A Gibberellin-Mediated DELLA-NAC Signaling Cascade Regulates Cellulose Synthesis in Rice

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Cellulose, which can be converted into numerous industrial products, has important impacts on the global economy. It has long been known that cellulose synthesis in plants is tightly regulated by various phytohormones. However, the underlying mechanism of cellulose synthesis regulation remains elusive. Here, we show that in rice (Oryza sativa), gibberellin (GA) signals promote cellulose synthesis by relieving the interaction between SLENDER RICE1 (SLR1), a DELLA repressor of GA signaling, and NACs, the top-layer transcription factors for secondary wall formation. Mutations in GA-related genes and physiological treatments altered the transcription of CELLULOSE SYNTHASE genes (CESAs) and the cellulose level. Multiple experiments demonstrated that transcription factors NAC29/31 and MYB61 are CESA regulators in rice; NAC29/31 directly regulates MYB61, which in turn activates CESA expression. This hierarchical regulatory pathway is blocked by SLR1-NAC29/31 interactions. Based on the results of anatomical analysis and GA content examination in developing rice internodes, this signaling cascade was found to be modulated by varied endogenous GA levels and to be required for internode development. Genetic and gene expression analyses were further performed in Arabidopsis thaliana GA-related mutants. Altogether, our findings reveal a conserved mechanism by which GA regulates secondary wall cellulose synthesis in land plants and provide a strategy for manipulating cellulose production and plant growth.

INTRODUCTION

Cellulose, comprising parallel β-1,4-glucans, is a major polymer of plant cell walls. It not only represents the most abundant sustainable resources in wide use in industrial production but also plays essential roles in plant growth. The molecular structure of cellulose is simple, but the amount, the degree of polymerization, the crystalline size, and the orientation vary across different cell types and developmental stages. Therefore, in land plants, cellulose, synthesized by cellulose synthases (CESAs) at the plasma membrane, has diverse physicochemical properties. CESA-related synthesis is tightly regulated (Somerville, 2006; Li et al., 2014). Multiple lines of genetic evidence have illustrated that different sets of CESAs form complexes required for primary and secondary wall cellulose production (Desprez et al., 2002; Doblin et al., 2002; Gardiner et al., 2003; Taylor et al., 2003). The corresponding genes, CESAs, are critical downstream targets in cellulose regulatory networks.

Significant effort has been focused on the transcriptional regulation of secondary wall biosynthesis. A detailed model consisting of transcription factors (TFs) from the NAC and MYB families has been established in the eudicot Arabidopsis thaliana (Zhong and Ye, 2007; Demura and Ye, 2010; Zhao and Dixon, 2011). Currently, through protein-DNA screening, an elaborate hierarchy network of TFs and secondary wall metabolic genes has been revealed in Arabidopsis, offering an opportunity for understanding the regulatory complexity of cell wall formation under abiotic stresses (Taylor-Teeples et al., 2015). However, the networks for grasses, which possess a distinct patterning and composition of the secondary wall, are largely unknown (Zhong et al., 2011; Handakumbura and Hazen, 2012). The CESA genes that are responsible for synthesizing cellulose, a major component of secondary walls, have been proposed to be regulated by many TFs. Surprisingly few direct regulators of CESAs have been identified. Arabidopsis MYB46 and its close homolog MYB83 have been reported to bind the promoter of secondary wall CESAs via the MYB46-responsive cis-regulatory element (Kim et al., 2012; Zhong and Ye, 2012). However, MYB46 and MYB83 may regulate overall secondary wall synthesis, because manipulating their expression alters the global cell wall composition of Arabidopsis, including the content of cellulose, hemicellulose, and lignin (Ko et al., 2009; Zhong and Ye, 2012; Kim et al., 2013). The myb46 myb83 double mutant showed severe abnormalities in secondary wall thickening and plant growth, suggesting that these TFs are central regulators of secondary wall biosynthesis in both fibers and vessels (Zhong et al., 2007a; McCarthy et al., 2009). Further studies have revealed that MYB46 and MYB83 are the direct targets of several secondary wall NAC regulators, including...
SECONDARY WALL ASSOCIATED-NAC DOMAIN PROTEIN1 (SND1), NAC SECONDARY WALL THICKENING PROMOTING FACTOR1 (NST1), VASCULAR-RELATED NAC DOMAINS (VND6), and VND7 (Ko et al., 2007; Zhong et al., 2007a, 2008; McCarthy et al., 2009). Mounting genetic proof has characterized these NAC TFs as “master switches” for secondary wall formation in Arabidopsis (Kubo et al., 2005; Zhong et al., 2006, 2007b; Yamaguchi et al., 2008). Although WALLS ARE THIN1 (WAT1) might be a regulator of SND1 and NST1 in fiber cells (Ranocha et al., 2010), the upstream signals for secondary wall cellulose synthesis regulation remain largely unknown.

Gibberellin (GA) is an important hormone for plant growth and development throughout the whole life cycle, including seed germination, stem elongation, floral transition, and fruit development. Genetic analyses of GA-deficient and GA-response mutants have revealed that the central step in GA action is to turn off the repressive effects of DELLAs (Peng et al., 1997; Itoh et al., 2002; Sasaki et al., 2002; Ueguchi-Tanaka et al., 2005; Harberd et al., 2009). In the presence of GA, the GA-GIBBERELLIN INSENSITIVE DWARF1 (GID1)-DELLA complex stimulates the interaction of DELLAs with an F-box protein, resulting in the degradation of DELLAs (Fu et al., 2002; Murase et al., 2008; Hirano et al., 2010; Sun, 2011) and consequently the activation of downstream-responsive processes. DELLA have been found to inhibit GA-promoted growth by interacting with key regulatory proteins and blocking their DNA binding or transactivation activities. The first proteins identified to interact with DELLAs are basic/helix-loop-helix-type TFs, which are PHYTOCHROME INTERACTING FACTORS (PIFs) that function in Arabidopsis hypocotyl elongation (de Lucas et al., 2008; Peng et al., 2008). BRASSINAZOLE RESISTANT1 (BZR1) and JASMONATE ZIM-DOMAIN are also DELLA-interacting proteins. These interactions integrate GA with various signals for coordinated regulation of plant growth and defense (Hou et al., 2010; Bai et al., 2012). Although studies of recently identified DELLA-interacting proteins have revealed the mechanisms of several GA-mediated responses (Arnaud et al., 2010; Cheminant et al., 2011; Feurtado et al., 2011; Yu et al., 2012; Marin-de la Rosa et al., 2014), much work is required to obtain a clear understanding of the numerous roles played by GA.

