats (SSR) loci was used. A total of 39 QTLs associated with five panicle-related traits were detected in the ILs with
Genetic Identification of Quantitative Trait Loci for Contents of Mineral Nutrients in Rice Grain

Ana Luisa Garcia-Oliveira, Lubin Tan, Yongcai Fu and Chuanqing Sun∗

(Department of Plant Genetics and Breeding and State Key Laboratory of Plant Physiology and Biochemistry, China Agricultural University Beijing 100094, China; National Centre for Evaluation of Agricultural Wild Plant (Rice Beijing 100094, China); and Beijing Key Laboratory of Crop Genetic Improvement and Genome of Ministry of Agriculture, Beijing 100094, China)

Abstract

In present study, Fe, Zn, Mn, Cu, Ca, Mg, P and K contents of 85 introgression lines (ILs) derived from a cross between an elite indica cultivar Teqing and the wild rice (Oryza rufipogon) were measured by inductively coupled argon plasma (ICAP) spectrometry. Substantial variation was observed for all traits and most of the mineral elements were significantly positive correlated or independent except for Fe with Cu. A total of 31 putative quantitative trait loci (QTLs) were detected for these eight mineral elements by single point analysis. Wild rice (O. rufipogon) contributed favorable alleles for most of the QTLs (26 QTLs), and chromosomes 1, 9 and 12 exhibited 14 QTLs (45%) for these traits. One major effect of QTL for zinc content accounted for the largest proportion of phenotypic variation (11%–19%) was detected near the simple sequence repeats marker RM152 on chromosome 8. The co-locations of QTLs for some mineral elements observed in this mapping population suggested the relationship was at a molecular level among these traits and could be helpful for simultaneous improvement of these traits in rice grain by marker assisted selection.

Key words: introgression lines; mineral elements; Oryza sativa; Oryza rufipogon; quantitative trait loci.


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Humans require at least 49 nutrients for their normal growth and development, and the demand for most nutrients are supplied by cereals, particularly rice due to its staple role (Welch and Graham 2004). Among these nutrients, mineral elements play numerous beneficial roles due to their direct or indirect effect in both plant and human metabolism and the deficiencies or insufficient intakes of these nutrients leads to several dysfunctions and diseases in humans. Studies have indicated widespread occurrence of deficiencies for mineral elements such as anemia for iron and osteoporoses for calcium in most developing countries as well as developed countries (Welch and Graham 1999). The numbers indicate that around two billion people suffer from iron deficiency, while prevalence of zinc deficiency is much harder to quantify due to the lack of a reliable and easy clinical assay (FAO 2004). In addition, other mineral deficiencies such as calcium are also associated with malnutrition and have reached worrying levels with data suggesting that roughly three million people over the age of 50 years suffer from osteoporosis (van Staa et al. 2001). Recent epidemiological studies found that whole-grain intake (such as brown rice), is linked to disease prevention against cancer, cardiovascular disease, diabetes and obesity (Slavin 2003).

In the past, much emphasis was placed on the enhancement of yield to increase the availability of food for resource-poor peoples. In the recent past, due to more awareness regarding the importance of mineral elements in human diets, breeders started to pay more attention to the improvement of nutrient qualities of major food grain crops especially mineral elements (Zhang et al. 2004). Many researchers have already studied genetic variation for mineral elements in cereal grains such as rice (Gregorio et al. 2000; Zhang et al. 2004), wheat (Cakmak et al. 2000; Ortiz-Monasterio and Graham 2000; Balint et al. 2001) and maize (Arnold and Bauman 1976; Arnold et al. 1977; Banziger and Long 2000) and reported the narrow genetic base
Arabidopsis Profilin Isoforms, PRF1 and PRF2 Show Distinctive Binding Activities and Subcellular Distributions

Feng Wang†, Yanping Jing‡, Zhen Wang‡, Tonglin Mao†, Jozef Šamaj§, Ming Yuan* and Haiyun Ren‡

