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LETTER TO THE EDITOR

A DELLA protein complex controls the arbuscular mycorrhizal symbiosis in plants

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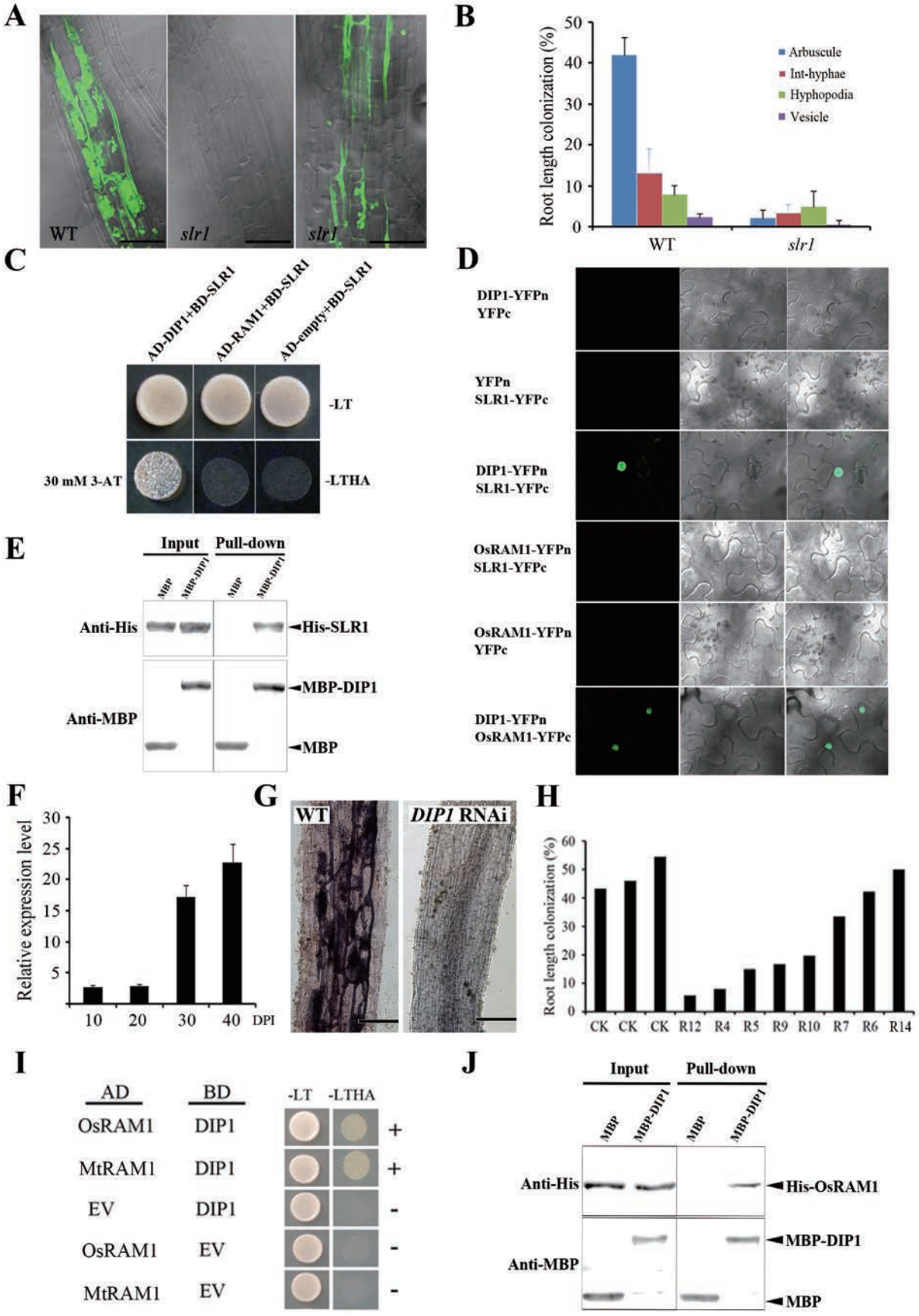
Dear Editor,