Understanding the role of GA in stem height determination sparked a great revolution in agriculture. The introduction of the semidwarfling genes Reduced height-1 and semi-dwarf1 (sd1) into cereal crops improved crop architecture and lodging resistance, leading to a huge increase in grain yields during the 1960s (Peng et al., 1999; Hedden, 2003). Cellulose also has major impacts on stem length and lodging resistance. However, the genetic link between GA signaling and cellulose synthesis remains elusive. It is unclear whether GA is an upstream signal for cellulose synthesis and, if so, how plants transmit GA signals downstream to the CESAs genes. Here, we identify a GA signaling cascade that modulates secondary wall cellulose synthesis in rice (*Oryza sativa*). SLENDER RICE1 (SLR1), a key repressor of GA signaling, interacts directly with NACs, which in turn inhibits an NAC-MYB-CESA signaling cascade. These findings reveal a conserved mechanism for the regulation of secondary wall cellulose synthesis in land plants.

RESULTS

The GA-DELLA Module Regulates Cellulose Synthesis

As the cellulose level is correlated with mechanical strength, we were curious to learn whether the lodging-resistant *sd1*, a GA-deficient rice mutant harboring a mutation in GA 20-oxidase2, has a higher amount of cellulose than the wild type. To avoid data discrepancies from varied developmental stages and growth habits of plants, all of the mutants analyzed in this study share the same genetic background with the corresponding wild-type plants and were used for comparison during growth stages matched to the wild type. We first compared the anatomical structure of the mature internodes of *sd1* and wild-type plants. Unexpectedly, the epidermal layer and sclerenchyma cell wall in *sd1* were significantly thinner than in the wild type (Figure 1; Supplemental Figure 1). Composition analysis revealed that *sd1* had reduced cellulose content (Figure 2A), indicating a potential link between GA signals and cellulose synthesis. To obtain further genetic evidence, another GA-deficient mutant (*dwarf18-Akibare-waisei dwarf [d18-AD]*) and two GA-response mutants (*gid1* and *slr1*) were investigated. *D18* encodes GA 3-oxidase2, an enzyme for GA biosynthesis (Sakamoto et al., 2004). Because the internodes in *d18-AD* are too short, we performed cellulose content and anatomical examination using the leaf sheaths. Anatomical analysis showed that *d18-AD* had fewer sclerenchyma cells and reduced thickness in the sclerenchyma cell walls, whereas *slr1*, which has a mutation in the rice *DELLA* gene, resulting in a GA-constitutive response (Itoh et al., 2002), had more sclerenchyma cells and increased wall thickness (Figures 1B to 1E; Supplemental Figure 1). These changes consistently resulted in decreased cellulose content in *d18-AD* and an increased cellulose level in *slr1* (Figure 2A). CESAs, CESes, and CESes are responsible for secondary wall cellulose synthesis (Tanaka et al., 2003). We then explored the expression levels of three secondary wall CESAs in these mutants by quantitative RT-PCR (qRT-PCR). These CESAs were downregulated to varying degrees in *sd1*, *d18-AD*, and *gid1* but were significantly upregulated in *slr1* (Figure 2B). *gid1* is a GA-insensitive mutant resulting from mutations in the gene encoding a soluble receptor for GA (Ueguchi-Tanaka et al., 2005). Most likely because the examined *gid1* is a weak allele, cellulose deficiency was not observed, although the wall thickness of sclerenchyma cells was slightly reduced and CESAs transcription was accordingly repressed (Figures 1D, 1E, 2A, and 2B).

To investigate whether GA can modulate cellulose synthesis in rice, we treated the wild-type rice plants with exogenous GA and examined CESAs expression in the internodes. The expression of CESAs was significantly upregulated by GA (Figure 2C). Rice PHYTOCHROME-INTERACTING FACTOR-LIKE PROTEIN1 (PIL1) and XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE8 (XTH8), two genes not responsive and responsive to GA treatment, respectively, were used as a negative and a positive control for the examination (Jan et al., 2004; Todaka et al., 2012). Our previous work showed that *bc11* is a missense mutant of CESAs and that overexpression of the mutated CESAs in *bc11* increased the cellulose content (Zhang et al., 2009). The heading-stage wild-type and *bc11* plants were treated with GA. Composition analysis revealed that both the GA-treated wild-type and
the bc11 plants had increased cellulose abundance (Figure 2D), indicating that GA can indeed promote CESA expression. All of these data suggested that GA and the SLR1-mediated signaling pathway are required for cellulose synthesis.

SLR1 Interacts with the Top-Layer TFs for Secondary Wall Formation

We then addressed whether the role of GA in regulating cellulose synthesis is direct or indirect. Because SLR1 is a key component of GA signaling and mediates the downstream responses through protein interactions (Davière and Achard, 2013), we explored whether a direct interaction exists between SLR1 and the TFs that regulate secondary wall formation. According to the hierarchical relationship of the Arabidopsis TFs responsible for secondary wall regulation (Zhong and Ye, 2007; Demura and Ye, 2010), we hypothesized that rice might have a similar transcription regulatory network, in which several NACs are located in the top layer. The candidate NACs were screened for coexpression with the secondary wall CESAs (Supplemental Table 1) and through homologous search with the identified Arabidopsis NACs (Supplemental Figure 2 and Supplemental File 1). We selected two NAC candidates (NAC29 and NAC31) that might be involved in secondary wall formation in rice to examine the interaction with SLR1. We first investigated whether SLR1 and the above two NACs are expressed in the same cell types. Parenchyma and sclerenchyma cells were harvested from young internodes via laser-capture microdissection and subjected to qRT-PCR analysis (Figure 3A). Although transcripts of SLR1 were detected in the parenchyma cells, they were also present in the sclerenchyma cells, where the two NACs were preferentially expressed (Figure 3B). Because CESA4 is predominantly expressed in sclerenchyma cells, it was used as a control to monitor the success of microdissection. Split-luciferase complementation assays then revealed an interaction between SLR1 and the two NACs in Nicotiana benthamiana leaves (Figures 3C and 3D). Using the transgenic rice plants that expressed green fluorescent protein (GFP)-tagged NAC29 or NAC31 (NAC29/31-GFP), we found that both NAC proteins were coimmunoprecipitated with SLR1 in vivo (Figure 3E). Yeast two-hybrid assays further illustrated that SLR1 interacts directly with both NACs via the C terminus (Figure 3F), which contains the GRAS domain required for repressor function (Itoh et al., 2002). To narrow down the interacting domain of NACs, NAC29 and NAC31 were split into an N-terminal DNA binding domain (BD) and a C-terminal activation domain. Bimolecular fluorescence complementation (BiFC) analysis in N. benthamiana revealed a direct interaction between SLR1 and the BDs of the two NACs in the nuclei of living plant cells (Figures 3G and 3H). Therefore, GA directly modulates cellulose synthesis via SLR1-NAC interaction.