(† State Key Laboratory of Plant Physiology and Biochemistry, China Agricultural University, Beijing 100094, China; ‡ College of Life Sciences, Beijing Normal University, Beijing 100875, China; § College of Biological Sciences and Biotechnology, Beijing Forestry University, Beijing 100083, China; § Institute of Cellular and Molecular Botany, Rheinische Friedrich-Wilhelms-University Bonn, Department of Plant Cell Biology, Kirschallee 1, D-53115 Bonn, Germany; * Institute of Plant Genetics and Biotechnology, Slovak Academy of Sciences, Akademicka 2, SK-95007, Nitra, Slovak Republic)

Abstract

Profilin is an actin-binding protein that shows complex effects on the dynamics of the actin cytoskeleton. There are five profilin isoforms in Arabidopsis thaliana L. However, it is still an open question whether these isoforms are functionally different. In the present study, two profilin isoforms from Arabidopsis, PRF1 and PRF2 were fused with green fluorescent protein (GFP) tag and expressed in Escherichia coli and A. thaliana in order to compare their biochemical properties in vitro and their cellular distributions in vivo. Biochemical analysis revealed that fusion proteins of GFP-PRF1 and GFP-PRF2 can bind to poly-L-proline and G-actin showing remarkable differences. GFP-PRF1 has much higher affinities for both poly-L-proline and G-actin compared with GFP-PRF2. Observations of living cells in stable transgenic A. thaliana lines revealed that 35S::GFP-PRF1 formed a filamentous network, while 35S::GFP-PRF2 formed polygonal meshes. Results from the treatment with latrunculin A and a subsequent recovery experiment indicated that filamentous alignment of GFP-PRF1 was likely associated with actin filaments. However, GFP-PRF2 localized to polygonal meshes resembling the endoplasmic reticulum. Our results provide evidence that Arabidopsis profilin isoforms PRF1 and PRF2 have different biochemical affinities for poly-L-proline and G-actin, and show distinctive localizations in living cells. These data suggest that PRF1 and PRF2 are functionally different isoforms.

Key words: actin filaments; Arabidopsis; endoplasmic reticulum; profilin isoforms; PRF1; PRF2.


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Profilin is an abundant, ubiquitous, small (12–15 kDa) actin-binding protein, which also interacts with poly-L-proline (PLP) and phosphoinositides, and plays an important role in the regulation of actin polymerization (Gibbon and Staiger 2000; Jockusch et al. 2007). Plants have several genes encoding highly divergent profilin isoforms (Staiger et al. 1993; Mittermann et al. 1996; Kovar et al. 2000; Kandasamy et al. 2002), which may have different characteristics. For example, different profilin isoforms in maize differ in biochemical properties such as affinities for adenosine triphosphate (ATP), actin, and poly-L-proline (Kovar et al. 2000), suggesting that isovariant dynamics among co-expressed plant profilin proteins may expand the responses of the actin cytoskeleton or buffer it against stress (Meagher et al. 1999).
Distribution of an Ankyrin-repeat Protein on the Endoplasmic Reticulum in *Arabidopsis*

Liqin Wei and Yan Li*

(State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100094, China)

Abstract

There are many ankyrin-repeat proteins in plant cells. However, the distribution and function of these proteins are mostly unclear. By reverse transcription-polymerase chain reaction, a gene encoding an ankyrin-like protein was cloned from *Arabidopsis* and named *AtANK1* (GenBank accession no. NM_120340). The 6-His-tagged *AtAnk1-N* fusion protein was affinity-purified and its rabbit polyclonal antibody was obtained. Immunoblotting with the purified anti-*AtAnk1-N* polyclonal antibody revealed that the relative molecular weight of the *AtANK1* protein was about 76 kDa. By immunofluorescence labeling and immuno-gold labeling with the purified anti-*AtAnk1-N* polyclonal antibody, coupled with confocal and transmission electron microscopy observation, *AtANK1* was found to be distributed on the membrane of the endoplasmic reticulum in *Arabidopsis* cells. Based on these results, we suggested that *AtANK1* might be involved in endoplasmic reticulum-related protein localization and sorting in plant cells.

Key words: ankyrin-repeat protein; *Arabidopsis*; endoplasmic reticulum; subcellular localization; western blotting.

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*Author for correspondence.
Tel: +86 10 6273 3396;
Fax: +86 10 6273 3491;
E-mail: <liyan@cau.edu.cn>.