Plants establish beneficial symbiotic associations with arbuscular mycorrhizal fungi, which colonize the root cortex, building specialized structures called arbuscules that facilitate nutrient exchange. The association occurs following plant recognition of lipochitooligosaccharides (LCOs) from mycorrhizal fungi, which activates the symbiosis signaling pathway prior to mycorrhizal colonization. Here we show that SLR1/DELLA, a repressor of gibberellic acid (GA) signaling, and its interacting partner protein are required for the mycorrhizal symbiosis. GA treatment inhibits mycorrhizal colonization and leads to the degradation of DELLAs. Consistently, rice lines mutated in *DELLA* are unable to be colonized by mycorrhizal fungi. DELLAs are members of the GRAS family of transcription factors. We further show that rice DELLA interacts with a second GRAS protein, DIP1 (DELLA Interacting Protein 1). DIP1 is also required for mycorrhizal colonization and in turn interacts with a previously characterized mycorrhizal GRAS protein, RAM1, that has been shown to directly regulate mycorrhizal-associated gene expression. We conclude that a complex of GRAS proteins, including DELLAs, is necessary for regulation of mycorrhizal-associated gene expression and thus colonization.

It has previously been shown by whole genome transcriptomic analysis that GA biosynthetic genes are induced during mycorrhizal colonization in rice [1]. Furthermore, GA has been shown to inhibit mycorrhizal colonization in *Pisum sativum* [2]. To understand the role of GA in this regulation, we sought to assess the function of GA during mycorrhizal colonization. *Oryzae sativa* ssp. *japonica* cv. *Zhonghua11* plants were infected with the arbuscular mycorrhizas (AM) fungus *Rhizophagus irregularis* and treated with 0.1 μM, 1 μM, 10 μM and 100 μM GA3. Consistent with previous studies, GA treatment greatly promoted the elongation of the shoot and root (Supplementary information, Figure S1). Interestingly, mycorrhizal colonization was severely impaired at 0.1 μM, 1 μM GA3, and was completely blocked at 10 μM and 100 μM GA3, suggesting that GA negatively regulates AM colonization (Supplementary information, Figure S2A). *AM1, AM3*, *AM10*, *AM14*, *AM15*, *AM34* and *PT11* were previously shown to be induced during mycorrhizal colonization of rice [3]. Consistent with the changes that we observed in mycorrhizal colonization, the expression of these AM-specific marker genes was repressed by GA treatment (Supplementary information, Figure S2B and S2C). To explore whether the effect of GA was limited to rice, we also tested the effect of GA treatment on the AM symbiosis in *Medicago truncatula*. Consistent with the results in rice, colonization levels were greatly reduced by 10 μM GA3 treatment in *M. truncatula* (Supplementary information, Figure S2E). These results suggest that GA negatively regulates the AM symbiosis in both monocotyledonous and dicotyledonous plants.

GA is perceived by GID1 and promotes the interaction between GID1 and SLR1 (the rice DELLA ortholog). GID1 interaction with SLR1 leads to polyubiquitination of SLR1 and its subsequent degradation [4]. The *gid1* mutant of rice is insensitive to GA and as a result, SLR1 accumulates in the nucleus [5]. Both wild-type and *gid1* mutant showed comparable levels of AM colonization; however, when treated with 10 μM GA, the AM colonization level in wild-type rice was greatly reduced, while colonization level in the *gid1* mutant was similar to that of control plants (Supplementary information, Figure S2D). Thus we conclude that GA signaling negatively regulates mycorrhizal colonization and that this regulation may be associated with the degradation of SLR1, but not associated with the impact on the performance of the mycorrhizal fungi.