Figure 1. Rice GA-Related Mutants Exhibited Altered Secondary Walls.

(A) to (C) Comparison of the cross sections of mature sd1-8 and slr1-6 internodes and 2-month-old d18-AD leaf sheaths with the corresponding wild-type plants. Brackets indicate the width of the epidermal sclerenchyma layer. Dashed lines and arrows indicate the alterations in sclerenchyma cells (SC) and secondary wall thickness. V, vascular bundles. Bars = 50 µm.

(D) Observation of the sclerenchyma cells from the internodes and leaf sheaths of the indicated GA-related mutants via scanning electron microscopy. Images are as follows: 1, cv Nanjing6 (wild type); 2, sd1-8; 3, gid1-20; 4, cv Zhonghua11 (wild type); 5, slr1-6; 6, cv Akibare (wild type); 7, d18-AD. Bars = 5 µm.

(E) Quantification of the thickness of sclerenchyma cell walls examined in (D). Error bars indicate se (n = 50). *P < 0.01 determined by Student’s t test.
Hierarchical TFs Regulate Secondary Wall Cellulose Synthesis in Rice

To determine whether the SLR1-interacting NAC29 and NAC31 are in the cellulose regulation pathways, NAC29- and NAC31-overexpressing (Ox) rice plants were generated. The transgenic plants had thick internodes and significantly increased cellulose content (Figure 4A; Supplemental Figures 3A and 3B). The total lignin amount and xylose content were not significantly altered (Supplemental Table 2). In addition, three secondary wall CESA s in the overexpressing plants were upregulated (Figure 4B). Taken together with the transactivation activity and subcellular localization data (Supplemental Figures 3C to 3F), NAC29 and NAC31 are CESA regulators.

The downstream components of NAC29 and NAC31 are often MYBs (Zhong et al., 2011). Coexpression analysis identified several secondary wall CESA coexpressed R2R3-type MYBs (Supplemental Table 1), most of which were GA-responsive (Supplemental Figure 4A). To identify which MYBs function downstream of NAC29 and NAC31, the expression levels of their respective genes were examined in the NAC29 Ox and NAC31 Ox rice plants by qRT-PCR. The upregulated levels indicated that certain MYBs are in the NAC29/31-mediated signaling pathways (Figure 4C). We chose MYB61 as a representative for further functional characterization. The MYB61 Ox plants had thick internodes, upward curved leaves, and significantly increased cellulose content (Figure 4D; Supplemental Figures 4B and 4C) but unchanged total lignin and xylose contents (Supplemental Table 2). Consistent with the increased cellulose content, MYB61 Ox had increased secondary wall thickness (Supplemental Figures 4D to 4F) and more secondary wall CESA transcripts and proteins (Figure 4E; Supplemental Figure 4G). Moreover, the MYB61-dominant repression plants that overexpressed a construct harboring an MYB61-SRDX fusion (Hiratsu et al., 2003) exhibited decreased cellulose content (Figure 4F). Thus, MYB61 indeed participates in the NAC-CESA regulatory pathway.

MYB61 Directly Activated by NAC29/31 Targets GAMYB Motifs of Secondary Wall CESAs

To find molecular support for the above signaling pathway, we addressed whether NAC29 and NAC31 regulate MYB61 transcription. Transactivation analysis revealed significant luciferase activities when the cauliflower mosaic virus (CaMV) 35S promoter driving either NAC29 or NAC31 was coexpressed with the MYB61 promoter driving luciferase reporter in rice protoplasts (Figure 5A), demonstrating that NAC29 and NAC31 activate MYB61 expression. Secondary wall-related NAC proteins often bind to the SNBE element in the targeted genes (Zhong et al., 2011; Handakumbura and Hazen, 2012). To determine whether the activation by NAC29 and NAC31 is direct, MYB61 promoter fragments containing two SNBE elements were examined in an electrophoretic mobility shift assay (EMSA). SNBE2 was bound by the recombinant NAC29 and NAC31 proteins fused to glutathione S-transferase (GST-NAC29 and GST-NAC31), which resulted in a mobility shift; GST alone, as a negative control, did not cause a mobility shift (Supplemental Figures 5A and 5B). The binding ability to SNBE2 was gradually suppressed by the addition of increasing amounts of unlabeled probes (Figure 5B) and was completely abolished when SNBE2 was mutated (Supplemental Figures 5C to 5E).
Thus, NAC29 and NAC31 bind to the SNBE motif and directly regulate MYB61 expression. We further investigated whether MYB61 can activate CESA transcription. After proving that MYB61 is a functional TF, MYB61 or MYB61 fused to the glucocorticoid receptor (MYB61-GR) was placed under the control of the CaMV 35S promoter (Figure 5C; Supplemental Figures 6A to 6C). Cotransferring rice protoplasts with the resulting constructs and a reporter driven by individual CESA promoters resulted in significant luciferase activity (Supplemental Figure 6D). In the GR-based inducible system, luciferase activity induced by the addition of dexamethasone (DEX) was completely quenched by treatment with the protein synthesis inhibitor cycloheximide (CHX) (Figure 5D), but the transcription of three secondary wall CESA s was activated by DEX even in the presence of CHX (Figure 5E), demonstrating that MYB61 directly induces the expression of secondary wall CESA s. EMSA was conducted to confirm the above result. MYB61 proteins fused to maltose binding protein (MBP-MYB61) were found to bind to the biotin-labeled CESA promoter fragments (Supplemental Figures 6E to 6G). To verify the interaction of MYB61 with CESA promoters in vivo, we performed a chromatin immunoprecipitation (ChIP) assay in wild-type and MYB61 Ox plants. MYB61-bound fragments enriched by immunoprecipitation with anti-MYB61 antibody were used for quantitative PCR.
Three CESA promoter fragments were significantly enriched in MYB61 Ox plants (Figure 5F). Sequence searching within the bound fragments identified a conserved element annotated as GAMYB, a motif bound by GA-responsive MYBs (Woodger et al., 2003). Then, 34-bp oligonucleotides containing a wild-type motif and a single base-mutated motif (GAMYB and GAMYBm; Supplemental Figure 6E) were synthesized. The EMSA revealed competition or abolition of binding with unlabeled GAMYB or GAMYBm (Figure 5G). Moreover, a transactivation assay performed by expressing three copies of a 20-bp wild-type core motif of GAMYB revealed a significantly higher activity than expressing the mutated version in protoplasts (Figures 5H and 5I). Therefore, GAMYB is an MYB61 binding element.