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Phosphorylation of Microtubule-associated Protein SB401 from Solanum berthaultii Regulates Its Effect on Microtubules

Bao-Quan Liu1,2†, Lifeng Jin1†, Lei Zhu1, Jiejie Li1, Shuli Huang1 and Ming Yuan1∗
(1 State Key Laboratory of Plant Physiology and Biochemistry, Department of Plant Sciences, College of Biological Sciences, China Agricultural University, Beijing 100094, China; 2 Department of Biological Engineering, College of Life Sciences, Dalian Nationalities University, Dalian 116600, China)

Abstract

We reported previously that the protein SB401 from Solanum berthaultii binds to and bundles both microtubules and F-actin. In the current study, we investigated the regulation of SB401 activity by its phosphorylation. Our experimental results showed that the phosphorylation of SB401 by casein kinase II (CKII) downregulates the activities of SB401, namely the bundling of microtubules and enhancement of the polymerization of tubulin. However, phosphorylation of SB401 had no observable effect on its bundling of F-actin. Further investigation using extract of potato pollen indicated that a CKII-like kinase may exist in potato pollen. Antibodies against CKII alpha recognized specifically a major band from the pollen extract and the pollen extract was able to phosphorylate the SB401 protein in vitro. The CKII-like kinase showed a similar ability to downregulate the bundling of microtubules. Our experiments demonstrated that phosphorylation plays an important role in the regulation of SB401 activity. We propose that this phosphorylation may regulate the effects of SB401 on microtubules and the actin cytoskeleton.

Key words: F-actin; microtubule-associated protein; microtubules; phosphorylation; Solanum berthaultii.

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Microtubule-associated proteins (MAPs) have been increasingly identified from plant cells and play important roles in various functions of microtubules (MTs) (Gardiner et al. 2001; Whittington et al. 2001; Hussey et al. 2002; Webb et al. 2002; Sedbrook et al. 2004; Fu et al. 2005; Wang et al. 2007; Huang et al. 2007). The regulation of MAP activities may be an effective way for the MT cytoskeleton to respond to signal transduction and to alter its functions.

It has been reported that the activities of MAPs are regulated by phosphorylation. For example, phosphorylation and dephosphorylation of AtMAP65-1 occurs during the cell cycle, and the phosphorylation reduces the binding and bundling of AtMAP65-1 to MTs (Smertenko et al. 2006). The mitogen-activated protein kinase (MAPK) cascade of tobacco plants is also involved in the phosphorylation of tobacco NtMAP65-1a (Sasabe et al. 2006). Similarly, NRK1/NTF6 phosphorylates the threonine residue at position 579 of NtMAP65-1a and downregulates its MT-bundling activity. This phosphorylation of NtMAP65-1a enhances the destabilization and turnover of MTs at the equator of the phragmoplast and thereby facilitates expansion of the phragmoplast.

The protein SB401 was first identified from the cDNA library of in vitro-germinated pollen from the diploid potato species Solanum berthaultii (Liu et al. 1997). Recently, we reported that SB401 binds to MTs and causes their bundling in vitro. In addition, SB401 binds to and bundles actin filaments. Hence, SB401 may function as a link between actin and the MT cytoskeleton (Huang et al. 2007). Nevertheless, our experiment showed that SB401 binds preferentially to MTs (Huang et al. 2007). The mechanism of regulation of the binding of SB401 to MTs or F-actin is unknown.

In the study reported herein, we obtained experimental results that showed that phosphorylation by casein kinase II (CKII)
Additive and Over-dominant Effects Resulting from Epistatic Loci Are the Primary Genetic Basis of Heterosis in Rice

Xiaojin Luo1,2,3,4, Yongcai Fu1,2,3, Peijiang Zhang5, Shuang Wu1,2,3, Feng Tian1,2,3, Jiayong Liu1,2,3, Zuofeng Zhu1,2,3, Jinshui Yang4 and Chuanqing Sun1,2,3

(1) Department of Plant Genetics and Breeding and State Key Laboratory of Plant Physiology and Biochemistry, China Agricultural University, Beijing 100193, China; (2) National Centre for Evaluation of Agricultural Wild Plant (Rice), Beijing 100193, China; (3) Beijing Key Laboratory of Crop Genetic Improvement and Genome of Ministry of Agriculture, Beijing 100193, China; (4) State Key Laboratory of Genetic Engineering, Morgan-Tan International Center for Life Sciences, School of Life Sciences, Fudan University, Shanghai 200433, China; (5) Institute of Rice Research, Anhui Academy of Agricultural Sciences, Hefei 230031, China