To assess whether DELLA proteins play a role during AM colonization, we analyzed the *slr1* mutant of rice and *SLR1-YFP* overexpression line. Unlike *Arabidopsis thaliana,* rice has only a single DELLA protein, SLR1, making analysis of the role of DELLAs easier in this species. Mycorrhizal colonization was severely impaired in the *slr1* mutant and was improved in *SLR-YFP* overexpression line, implying a direct role for this DELLA


A
WT
slr1
slr1
B
Root length colonization (%)
Arbuscule
Int-hyphae
Hyphopodia
Vesicle
WT
slr1
C
AD-DIP1+BD-SLR1
AD-RAM1+BD-SLR1
AD-empty+BD-SLR1
-LT
30 mM 3-AT
-LTHA
D
DIP1-YFPn
YFPc
YFPn
SLR1-YFPc
DIP1-YFPn
SLR1-YFPc
OsRAM1-YFPn
SLR1-YFPc
OsRAM1-YFPn
YFPc
DIP1-YFPn
OsRAM1-YFPc
E
Input
Pull-down
MBP
MBP-DIP1
Anti-His
His-SLR1
Anti-MBP
MBP-DIP1
MBP
F
Relative expression level
DPI
G
WT
DIP1 RNAi
H
Root length colonization (%)
CK CK CK R12 R4 R5 R9 R10 R7 R6 R14
I
AD
BD
-LT
-LTHA
OsRAM1 DIP1 +
MtRAM1 DIP1 +
EV DIP1 -
OsRAM1 EV -
MtRAM1 EV -
J
Input
Pull-down
MBP
MBP-DIP1
Anti-His
His-OsRAM1
Anti-MBP
MBP-DIP1
MBP

protein during AM colonization (Figure 1A, 1B and Supplementary information, Figure S3). This is surprising as DELLA proteins are generally considered to be negative regulators, yet during AM colonization *SLR1* is essential. The fact that GA promotes SLR1 degradation (Supplementary information, Figure S2F) and that SLR1 is essential for AM colonization, explains why GA treatment negatively regulates AM colonization.

The *slr1* mutant showed greatly reduced AM colonization, with reductions in internal hyphae, arbuscules and vesicles (Figure 1A, 1B). However, hyphopodia (the infection structures on the root surface) formation appeared to be unaffected in *slr1* (Figure 1B). Consistent with the defect in AM colonization, the induction of the AM-specific genes *AM1*, *AM3*, *AM11*, *AM14*, *AM15*, *AM34* and *PT11* was severely suppressed in *slr1* (Supplementary information, Figure S4A and S4B). Interestingly, *SLR1* was slightly induced at 30 and 40 days post inoculation (DPI) with *R. irregularis* (Supplementary information, Figure S4C), consistent with its role in the AM colonization.

To further investigate the function of SLR1 in the mycorrhizal signaling pathway, we searched for its interacting proteins using a yeast two-hybrid (Y2H) screen. We identified an interacting protein (LOC_Os12g06540) from this screen (Figure 1C), which interestingly encoded a GRAS-domain protein (Supplementary information, Figure S5). SLR1 itself is a member of the GRAS family of transcriptional regulators. We named this new GRAS protein, DELLA Interacting Protein 1 (DIP1). We further confirmed the SLR1-DIP1 interaction using bimolecular fluorescence complementation (BiFC) analysis in *Nicotiana benthamina*, which revealed an interaction between these two proteins in the nucleus (Figure 1D). We further validated this interaction using pull-down asssy with heterologously expressed proteins and detected a clear interaction (Figure 1E).

Detailed expression analysis showed that *DIP1* was induced at 30 DPI with *R. irregularis* and was highly expressed at 40 DPI (Figure 1F). This expression pattern is suggestive of a role for *DIP1* in the mycorrhizal association. Unfortunately, rice T-DNA insertion lines were not available in *DIP1*. Therefore, to assess whether *DIP1* functioned during AM colonization, we generated transgenic rice lines expressing a *DIP1* hairpin allowing RNA-mediated interference (RNAi) of *DIP1*. We observed that *DIP1* expression levels were reduced in several RNAi lines and those lines with significant *DIP1* reductions showed defects in AM colonization (Figure 1G, 1H). The expression level of *DIP1* in the RNAi lines correlated well with the AM colonization levels (Figure 1H and Supplementary information, Figure S6), implying a role for *DIP1* during AM colonization.