Taken together, the above studies have identified one regulatory pathway, NAC29/31-MYB61-CESA, for secondary wall cellulose synthesis in rice.

The Interaction between SLR1 and NAC29/31 Blocks the NAC-MYB61-CESA Regulatory Pathway

We then addressed the effect of the SLR1-NAC29/31 interaction on the identified signaling cascade. Because NAC29 and NAC31 interact with SLR1 via the BD, their ability to bind the MYB61 promoter may be affected. Transactivation analysis showed that luciferase activity in the cells coexpressing a reporter containing the MYB61 promoter driving luciferase and an effector containing NAC29 or NAC31 was significantly repressed by the additional coexpression of SLR1. This repression was suppressed by the application of exogenous GA (Figures 6A and 6B), demonstrating that SLR1 inhibits the NAC29/31-mediated regulatory pathway. Additional proof of this sequestration role was derived from EMSAs. Affinity-purified SLR1 extracted from N. benthamiana leaves agroinfiltrated with the FLAG-SLR1 construct significantly abolished the binding of rice NAC29 and NAC31 to the MYB61 promoter (Figure 6C). The effect of the NAC29/31-SLR1 interaction on secondary wall CESA s was further determined by examining their transcripts in rice protoplasts expressing the CaMV 35S promoter driving NAC29 or NAC31 and SLR1. The upregulated CESA levels in cells expressing NAC29 or NAC31 were attenuated by the additional coexpression of SLR1. The application of GA again activated the transcription of CESAs (Figure 6D). Altogether, the in vitro and in vivo evidence indicate that SLR1 directly interacts with NAC29 and NAC31 to sequester these factors and inhibits

Figure 4. Identifying the TFs Involved in Secondary Wall Cellulose Synthesis in Rice.

(A), (D), and (F) Measurement of the cellulose content in NAC29 Ox and NAC31 Ox (A), MYB61 Ox (D), and MYB61-SRDX rice plants (F). AIR, alcohol-insoluble residues.
(B), (C), and (E) qRT-PCR examination of the expression of three secondary wall CESA s (B) and GA-inducible MYBs (C) in NAC29 Ox and NAC31 Ox and three secondary wall CESA s in MYB61 Ox (E). TP1 was used as an internal control.
Error bars indicate the std of three biological repeats. *P < 0.01 determined by Student’s t test.
Figure 5. Identifying the NAC-MYB61-Secondary Wall CESA Regulatory Pathway.

(A) Luciferase activities in rice protoplasts cotransfected with the constructs shown above. The transactivation activity was monitored by assaying the luciferase activity in the rice protoplasts, with the ones transfected with an empty effector construct defined as 1.

(B) EMSA showing the competing binding of the NACs to SNBE fragments with an increasing amount of unlabeled DNA probe (10-fold [+]) and 50-fold [++]).

(C) Diagrams of the effector and reporter constructs used in (D) and (E).

(D) Luciferase activities in protoplasts cotransfected with the constructs shown in (C) in the presence or absence of 10 μM DEX and/or 2 μM CHX.

(E) qRT-PCR examination of the transcripts of three secondary wall CESAs in the protoplasts expressing the effector shown in (C). TP1 was used as an internal control. The luciferase activity (D) and expression level of CESAs (E) in the protoplasts without DEX treatment were set to 1.

(F) ChIP-quantitative PCR analysis showing MYB61 binding to the CESA promoter sequences in vivo compared with samples without antibody application. Actin1 was used as a negative control.
their binding to MYB61, which consequently blocks CESA transcription.

This Signaling Machinery Is Required for Internode Development

Next, the above regulatory machinery was verified in a natural physiological event. The rice internode contains abundant secondary walls and is a typical location for GA action. Therefore, we collected the developing second internodes from wild-type plants when they were 9 cm in length and divided the internodes into nine sections at 1-cm intervals. Anatomical and compositional analyses revealed that the secondary walls were gradually deposited in the sclerenchyma cells from the bottom up (Supplemental Figure 7), in accordance with the feature of intercalary growth, a lengthwise growth due to the activity of intercalary meristems in monocot plants. Cell elongation almost ceased after section 3 upward from the bottom (Figure 7A). Secondary wall, including cellulose and lignin, initiated formation at section 2 and rapidly accumulated above this section (Figure 7B). Therefore, rice internode development includes elongation (sections 1 to 3) and secondary wall formation (sections 2 to 9). To define GA’s physiological roles in the two processes, the endogenous GA levels were examined. Because section 9 is at the mature stage and the relevant genes are inactive, the examinations were performed in the lower eight sections. The content profiles of GA1, GA19, GA20, GA24, and GA53 in the developing internodes were similar: the concentration was highest in section 1.
and dropped to a stable level in sections 3 to 8 (Figure 7C). GA$_4$, GA$_9$, and GA$_{12}$ were difficult to detect except in section 1, where the concentrations of GA$_4$ and GA$_9$ were 0.13 ± 0.02 and 0.09 ± 0.02 ng/g fresh weight, respectively. The above anatomical and compositional data on each section indicate that the relatively high GA level in the lower sections is essential for cell elongation and the initiation of secondary wall synthesis; the low GA level in the upper sections may be required to maintain cellulose synthesis therein at a certain level.

To verify this conclusion, we investigated the expression patterns of the components in the NAC29/31-MYB61-secondary wall CESA signaling pathway by qRT-PCR. The transcripts of SLR1, NAC29, and NAC31 peaked in section 2, consistent with the finding that this stage involves the onset of secondary wall cellulose synthesis; the peak of MYB61 and secondary wall CESAs occurred in section 3 or 4 (Figure 7D). The slightly lagging peaks of downstream components verified the hierarchical relationship of this signaling cascade. After being activated in sections 2 and 3, this signaling pathway was gradually repressed through sections 3 to 8, where GA$_4$ was maintained at a steady low level. Therefore, it was hypothesized that this signaling pathway is activated by a relatively high GA level in the early stages of internode development and is gradually repressed by a steady low level of GA in the later developing stages. To confirm this speculation, we further compared the expression pattern of MYB61 and CESAs in the internodes of the wild type and sd1, as sd1 is deficient in GA biosynthesis and has been widely used in rice breeding. The overall expression patterns of MYB61 and CESAs in sd1 were lower than in the wild type, regardless of the activation and repression stages (Figure 7E). All of these results suggest that this signaling transduction pathway is modulated by varied GA levels in the early and late stages of internode development.