Abstract

A set of 148 F3 recombinant inbred lines (RILs) was developed from the cross of an indica cultivar 93-11 and japonica cultivar DT713, showing strong F1 heterosis. Subsequently, two backcross F1 (BCF1) populations were constructed by backcrossing these 148 RILs to two parents, 93-11 and DT713. These three related populations (281BCF1 lines, 148 RILs) were phenotyped for six yield-related traits in two locations. Significant inbreeding depression was detected in the population of RILs and a high level of heterosis was observed in the two BCF1 populations. A total of 42 main-effect quantitative trait loci (M-QTLs) and 109 epistatic effect QTL pairs (E-QTLs) were detected in the three related populations using the mixed model approach. By comparing the genetic effects of these QTLs detected in the RILs, BCF1 performance and mid-parental heterosis (HMP), we found that, in both BCF1 populations, the QTLs detected could be classified into two predominant types: additive and over-dominant loci, which indicated that the additive and over-dominant effect were more important than complete or partially dominance for M-QTLs and E-QTLs. Further, we found that the E-QTLs detected collectively explained a larger portion of the total phenotypic variation than the M-QTLs in both RILs and BCF1 populations. All of these results suggest that additive and over-dominance resulting from epistatic loci might be the primary genetic basis of heterosis in rice.

Key words: additivity; epistasis; heterosis; over-dominance; quantitative trait locus.


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Rice is the staple food for more than half of the world's population. Rice production has been further enhanced after the "Green Revolution" due to the successful exploitation of heterosis since the late 1970s (Yuan 1992; Khush 2001). However, study of the genetic basis of heterosis dropped behind exploitation of heterosis long ago. Though many theories, such as dominance (Bruce 1910; Keeble and Pellew 1910; Jones 1917), over-dominance (Shull 1908, East 1936) and epistasis (Stuber 1994; Goodnight 1999), have been proposed to explain the genetic basis of heterosis, its mechanism has not been elucidated entirely yet.

Recent advances in genome research involving a number of molecular-marker techniques and the availability of high-density molecular linkage maps, together with developments in analytical methods (Lander and Botstein 1989; Zeng 1994), facilitated the analysis of the genetic basis of quantitative traits. Many quantitative trait loci (QTLs) mapping studies were
Comparative Proteomic Analysis of Arabidopsis Mature Pollen and Germinated Pollen

Junjie Zou1, Lianfen Song1, Wenzheng Zhang1, Yi Wang1, Songlin Ruan1,2 and Wei-Hua Wu1

(1 State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100193, China; 2 Institute of Biotechnology, Hangzhou Academy of Agricultural Sciences, Hangzhou 310024, China)

Abstract

Proteomic analysis was applied to generating the map of Arabidopsis mature pollen proteins and analyzing the differentially expressed proteins that are potentially involved in the regulation of Arabidopsis pollen germination. By applying 2-D electrophoresis and silver staining, we resolved 499 and 494 protein spots from protein samples extracted from pollen grains and pollen tubes, respectively. Using the matrix-assisted laser desorption ionization time-of-flight mass spectrometry method, we identified 189 distinct proteins from 213 protein spots expressed in mature pollen or pollen tubes, and 75 new identified proteins that had not been reported before in research into the Arabidopsis pollen proteome. Comparative analysis revealed that 40 protein spots exhibit reproducible significant changes between mature pollen and pollen tubes. And 21 proteins from 17 downregulated and six upregulated protein spots were identified. Functional category analysis indicated that these differentially expressed proteins mainly involved in signaling, cellular structure, transport, defense/stress responses, transcription, metabolism, and energy production. The patterns of changes at protein level suggested the important roles for energy metabolism-related proteins in pollen tube growth, accompanied by the activation of the stress response pathway and modifications to the cell wall.

Key words: 2D-polyacrylamide gel electrophoresis; Arabidopsis thaliana; matrix-assisted laser desorption ionization time-of-flight mass spectrometry; pollen; pollen tube; proteome.