GRAS-domain proteins have already been shown to function during AM associations, with RAM1 playing a major role [6], and NSP1/NSP2 playing minor roles [7-8]. We found that Os*RAM1* can complement Medicago *ram1*, indicating that Os*RAM1* served the same function as Mt*RAM1* during mycorrhizal colonization (Supplementary information, Figure S7). Considering that we have identified two additional GRAS proteins functioning in the mycorrhizal association, we wished to ask whether these two proteins interacted with those previously defined mycorrhizal GRAS proteins. We found that DIP1, but not SLR1, interacted with OsRAM1 and MtRAM1 (Figure 1C, 1I). The interaction between DIP1 and OsRAM1 was validated by BiFC and the interaction occurred in the nucleus (Figure 1D). We further confirmed this interaction using pull-down assays (Figure 1J). Taken together, our results imply that a large complex of GRAS-domain proteins functions in the AM symbiosis with at least SLR1, DIP1 and RAM1 interacting together. As MtRAM1 has been shown to bind to the promoter of an AM-induced gene [6, 9], we propose that

Figure 1 DELLA protein complex is required for the mycorrhizal symbiosis. **(A)** Confocal images of *R. irregularis* colonization stained with wheat germ agglutinin (WGA)-conjugated Alexa Fluor 488 at 6 weeks post inoculation. Left, middle and right panels show normal arbuscule development in wild-type, no colonization in *slr1* and occasional colonization events in *slr1* mutant. Scale bars, 150 μm. **(B)** Internal root length colonization of *slr1* mutants at 6 weeks post inoculation with *R. irregularis*. The experiment was repeated three times and representative data is shown. Error bars show standard errors. **(C)** Interactions between SLR1 and DIP1 in a Y2H assay. **(D)** Protein-protein interactions in *N. benthamiana* leaves revealed by BiFC. YFP fluorescence of leaves co-transformed with *SLR1-YFPc* (C-terminal half of YFP) or Os*RAM1-YFPc* and *DIP1-YFPn* (N-terminal half of YFP). No YFP fluorescence in OsRAM1-YFPn/YFPc, OsRAM1-YFPn/SLR1-YFPc, DIP-YFPn/YFPc and YFPn/SLR1-YFPc. **(E)** The MBP-DIP1 fusion protein interacts with His-SLR1 in pull-down assays using heterologously expressed proteins in *E. coli*. **(F)** A time course (10-40 DPI) of relative expression of *DIP1* following *R. irregularis* inoculation. These experiments were replicated two times. Bars represent standard error. **(G)** Ink stained roots of rice reveal a greatly reduced level of infection in the *DIP1* RNAi lines at 6 weeks post inoculation. Scale bars, 150 μm. **(H)** Quantification of *R. irregularis* colonization levels in 8 independent *DIP1* rice RNAi lines at 6 weeks post inoculation (CK, empty vector control). **(I)** Interactions between DIP1, OsRAM1 and MtRAM1 are shown in a Y2H assay. **(J)** The MBP-DIP1 fusion protein can interact with His-OsRAM1 in pull-down assays with heterologously expressed proteins in *E. coli*. In **(C**, **I)**: BD, binding domain; AD, activation domain. -LT, SD/-Leu-Trp; -LTHA, SD/-Leu-Trp-His-Ade. 30 mM 3-AT (3-aminotriazole) was used to suppress protein autoactivation.

this GRAS-domain protein complex is directly associated with mycorrhizal gene expression.

The mycorrhizal symbiosis is extremely ancient and was found in the earliest land plants, suggesting that the role of *DELLAs* in the AM symbiosis is probably very ancient. A role for *SLR1* during the AM symbiosis provides a direct link between GA levels and AM colonization. The fact that *SLR1* is necessary for AM colonization explains why GA treatment inhibits AM infection: GA-induced degradation of SLR1 will limit AM signaling. Linking AM signaling with a component of GA signaling should allow direct hormonal regulation of AM colonization. Thus, GA, acting as a general signal during plant development, can regulate the AM association, presumably in accordance with the developmental status of the plant. DELLAs act as a node for crosstalk during nutrient responses, abiotic stress, light perception and sigaling of several interacting hormones including auxin, ethylene, abscisic acid and brassinosteroids. The colonization by AM is tightly regulated by the host in response to its local environment and our results suggest that this is at least partly achieved by integrating GA signaling and AM signaling through DELLAs, which function directly in AM colonization.

Detailed methods are described in the Supplementary information, Data S1.

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(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)