**Figure 7.** Examination of the GA-Mediated NAC29/31-MYB61-CESA Signaling Cascade in Developing Rice Internodes.

(A) Measurement of cell length in the longitudinal direction in the developing internodes divided into nine sections, as shown in Supplemental Figure 7. Error bars indicate SD (n = 30).

(B) Total yield of cellulose and lignin in each section of internodes as described above. Error bars indicate SD (n = 50).

(C) Endogenous GA levels in each section of internodes as described above. F.W., fresh weight.

(D) and (E) qRT-PCR analyses of the expression levels of genes in the signaling pathway in each internode section from cv Nipponbare (D) and from cv Nanjing6 (wild type) and sd1 (E) plants. TPI was used as an internal control. The expression level in section 1 was considered as 1 in (D).

The numbers on the x axes indicate the internode sections from the bottom up. Error bars indicate the SD of three biological replicates.
levels, which regulate cellulose synthesis during internode development.

The Identified Signaling Machinery Is Conserved in Plants

To determine whether this signaling pathway is present in other plant species, the role of GA in cellulose synthesis was examined in Arabidopsis, a model plant with GA functional components identical to the ones in rice. We found that treating Arabidopsis plants with exogenous GA induced the expression of secondary wall CESAs and hierarchical TFs (Figure 8A; Supplemental Figure 8A). Genetic evidence was further provided by the examination of gene expression and cellulose synthesis in GA-related Arabidopsis mutants. Three secondary wall CESAs were upregulated in the double mutants lacking two DELLA genes, GAI and RGA (gai-t6 rga-24); however, this upregulation was attenuated in a triple mutant (gai-t6 rga-24 ga1-3), which harbors an additional mutation in CPS (ga1-3), a gene encoding an enzyme for GA biosynthesis (Figure 8B). The three CESAs were downregulated in the DELLA gain-of-function mutant gai-1 (Figure 8B). Consistent with the gene expression data, DELLA loss-of-function mutants exhibited increased secondary walls in interfascicular fibers and xylem vessels, whereas the DELLA gain-of-function mutant gai-1 and the GA-deficient mutant ga1-3 tended to exhibit compromised secondary wall deposition in fiber and vessel cells (Figure 8C). Chemical analysis revealed corresponding alterations in cellulose content in the above mutants (Figure 8D). Furthermore, split-luciferase complementation assays showed interaction between the Arabidopsis DELLA protein RGA and SND1, an NAC TF for secondary wall formation in N. benthamiana leaves (Figure 8E). Therefore, GA signals and DELLA-mediated signaling are required for the regulation of secondary wall cellulose synthesis in Arabidopsis.

Because GAMYB motifs are critical for GA signal transduction, the promoter regions of the primary and secondary wall CESAs in Arabidopsis and poplar (Populus trichocarpa) were examined. The presence of at least one GAMYB motif in At-CESAs and Ptr-CESAs (Supplemental Table 3) and the action of poplar CESAs in response to GA treatment (Supplemental Figure 8B) indicated that this signaling cascade for cellulose synthesis is conserved in land plants.

DISCUSSION

GA Regulates Secondary Wall Cellulose Synthesis via an SLR1-NAC Signaling Cascade

Cellulose is a basic structural polymer of plant cell walls. Its synthesis is thought to be highly regulated by various hormones

Figure 8. GA Promotes Cellulose Synthesis in Arabidopsis.

(A) Inducing the expression of Arabidopsis secondary wall CESAs in the inflorescence stems from wild-type plants treated for 6 h with 10 μM GA3 (+GA) compared with control plants (−GA).

(B) qRT-PCR analyses showing altered secondary wall CESA expression levels in the inflorescence stems from the wild type and GA-related mutants. dellaΔΔΔ, gai-t6 rga-24; ga1-3 dellaΔΔΔ, ga1-3 gai-t6 rga-24.

(C) Observation of interfascicular fiber (If) and xylem vessel (Xv) cells from cross sections of the interfascicular stems from the wild type and GA-related mutants. Bars = 50 μm.

(D) Measurement of cellulose content in the inflorescence stems from the wild type and GA-related mutants. AIR, alcohol-insoluble residues. *P < 0.01 determined by Student’s t test.

(E) Split-luciferase complementation assay showing the interaction between Arabidopsis RGA and SND1 in N. benthamiana leaves agroinfiltrated with the constructs shown at top left. BF, bright field. Bar = 1 cm.

Arabidopsis UBQ10 was used as an internal control in (A) and (B). Error bars indicate the SD of three biological repeats.
and environmental signals, fulfilling its roles in different cell types and at distinct developmental stages (Xie et al., 2011; Zhong et al., 2011). Previous studies have identified a few TFs that function in the transcriptional regulation of cellulose (Ko et al., 2007; Demura and Ye, 2010; Zhong and Ye, 2012; Kim et al., 2013). However, the underlying mechanism, especially a complete signal transduction pathway, has been unclear. In this study, we reveal a role for GA and the mechanism of GA-regulated cellulose synthesis. Multiple lines of evidence from genetic, biochemical, and gene expression analyses point to a complete GA signaling cascade (Figure 9), in which GA signals are transmitted through the interaction between SLR1, a key repressor of GA signaling (Itoh et al., 2002), and NACs, the top-layer master switches for the transcriptional regulation of secondary wall formation (Zhong and Ye, 2007). In the presence of GA, the GA-induced degradation of SLR1 releases NACs, enabling these factors to bind to and upregulate the downstream target MYB61 and consequently enhancing the transcription of three secondary wall CESA genes (Figure 9). Critical biological proof for this regulatory machinery came from the examination of GA content and the expression of key regulators in developing rice internodes, where the behavior of this signaling pathway is correlated with the endogenous GA levels. Moreover, this signaling mechanism was found to be conserved in land plants, as evidenced by the following observations: the Arabidopsis DELLA protein RGA interacts with SND1; the Arabidopsis GA-related mutants exhibit alterations in CESA expression and cellulose synthesis; and the CESA genes from Arabidopsis and poplar were GA-inducible and have at least one GAMYB element in their promoter regions. GAMYB binding proteins are TFs belonging to a distinct subclass in the MYB superfamily, which is widely present in plant species, and in which many members are required for modulating cell wall biosynthesis (Woodger et al., 2003; Zhong and Ye, 2007). Therefore, the GA-response pathway mediated by GAMYB binding TFs, such as MYB61, is applicable to other plant species.