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Sexual reproduction of flowering plants comprises several sequential steps from pollination to fertilization. Pollen landing on a stigma is the first step, followed by pollen hydration, germination, and pollen tube growth through intercellular spaces in the pistil. When the pollen tube reaches the embryo sac of the ovary, it delivers sperm cells for double fertilization (Franklin-Tong 1999a; McCormick 2004). The critical steps in this continuous process include pollen germination on the stigma and pollen tube growth. The investigation of the regulatory mechanisms for pollen germination and tube growth is important for fundamental studies of fertility and reproduction in flowering plants. In addition, pollen germination is an ideal model system for the investigation of important issues in cell biology, such as polarized tip growth, cell-cell interactions, and signal transduction (Franklin-Tong 1999b).

Increasing efforts have been made to investigate the genetic and molecular mechanisms of pollen germination and tube growth, and at least 150 genes involving in pollen development and pollen tube growth have been studied (Twell 2002). Functional composition analysis of the Arabidopsis pollen transcriptome has revealed that the mRNAs specifically or preferentially presented in pollen mainly encode proteins potentially involved in cell wall metabolism, vesicle transport, cytoskeleton, and signaling (Honys and Twell 2003; Honys and Twell 2004; Pina et al. 2005; Wang et al. 2008). These results may reflect the functional specialization of mature pollen in the commitment of germination and tube growth. However, gene expression at the mRNA expression level lacks a direct correlation with protein level and activity (Greenbaum et al. 2003). Although mature pollen grains may contain pre-synthesized mRNA for
Regulation of OsSPX1 and OsSPX3 on Expression of OsSPX domain Genes and Pi-starvation Signaling in Rice

Zhiye Wang, Han Hu, Hongjie Huang, Ke Duan, Zhongchang Wu and Ping Wu

(State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou 310058, China)

Abstract

The rice (Oryza sativa L.) genome contains at least six genes exclusively with an SPX (SYG1/PHO81/XPR1) domain at the N-terminal, designated as OsSPX1-6. Here we report the diverse expression patterns of the OsSPX genes in different tissues and their responses to Pi-starvation. Among them, five genes, OsSPX1, 2, 3, 5 and 6 are responsive to Pi-starvation in shoots and/or in roots. The subcellular localization analysis indicates that OsSPX1 and OsSPX2 is exclusively located in nucleus, OsSPX3 in the cytoplasm, and OsSPX4 is a membrane localization protein. OsSPX1 regulates OsSPX2, 3 and 5 at the transcription level and is positively involved in the responses of the genes to Pi-starvation. Overexpression of OsSPX3 downregulates OsSPX5 in shoots under Pi-sufficiency. OsSPX3 negatively regulates the PSI (Pi-starvation induced) gene, OsIPS1 and is involved in the responses of miR399 and OsPHO2 to Pi-starvation. Our results suggest that OsSPX1 may be a regulator involved in the transcriptions of OsSPX2, 3 and 5. OsSPX3 plays a role in OsIPS1/miR399 mediated long distance regulation on OsPHO2. Our results also indicate that OsSPX3 is involved in plant tolerance to Pi-starvation stress.

Key words: Oryza sativa; SPX domain gene; expression regulation; Pi-signaling.


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The SPX (SYG1/PHO81/XPR1) domain at the N-termini of various proteins was defined following that of SYG1 (suppressor of yeast gap1), PHO81 (cyclin-dependent kinase) in yeast (Spain et al. 1995; Lenburg and O’Shea 1996) and the human protein XPR1 (the xenotropic and polytropic retrovirus receptor) (Battini et al. 1999). ScPHO81 is a cyclin-dependent kinase (CDK) inhibitor that is induced by Pi starvation, interacting with cyclin ScPHO80 to repress the activity of CDK ScPHO85, thus promoting the expression of ScPHO5 and enhancing yeast tolerance to Pi starvation (Lenburg and O’Shea 1996). More recently, the function of ScPHO81 as a critical protein for internal phosphate sensing and for signaling through the low orthophosphate affinity carriers has been known (Pinson et al. 2004). Several SPX domain genes in plants were found to be involved in responses to environmental cues or internal regulation of nutrition homeostasis. Barley IDS4 (iron-deficiency specific clone 4) contains part of the SPX domain and is preferentially expressed in Fe-deficient roots (Nakanishi et al. 1993). Arabidopsis PHO1, harboring both SPX and EXS (ERD1/XPR1/SYG1) domains, plays a role in loading root Pi into the xylem vessels. Loss of AtPHO1 function in pho1 mutants results in Pi deficiency in above-ground tissues (Poirier et al. 1991; Hamburger et al. 2002; Wang et al. 2004). AtSHB1 (SHORT HYPOCOTYL UNDER BLUE 1) protein containing SPX and EXS domains has been reported to be a sensor of blue light and involved in seed development (Kang et al. 2006; Zhou et al. 2009).

