Binding of GID1 to DELLAs promotes dissociation of GAF1 from DELLA in GA dependent manner

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Gibberellins (GAs) are important phytohormones for plant growth and development. DELLA proteins play crucial roles in GA signaling. In this study, we investigate the relationship between non-proteolytic regulation of DELLA and GA signaling via DELLA-GAF1 complex using modified yeast two-hybrid system.

Gibberellins (GAs) promote seed germination, root growth, stem elongation, leaf expansion, flower induction and the development of flowers, fruits, and seeds.1,2 The endogenous levels of GAs are fine-tuned by feedback control at several steps in their metabolic pathway, including GA 20-oxidase and GA 3-oxidase.2,3 GA feedback regulation has been shown to depend on GA signaling components, including the GA receptors GA-INSENSITIVE DWARF1 (GID1), the F-box proteins SLEEPY1 (SLY1) and DELLA. Bioactive GAs are recognized by GID1, and the GID1-DELLA complexes are recognized and ubiquitinated by the SCFSLY complex. The sleepy1 (sly1) F-box mutant exhibits dwarfism and low-germination phenotypes due to high accumulation of DELLA. Overexpression of GID1 in the sly1 mutant partially rescues these phenotypes without degradation of DELLA suggesting that proteolysis is not the only mechanism of non-proteolytic regulation of DELLA.

Recently we identified a DELLA binding transcription factor, GAI-ASSOCIATED FACTOR1 (GAF1). GAF1 also interacts with co-repressor TOPLESS RELATED (TPR) in nuclei. DELLAs and TPR act as coactivator and corepressor of GAF1, respectively. GAs convert the GAF1 complex from transcriptional activator to repressor via degradation of DELLAs. The overexpression of ΔPAM, lacking of DELLA binding region of GAF1, partially rescue dwarf phenotypes of GA deficient or GA insensitive mutant. In this study, we investigate the relationship between non-proteolytic regulation of DELLA and GA signaling via DELLA-GAF1 complex using modified yeast two-hybrid system.

The titration of transcriptional activators by DELLA partly explains GA-dependent transcriptional activation and how plants integrate environmental stimuli and GA signals to optimize growth and development. However, genes encoding GA 20-oxidase and GA 3-oxidase are down regulated by GA via feedback regulation. Genome-wide analysis revealed that the effect of GA on gene expression is predominantly through repression.10,11 These observations cannot be explained by the conventional titration model. Thus other molecular mechanisms underlying GA-dependent transcriptional regulation must exist.

Recently we identified a DELLA binding transcription factor, designated GAI-ASSOCIATED FACTOR1 (GAF1) and revealed a new role of DELLAs as transcriptional coactivator. GAF1 also interacts with corepressor TOPLESS RELATED (TPR). DELLA and TPR act as coactivator and corepressor of GAF1, respectively (Fig. 1).12 The DELLA turn on or off two sets of GA-regulated genes by titration and co-activation, providing a novel mechanism for integrative regulation of plant growth and GA homeostasis. To investigate the biological role of DELLA binding to GAF1, we generated transgenic plants expressing a mutant version of GAF1 that cannot bind DELLA (ΔPAM) under the control
of the CaMV 35S promoter in the ga1–3, a GA deficient mutant or gai-1, a GA insensitive mutant. The overexpression of ΔPAM in ga1–3 or gai-1 partially rescue dwarf and low-germination phenotypes without affecting DELLA levels.12 ΔPAM protein acts as a constitutive repressor with TPR and promotes GA signaling (Fig. 1).

The sly1 mutant exhibited seed dormant, dwarf and infertile phenotypes associated with high level accumulation of DELLA due to lack of DELLA proteolysis.15,16 Although the amounts of accumulated DELLAs in sly1 mutant are higher than those of GA deficient mutant ga1–3 or GA insensitive gid1a gid1b gid1c triple mutant, sly1 exhibited less severe dwarf and low-germination phenotypes than ga1–3 or gid1a gid1b gid1c. Moreover GID1 overexpression can partially rescue the dwarf and low-germination phenotypes of sly1 mutant in a GA-dependent manner. This observation suggests the existence of non-proteolytic repression of DELLA that is hidden in the wild-type genetic background because DELLA are rapidly degraded in response to GAs. How do GAs promote GA signaling without degradation of DELLAs in sly1? One possible mechanism for the non-proteolytic repression of DELLA is that GAs promote dissociation of the complex between DELLA and transcription factors, including GAF1. The dissociated GAF1 from DELLAs could form a repressor complex with TPR and promote GA signaling.

To examine the effect of GA-GID1 binding to DELLA for the interaction between DELLA and GAF1, we carried out modified yeast two-hybrid assay. As expected, the binding of GID1 to GAL4AD-DELLA reduced the interaction between GAL4AD-DELLA and GAL4BD-GAF1 in a GA-dependent manner (Fig. 2). Every AtGID1-DELLAs interaction was detected in a GA-dependent manner, although the interaction of AtGID1b with some DELLAs was weakly detected without GA in yeast.15,16 We could not detect the reduction of β-galactosidase activity in the absence of GAs in yeast.15,16 It indicated that the dissociation of GAF1 from DELLAs by the binding of GID1 to DELLA motif in yeast, we used GAL4 activation domain fused to DELLAs as prey protein. GAF1 interact with DELLAs, GAI and RGA, in yeast. The binding of GID1 promote the dissociation of GAF1 from DELLA in presence GA condition. Data are means±SD, n = 3 “vec” indicates empty vector using as negative control. Asterisks represent Student’s t test significance compared with BD-GAF1 (*P < 0.05).
Thus, these observations suggest that GA-GID1 promote GA signaling through the dissociation of GAF1 from DELLAs without degradation of DELLAs in sly1 genetic background (Fig. 3). This hypothesis is consistent with the previous observation that the overexpression of GID1 suppresses the phenotypes of sly1 in a GA dependent manner. The SAW domain of DELLAs is also necessary for the interaction with BZR1. Further analysis of the effect of GA-GID1 binding for the interaction between DELLAs and other interaction proteins provides new insight for the mechanism of non-proteolytic regulation of DELLA.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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