**SLR1 May Be a Key Component That Integrates GA with Other Signals in Cellulose Synthesis Regulation**

In addition to GA, other hormonal and environmental stimuli are involved in the modulation of cellulose synthesis. Brassinosteroid and light have been found to promote Arabidopsis hypocotyl elongation and primary wall CESA gene transcription (Leivar and Quail, 2011; Xie et al., 2011). WAT1, which was characterized as a vacuolar auxin transporter, has been found to play a role in secondary wall formation in fiber cells (Ranocha et al., 2013). However, the complete signaling pathways linking CESAs and BZR1, PIFs, or WAT1 have been unclear. On the other hand, an increasing number of studies have revealed a comprehensive view of DELLA action by identifying the physical interactions with several types of TFs (Marín-de la Rosa et al., 2014), which are thought to form a central command system for integrating various signals (Davière and Achard, 2013). Previous work in Arabidopsis has revealed the interaction between DELLA and PIFs/BZR1 (de Lucas et al., 2008; Feng et al., 2008; Bai et al., 2012). In this signaling cascade, SLR1 interacts with secondary wall NACs. Therefore, identification of the interdependent relationship

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**Figure 9. Regulatory Model for Secondary Wall Cellulose Formation in Rice.**

SLR1 interacts with secondary wall NACs to repress their DNA binding activity. In the presence of GA, GA triggers the proteasomal degradation of SLR1 and frees the NACs to activate the expression of downstream MYB and secondary wall CESAs, which consequently promotes cellulose synthesis. MT, microtubules; PM, plasma membrane.
between NACs and the other SLR1-interacting TFs will provide a better mechanistic understanding of cellulose synthesis in response to different signals.

Potential Value of This Regulatory Pathway in Crop and Biomass Production

The widely known physiological role of GA is to promote plant height. Therefore, one common phenotype of many GA-related mutants is the altered stem length (Sakamoto et al., 2004). The major contribution of GA to the Green Revolution is based on this role, relying on its function in cell expansion and proliferation (Peng et al., 1997; Sasaki et al., 2002; Achard et al., 2009; Lee et al., 2012; Mimura and Itoh, 2014). The role for GA in regulating secondary wall cellulose synthesis, which also results from promoting sclerenchyma wall thickening and the proliferation of sclerenchyma cells, extends its function directly to lodging resistance. Combining the results of anatomical analysis and GA content in the developing rice internodes, we conclude that cell elongation and secondary wall formation are modulated by varied GA levels via different SLR1-mediated signaling pathways. Our study here promotes an in-depth consideration of these Green Revolution genes, addressing how GA coordinate modulates cell elongation and secondary wall synthesis during internode development. Identifying the SLR1-mediated downstream pathways may be a critical way to manipulate both agronomic traits.

More importantly, cellulose is the most valuable type of biomass. Owing to its economic importance, much attention has been paid to the manipulation of cellulose properties. The activation of this GA-mediated signaling cascade in rice plants promotes cellulose synthesis without significantly altering other major cell wall components. This finding was distinguished from the results of overexpressing the Arabidopsis CESA regulatory genes, such as SND1, MYB46, and MYB83, in which the overall secondary wall synthesis was altered (Zhong et al., 2006; Ko et al., 2009; Zhong and Ye, 2012; Kim et al., 2013). This discrepancy indicates that the secondary wall regulatory network in monocot plants may be distinct from the one in dicots in certain respects. In this study, although total lignin content was not changed in the transgenic plants, we cannot exclude the possibility that their lignin composition was altered, as Os-NAC31 and Os-MYB61 were reported to regulate Os-MYB46 and Os-CINNAMYL ALCOHOL DEHYDROGENASE2 expression (Zhong et al., 2011; Hirano et al., 2013). Moreover, cellulose biofuel is a type of sustainable energy source that has gained popular interest due to its potential benefit to the global environment. In the future, the economical use of this biofuel will depend on whether we can manipulate cellulose biosynthesis (Li et al., 2014). This study showed that cell wall residues from TF-overexpressing plants exhibited increased cellulose content and improved saccharification efficiency (Supplemental Figure 8D), suggesting the intriguing value of this regulatory pathway in biomass production.

METHODS

Plant Materials and Growth Conditions

The rice plants (Oryza sativa) used in this study, including the wild-type plants, the GA-related mutants sd1-8 (E386K), gid1-20 (A110T), d18-AD, and slr1-6 (L246P), and the relevant transgenic plants, were grown in the experimental fields at the Institute of Genetics and Developmental Biology in Beijing and Sanya in Hainan Province during the natural growing seasons. sd1-8 and gid1-20 are near-isogenic lines of cv Nanjing6, an indica cultivar. slr1-6 and d18-AD are of cv Zhonghua11 and cv Akibare, japonica cultivars, respectively. The Arabidopsis thaliana GA-related mutants, including gai-1, gai-t6 rag-24 (double mutant), and gai-3 gai-t6 rga24 (triple mutant), are of the Landsberg erecta ecotype. The typical phenotype of GA-related mutants is altered stem length.

Phylogenetic Analysis

The secondary wall-related NAC transcription factors in rice were analyzed by BLAST searching the rice genome database (http://rice.plantbiology.msu.edu) against Arabidopsis SND1 and VND6/7. An unrooted tree of the secondary wall-related NACs in rice and Arabidopsis was generated using MEGA5 software (Tamura et al., 2011) with 1000 bootstrap replications.

Generation of Transgenic Rice Plants

For generation of the TF-overexpressing rice plants, the full-length cDNA sequences (CDSs) of the TFs were amplified by PCR using the primers listed in Supplemental Table 4 and inserted into the pCAMBIA 1300 vector (Cambia) between the rice ubiquitin promoter and the nopaline synthase terminator via BamHI and KpnI/SpeI digestion. For generation of the MYB61-SRDX plants, a sequence-confirmed MYB61 CDS was fused with the SRDX sequence and inserted into the pCAMBIA 1300 vector (Cambia) between the rice ubiquitin promoter and the nopaline synthase terminator via BamHI and KpnI digestion. The resulting constructs were transfected into Agrobacterium tumefaciens EHA105 and introduced into the wild-type varieties cv Nipponbare and cv Zhonghua11, respectively (Hiei et al., 1994).