Twenty genes in Arabidopsis with the SPX domain were identified based on Arabidopsis genome sequence data (Wang et al. 2004). The 20 genes were grouped into four sub-families. Three sub-families, with a total of 16 members, encode proteins with the SPX domain and an extra conservative domain. The other four members (At5g20150, At2g26660, At2g45130 and At5g15330) form a unique sub-family with no conservative region of the Pfam-A type other than the SPX domain. Recently, four genes not encoding any conservative region other than a SPX domain in Arabidopsis were characterized and were

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A Genome-wide Functional Characterization of Arabidopsis Regulatory Calcium Sensors in Pollen Tubes

Liming Zhou1,2, Ying Fu1,2∗ and Zhenbiao Yang2,3∗

(1 State Key Laboratory of Plant Physiology and Biochemistry, Department of Plant Sciences, College of Biological Sciences, China Agricultural University, Beijing 100193, China; 2 China Agricultural University (CAU)-University of California, Riverside (UCR) Joint Center for Biological Sciences and Biotechnology, College of Biological Sciences, China Agricultural University, Beijing 100193, China; 3 Center for Plant Cell Biology and Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA)

Abstract

Calcium, an ubiquitous second messenger, plays an essential and versatile role in cellular signaling. The diverse function of calcium signals is achieved by an excess of calcium sensors. Plants possess large numbers of calcium sensors, most of which have not been functionally characterized. To identify physiologically relevant calcium sensors in a specific cell type, we conducted a genome-wide functional survey in pollen tubes, for which spatiotemporal calcium signals are well-characterized and required for polarized tip growth. Pollen-specific members of calmodulin (CaM), CaM-like (CML), calcium-dependent protein kinase (CDPK) and calcineurin B-like protein (CBL) families were tagged with green fluorescence protein (GFP) and their localization patterns and overexpression phenotypes were characterized in tobacco pollen tubes. We found that several fusion proteins showed distinct overexpression phenotypes and subcellular localization patterns. CDPK24-GFP was localized to the vegetative nucleus and the generative cell/sperms. CDPK32-GFP caused severe growth depolarization. CBL2-GFP and CBL3-GFP exhibited dynamic patterns of subcellular localization, including several endomembrane compartments, the apical plasma membrane (PM), and cytoskeleton-like structures in pollen tubes. Their overexpression also inhibited pollen tube elongation and induced growth depolarization. These putative calcium sensors are excellent candidates for the calcium sensors responsible for the regulation of calcium homeostasis and calcium-dependent tip growth and growth oscillation in pollen tubes.

Key words: Arabidopsis; calcium sensor; calcium signaling; Rho-related GTPase from plants; subcellular localization; tip growth.


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The pollen tube provides an excellent model system for the investigation of calcium signaling in plant cells because it forms an oscillating tip-focused gradient that is important for polarized tip growth, directional pollen tube elongation and growth oscillation (Krichevsky et al. 2007). It has been shown that artificial dissipation of Ca2+ gradient seriously inhibits tip growth of pollen tubes, whereas elevation of internal Ca2+ levels induces bending of the growth axis toward the zone of higher cytoplasmic calcium concentration ([Ca2+]cyt) (Rathore et al. 1991; Pierson et al. 1994; Malho and Trewavas 1996; Hepler 1997). Meanwhile, high [Ca2+]cyt promotes F-actin disassembly, blocks cytoplasmic streaming, and is critical for self-incompatibility-induced growth arrest in pollen (Yokota et al. 1999; Geitmann et al. 2000; Blankafir 2002). An important question about calcium signaling is how a particular calcium signal, such as the tip-focused [Ca2+]cyt gradient and oscillation, is generated and regulated. [Ca2+]cyt elevation is thought to require tip-localized calcium influxes. Pollen-expressed Rho-family small GTPases, Rho-related GTPase from plants (ROPs), which are also localized as a tip-high gradient in the apical plasma membrane (PM) and required for pollen tube tip growth, have been implicated in the formation of the tip-focused calcium gradient through the regulation of calcium influxes (Li et al. 1999; Zheng and...
**GNOM-LIKE 2, Encoding an Adenosine Diphosphate-Ribosylation Factor-Guanine Nucleotide Exchange Factor Protein Homologous to GNOM and GNL1, is Essential for Pollen Germination in Arabidopsis**

Dong-Jie Jia¹, Xi Cao¹, Wei Wang¹, Xiao-Yun Tan¹, Xue-Qin Zhang¹, Li-Qun Chen¹ and De Ye¹,²

(¹ State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100193, China; ²National Center for Plant Gene Research, Beijing 100101, China)

**Abstract**

In flowering plants, male gametes are delivered to female gametophytes by pollen tubes. Although it is important for sexual plant reproduction, little is known about the genetic mechanism that controls pollen germination and pollen tube growth. Here we report the identification and characterization of two novel mutants, gnom-like 2-1 (gnl2-1) and gnl2-2 in Arabidopsis thaliana, in which the pollen grains failed to germinate in vitro and in vivo. GNL2 encodes a protein homologous to the adenosine diphosphate-ribosylation factor-guanine nucleotide exchange factors, GNOM and GNL1 that are involved in endosomal recycling and endoplasmic reticulum-Golgi vesicular trafficking. It was prolifically expressed in pollen grains and pollen tubes. The results of the present study suggest that GNL2 plays an important role in pollen germination.

**Key words:** adenosine diphosphate-ribosylation factor-guanine nucleotide exchange factor; Arabidopsis; gametophyte; GNL2; male gametophyte-defective; pollen.


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Full Length Research Paper

Expression analysis of four flower-specific promoters of *Brassica* spp. in the heterogeneous host tobacco

An-Qi Geng, Zhan-Jun Zhao, Xuan-Li Nie and Xing-Guo Xiao

State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing, 100094 China.

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The 5'-flanking region of ca. 1200 bp upstream of the translation start site (TSS) of a putative cell wall protein gene was cloned from *Brassica campestris*, *B. chinensis*, *B. napus* and *B. oleracea*, and transferred to tobacco via *Agrobacterium*-mediation after fused to promoter-less beta-glucuronidase (GUS) reporter gene. Histochemical GUS staining and fluorometric quantification of the transgenic tobacco showed that all four promoters conferred GUS expression in petal, anther, pollen and stigma of the flower, not in any vegetative organs or tissues of the plants. A series of 5'-end deletion of the promoter from *B. napus* disclosed that the region -104 to -17 relative to TSS was sufficient to confer flower-specific expression, and the region -181 to -161 played a key role in maintaining strong driving power of the promoter. Besides, several enhancer and suppressor regions were also identified in the promoter.

**Key words**: Flower-specific promoter, floral-specific promoter, *Brassica campestris*, *Brassica chinensis*, *Brassica napus*, *Brassica oleracea*, minimal promoter, enhancer, suppressor, coordinated expression.

INTRODUCTION

Genetic engineering of plants does require not only appropriate target gene but also highly specific promoter with regard to specific spatial and/or temporal expression of the target gene and decrement of the burden of plant growth and biosafety debate. A grand body of organ- and/or tissue-specific promoters, therefore, were cloned, characterized and explored, such as leaf-specific promoter (Gowik et al., 2004), phloem-specific promoter (Husebye et al., 2002), root-specific promoter (Yamamoto et al., 1991), fruit-specific promoter (Pear et al., 1989), pollen-specific promoter (Rogers et al., 2001) and flower-specific promoter (van Tunen et al., 1988). Among organ- and/or tissue-specific promoters, the flower-specific promoter and floral organ-specific promoter have been extensively studied and their cis-acting elements dissected, because that flower serves sexual reproduction and has great market in horticulture worldwide.