Microscopy

The fresh hand-cut cross sections of second internodes from the mature rice plants of sd1-8, gid1-20, slr1-6, and their corresponding wild type, as well as the cross sections of leaf sheaths from 2-month-old wild type and d18-AD plants, were prepared with razor blades. The fresh hand-cut cross sections of the mature inflorescence stems were also prepared from the Arabidopsis GA-related mutants and the corresponding wild-type plants. The autofluorescent signals of cell walls were viewed and photographed at an excitation wavelength of 450 to 480 nm with a fluorescence microscope (Zeiss). For the scanning microscopic analysis, the internodes and leaf sheaths of GA-related mutants were cut and fixed in 4% paraformaldehyde (Sigma-Aldrich). After dehydoration through a gradient of ethanol, the samples were sprayed with gold particles and observed with a scanning electron microscope (S-3000N; Hitachi). For the transmission electron microscopic analysis, the internodes from cv Nipponbare were cut and fixed in 2.5% (w/v) glutaraldehyde. The samples were then embedded with the Spurr Kit (Sigma-Aldrich) and sectioned with an Ultracut E ultramicrotome (Leica). The sections were stained and observed with a transmission electron microscope (H-7500; Hitachi) operated at 80 kV. For laser-capture microdissection, the young internodes from cv Nipponbare were cut and fixed in glacial acetic acid and ethanol (1:3). After they were embedded in wax (Sigma-Aldrich), the internodes were sectioned and applied for cell harvesting with an LMD 7000 (Leica) laser microdissection system. The harvested samples were subjected to RNA isolation with an RNaseasy micro kit (Qiagen) and to qRT-PCR analysis.

qRT-PCR and ChiP-Quantitative PCR Assays

The second internodes or leaf sheaths were harvested from heading-stage wild-type, sd1-8, gid1-20, slr1-6, and d18-AD plants and subjected to total RNA isolation. Four-week-old inflorescence stems of wild-type
Arabidopsis plants and the development-matched stems of GA-related mutants were also harvested and subjected to RNA isolation. Concert Plant RNA Reagent (Invitrogen) and TRIzol Reagent (Invitrogen) were used to isolate the total RNA. qRT-PCR was performed on a cycler apparatus (Bio-Rad CFX96) with the FastStart Universal SYBR Green Master (Roche). To examine the effect of GA on cellulose synthesis, heading-stage rice plants, 4-week-old Arabidopsis in the Landsberg ecotype, and 4-week-old poplar (Populus tomentosa) seedlings were treated with 10 μM GA4 for 6 or 9 h. The young parts of rice internodes and Arabidopsis and poplar stems were subjected to RNA isolation and gene expression examinations. Primers for the expression analysis are summarized in Supplemental Table 5. For ChIP-quantitative PCR analysis, formaldehyde cross-linked chromatin DNA was isolated from leaf sheaths of 2-month-old wild-type and MYB61 Ox plants. Immunoprecipitation was performed using 5 μl of anti-MYB61 antibody with a 1:100 dilution (Immunoway). The generated ChIP DNA was used as a template for PCR amplification (Supplemental Table 5) using a cycler apparatus (Bio-Rad CFX96) with the FastStart Universal SYBR Green Master (Roche). Enrichment folds of MYB61-bound DNA fragments were calculated by comparing the samples with a sample without antibody applied. The data are presented as means ± SD of three biological repeats.

Protein Interactions

The split-luciferase complementation assay was performed as described (Chen et al., 2008). The images were acquired using Indigo software. For coimmunoprecipitation analysis, leaf sheaths from 2-month-old NAC29-GFP and NAC31-GFP transgenic plants were harvested and homogenized in immunoprecipitation buffer (25 mM Tris-Cl, pH 7.5, 2 mM EDTA, 150 mM NaCl, 0.1% Triton X-100, 10% glycerol, and 1× protease inhibitor). The samples were purified through anti-GFP immobilized protein A agarose beads (Pierce Biotechnology) and were applied for immunoblot analysis using anti-SLR1 and anti-GFP antibody with a 1:1000 dilution (Invitrogen). The second hybrid assays, full-length SLR1 and the N-terminal and C-terminal parts of SLR1 were amplified (Supplemental Table 4) and fused with GAL4 BD in the pDEST32 vector (Invitrogen). Interactions in yeast were tested on SD/-Trp/-Leu/-His/-Ade/3AT (25 mM) medium. For the BIFG analysis, the cDNA of SLR1 and the N and C termini of NAC29 and NAC31 were amplified (Supplemental Table 4) and cloned into serial pSPY vectors (Wada et al., 2008) containing either N- or C-terminal enhanced yellow fluorescent protein fragments via Gateway cloning technology. The resulting constructs were then introduced into Agrobacterium strain C58 and coinfiltated into the abaxial surface of the leaves of 4-week-old Nicotiana benthamiana plants according to Chen et al. (2008). Fluorescence was observed with a confocal laser-scanning microscope (TCS SP5; Leica).

Transactivation Assay

The CDSs of SLR1, NAC29, NAC31, and MYB61 and the promoter regions (upstream of the ATG) of MYB61 and three secondary wall CESA genes were amplified (Supplemental Table 4) and cloned into the effector (35S-transcription factor) and reporter (promoter-luciferase) vectors (Promega) between HindIII/SacI and KpnI cleavage sites. The resulting effector and reporter constructs were cotransfected into protoplasts prepared from 2-week-old rice seedlings or 4-week-old Arabidopsis leaves (Ko et al., 2009). The Renilla luciferase gene driven by the CaMV 35S promoter was also cotransfected to determine the transfection efficiency. Luciferase activities were measured with a dual-luciferase reporter assay system (Promega). For the inducible system, the CDS of MYB61 fused with the GR domain was inserted into the p2GW7-35S-GR vector (Aoyama and Chua, 1997; Zhao et al., 2010). The rice protoplasts transfected with the effector and reporter constructs were treated with 10 μM DEX (Sigma-Aldrich) for 6 h, leading to MYB61-GR translocation into the nuclei. The transfected rice protoplasts were treated with 2 μM CHX for 30 min to inhibit new protein synthesis prior to the addition of DEX. The transfected protoplasts were subsequently subjected to qRT-PCR or dual-luciferase activity analyses. To examine the transactivation activity of GAMYB, three copies of the 20-bp GAMYB (GGGCACAAACCGCGCGTCCG) or GAMYBm (GGGCACGAGAAGCGCC- GTTCCG) of the CESA4 promoter were synthesized and inserted into the reporter vector and transfected into Arabidopsis protoplasts. Luciferase activities were measured as described above. To investigate the effects of SLR1-NAC29/31 interaction, protoplasts cells coexpressing the indicated reporters and effectors were treated with 10 μM GA4 for 6 h. Then, the protoplast cells were subjected to qRT-PCR and luciferase activity assays. The data are presented as means ± SD of three biological repeats.