The flower has a complex structure consisting of different floral organs such as sepal, petal, stamen and carpel in flowering plants. Almost all floral organs have been subject of floral bioengineering for extending the shelf life, developing new fragrances, breaking color barrier, modifying male and/or female fertility and improving resistance etc. and the bioengineering depends upon, in great extent, flower-specific and/or floral organ-specific promoters and their key cis-acting elements. van der Meer et al. (1990) reported that a 67 bp promoter region of petunia chalcone synthase (*chs*) gene could direct flower-specific expression and the TACPyAT repeats in the region was important in the specific regulation of the gene in petunia. For bean *chs* promoter, presence of two cis-acting elements in close proximity to the TATA box was essential for high petal-specific expression of the promoter in transgenic tobacco and point mutation in the H-box element (CCTACC) and G-box element (CACGTG) dramatically decreased *chs15::GUS* fusion gene expression in the floral tissue (Faktor et al., 1996). The region between -1800 bp and -800 bp of the promoter of petunia EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) gene was sufficient for petal-specific expression in petunia, but it showed very low expression in the petals of transgenic tobacco (Benfey and Chua, 1989).
Enzymes and genes involved in the betalain biosynthesis in higher plants

Xiao-Hong Han, Zhao-Jian Gao and Xing-Guo Xiao

State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing, 100094 China.

Betalains, a class of water-soluble nitrogen-containing pigments, replace anthocyanins and serve the analogous functions in 13 families of the order, Caryophyllales. They modulate the attractive appearance of plants and protect them against destructive oxidative damage. Their antioxidant roles, radical scavenging properties in human health and their potential uses in food and pharmaceutical industries have made significant progress achieved in the detection, purification, quantification, structure elucidation of betalains, and in particular in the understanding of biosynthetic pathways of the pigments, the enzymes and their genes involved in the pathways. In this paper, major progress in betalain biosynthesis and the enzymes and genes involved in the biosynthetic pathways in higher plant are reviewed, and the perspectives discussed.

Key words: Betalain, biosynthesis, tyrosinase, DOPA dioxygenase, glucosyltransferase, betacyanin, betaxanthin

INTRODUCTION

Plant pigments are generally classified into 4 major groups: anthocyanins, betalains, carotenoids and chlorophylls. Betalains, as anthocyanins, are responsible mainly for the attractive natural display of flower, fruit, and storage root colour. They are water-soluble, nitrogen-containing pigments and comprise two groups of colour different pigments: the red-violet betacyanins and the yellow betaxanthins. The betacyanins are further divided into four sub-groups; and the betaxanthins, three groups (Strack et al., 2003) (Figure 1). Each subgroup contains several compounds except the subgroup, amaranthin in the betacyanins which has only one number (Figure 1).

Although being responsible for the attractive colour of flowers, fruits, and occasional vegetative tissues, betalains, unlike anthocyanins, are present only in 13 families of the order, Caryophyllales and in some higher fungi such as fly agaric (Amanita muscaria) (Steglich and Strack, 1990; Strack et al., 2003; Grotewold, 2006; Moreno et al., 2008). More interestingly, betalains are mutually exclusive with anthocyanins in the angiosperms, and the betalains and anthocyanins have never been observed in the same plants (Mabry and Dreiding, 1968; Kimler et al., 1970; Clement and Mabry, 1996; Lee and Collins, 2001; Strack et al., 2003; Stintzing and Carle, 2004a, Cai et al., 2005; Grotewold, 2006; Moreno et al., 2008), for which the reason remains mysterious.

In natural plant, betalains play important roles in physiology, optical attraction for pollination and seed dispersers (Piattelli, 1981). They also function as reactive oxygen species (ROS) scavengers, protect plants from damages caused by wounding and bacterial infiltration as seen in red beet (Beta vulgaris subsp. vulgaris) (Sepúlveda-Jiménez et al., 2004), function as UV-protector in ice plant (Mesembryanthemum crystallinum) (Vogt et al., 1999a). In human sociality, since synthetic dyes are becoming more and more critically assessed by the consumer, betalains, as food additives, receive more and more attention with the growing interest in the use of natural pigments for food colouring. As food additives, in particular as low acid food additives, betalains do not only improve food appearance, but also contribute to consumer health. It has been reported that betalains are a class of compounds with antioxidant and radical scavenging properties (Escribano et al., 1998; Pedreño and Escribano, 2000;