EMSA

The amplified CDSs of MYB61, NAC29, and NAC31 genes (Supplemental Table 4) were fused in-frame with MBP (New England Biolabs) or GST (Invitrogen) tags and transformed into Escherichia coli Rosetta (Novagen). MBP-MYB61 and GST-NAC29/31 recombinant proteins were purified and incubated with the biotin-11-UTP-labeled DNA fragments, including the CESAs and MYB61 promoters and the synthesized GAMYB and SNBE oligonucleotides, for 30 min in the EMSA binding buffer (Thermo). The DNA signals were detected by chemiluminescence (Thermo). For the competition assays, unlabeled oligonucleotides (10- and 50-fold of labeled probes) were added to the EMSA reactions. To examine the effect of SLR1 on the abilities of NAC29/31 binding to MYB61, a 35S-FLAG-SLR1 construct was prepared and introduced into N. benthamiana leaves by Agrobacterium infiltration. The FLAG-SLR1 proteins were extracted from the transfected leaves and purified by incubation with anti-FLAG M2 affinity gel (Sigma-Aldrich). Different amounts of the purified FLAG-SLR1 proteins were added to EMSA reactions. The reaction mixtures were separated by SDS-PAGE, and the signals were analyzed as described above.

Cell Wall Composition Analyses

The second internodes from the mature wild-type plants, sd1-8, gid1-20, and slr1-6 mutants, and the relevant transgenic plants after seed harvest and the leaf sheaths of 2-month-old wild-type and d18-AD plants were collected for cell wall residue preparation. The Arabidopsis cell wall residues were prepared from the lower part of inflorescence stems of mature wild-type plants and GA-related mutants. To examine the impact of GA on cellulose synthesis, heading-stage wild-type and bcl1 plants were treated with 10 μM GA4 until seed harvest. The second internodes from the treated and control plants were collected to prepare cell wall residues. The cell wall samples were treated with alcohol to obtain the insoluble residues. Monosaccharide composition was determined by gas chromatography-mass spectrometry (Agilent) as described previously (Zhang et al., 2012). For crystalline cellulose analysis, the remains after trifluoroacetic acid treatment were hydrolyzed in Updegraff reagent. The cooled pellets were washed and hydrolyzed with 72% sulfuric acid. The cellulose content was quantified by the anthrone assay (Updegraff, 1969). The lignin content was measured using the acetyl bromide method (Foster et al., 2010). The data are presented as means ± SD of three or four biological repeats.

Examination of Endogenous GAs

The 9-cm second internodes were collected from cv Nipponbare at the heading stage and divided into nine sections at 1-cm intervals. Sections 1 to 8 were applied for endogenous GA measurement as described (Li et al., 2011). In brief, 3-g internode section tissues were frozen and ground in liquid nitrogen and extracted in the buffer containing 80% (v/v) methanol at 4°C for 12 h. The internal standards, such as [3H]GA1 (1.00 ng/g), [3H]GA2 (2.00 ng/g), [3H]GA3 (2.00 ng/g), [3H]GA4 (6.00 ng/g), and [3H]GA8 (4.00 ng/g), were added to the plant samples during extraction. After
Cell Wall Saccharification Assay
To examine the enzyme activity of the commercial cellulase mixture (Novozymes), the filter-paper unit was used to determine the cellulase units. Fifty milligrams of filter paper was incubated with 900 μL of distilled water for 1 h at 100°C. After the hot-water treatment, 100 μL of 0.5 M citrate buffer, pH 4.8, was added into the tubes containing different amounts (0.01, 0.1, 1, and 10 μL) of cellulase mixture. The free sugars were assayed using the dinitrosalicylic acid method after incubation in a 50°C shaker for 1 h (Ghose, 1987). The absorbance at 540 nm was measured on a UV light-species and subjected to the saccharification assay as described above. The data are presented as means ± SD of four biological repeats.

Accession Numbers
Sequence data from this article can be found in the GenBank/EMBL libraries under the following accession numbers: Os01g66100 (SD1), Os01g08220 (D18), Os05g33730 (GID1), Os03g49990 (SLR1), Os01g18240 (MYB61), Os08g02300 (NAC29), Os08g01330 (NAC31), Os01g54620 (CESA4), Os10g32980 (CESA7), Os09g25490 (CESA9), AT5G62380 (VND6), AT1G71930 (VND7), AT1G32770 (SND1), and AT2G01570 (RGA).

Supplemental Table 4. The Primers Used for Generation of Transgenic Rice Plants and Relevant Analyses in This Study.

Supplemental Table 5. qRT-PCR and ChiP-PCR Primers Used in This Study.

Supplemental File 1. Alignments Used to Produce the Phylogenetic Tree Shown in Supplemental Figure 2.

ACKNOWLEDGMENTS
We thank W. Yang (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences) for help with laser microdissection, X.W. Deng and H. Chen (School of Life Sciences, Peking University) for providing the BiFC vectors, J. Wei (Beijing Academy of Agriculture and Forestry Sciences) for providing the poplar seedlings, and Q. Qian (China National Rice Research Institute, Chinese Academy of Agricultural Sciences) for providing the d18-AD mutan. This study was supported by the Ministry of Sciences and Technology of China (Grant 2012CB114501), the National Natural Science Foundation of China (Grants 31125019 and 91417303), the Ministry of Agriculture of China for Transgenic Research (Grant 2008ZX08009-003), and the State Key Laboratory of Plant Genomics.

AUTHOR CONTRIBUTIONS
Y.Z. and X.F. together designed the experiments. D.H. performed transactivation assay, yeast two-hybrid assay, EMSA, and cell wall composition analysis in rice and Arabidopsis. S.W. performed qRT-PCR, split-luciferase complementation assay, transactivation assay, EMSA, and GA content analysis. B.Z. analyzed motifs in promoter regions and helped with critical discussion of the work. K.S.-G. performed coimmunoprecipitation assay and gene expression analysis in Arabidopsis. Y.S. performed subcellular localization of TFs and BiFC assay. D.Z. performed ChiP-quantitative PCR and laser-capture microdissection assays. X.L. performed rice transformation. K.W. screened and cultivated the rice and Arabidopsis GA mutants. Z.X. performed field cultivation. Y.Z. performed anatomical analysis and wrote the article.

Received January 6, 2015; revised April 17, 2015; accepted May 6, 2015; published May 22, 2015.

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