論 文 の 内 容 の 要 旨

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論文題目: **Studies on Arabidopsis heterotrimeric G protein β subunit (AGB1) and its interaction partners**
（シロイヌナズナのヘテロ三量体 G タンパク質βサブユニット（AGB1）及びその相互作用因子に関する研究）

Heterotrimetic G proteins (consisting of subunits Gα, Gβ, and Gγ) are signaling molecules widely found in eukaryotic organisms. In plants, G proteins play roles in signal transduction of phytohormones such as auxin and abscisic acid (ABA). Molecular mechanisms of G protein-mediated signaling have been well characterized in animals, but the numbers and kinds of components of G protein-mediated signaling are different between animals and plants, raising the possibility that plants have plant-specific mechanisms for G protein-mediated signaling. Here, studies on *Arabidopsis thaliana* Gβ (AGB1) and its interaction partners are described. The AGB1-interaction partners focused on are: (1) signal transduction of a phytohormone, brassinosteroid (BR); (2) VIP1, a bZIP protein which physically interacts with AGB1.

1. AGB1 regulates brassinosterid signaling independently of BZR1

An AGB1-null mutant, *agb1-2*, is known to be hyposensitive to BR in seed germination. Moreover, both *agb1-2* and another AGB1-null mutant, *agb1-1*, have

the phenotypes similar to the phenotypes of mutants that have defects in BR signaling (e.g. rounder leaves and higher ABA sensitivities). An established model of BR signaling consists of specific types of protein kinases, protein phosphatases, and transcription factors, but it is still unclear whether AGB1 interacts with those components of BR signaling.

A key transcription factor downstream of BR, BZR1, and its gain-of-function mutant, bzr1-1, were overexpressed in an AGB1-null mutant, *agb1-1*, to examine their effects on the *agb1* phenotypes, and to examine whether AGB1 regulates the functions of BZR1. Because the amino acid sequence of AGB1 contains 17 putative modification motifs of Glycogen synthase kinase 3/SHAGGY-like protein kinases (GSKs), which phosphorylate BZR1 and thereby regulate BR signaling, the interaction between AGB1 and one of Arabidopsis GSKs, BIN2, was examined.

Expression of bzr1-1 alleviated the inhibitory effects of a BR biosynthesis inhibitor, brassinazole, on hypocotyls elongation in both wild type (WT) and *agb1-1*. Overexpression of BZR1 alleviated the inhibitory effects of ABA on cotyledon greening in both WT and *agb1-1*. AGB1 interacted with BIN2 in vitro. However, AGB1 did not affect the phosphorylation state of BIN2, and was not phosphorylated by BIN2 in vitro. AGB1 did not affect the phosphorylation state of BZR1 in vivo, either. These results suggest that AGB1 interacts with BIN2, but regulates BR signaling in a BZR1-independent manner.

2. Studies on an AGB1-interacting protein, VIP1

2.1. VIP1 is a regulator of osmosensory signaling

A yeast two-hybrid (Y2H) screen using the Arabidopsis leaf cDNA library identified a bZIP protein, VIP1, as a potential AGB1-interacting protein. During an investigation of the subcellular localization of VIP1, it was discovered that the nuclear localization of VIP1 is rapidly enhanced in response to rehydration. This interesting finding tempted the author to examine the interaction between VIP1 and two ABA-inactivating genes, *CYP707A1* and *CYP707A3* (*CYP707A1/3*), which are known to be induced by rehydration.

The subcellular localization of VIP1 was examined by expressing GFP-fused VIP1 (VIP1-GFP) in Arabidopsis. The interactions between VIP1 and

CYP707A1/3 promoters were examined by a transient reporter assay, gel shift assays, and chromatin immunoprecipitation (ChIP). The expressions of *CYP707A1/3* in a VIP1-null mutant, *vip1*, and VIP1-GFP-expressing plants were also examined.

VIP1-GFP signals in the nucleus were enhanced within 10 minutes, and then gradually weakened when plants were submerged in a hypotonic solution. The time course of the changes of the subcellular localization of VIP1-GFP was consistent with the time course of *CYP707A1/3* inductions during rehydration. The transient reporter assay, the gel shift assays, and the ChIP assay all showed that VIP1 interacts with the *CYP707A1/3* promoters, supporting the idea that VIP1 regulates the *CYP707A1/3* expressions in osmosensory signaling. The gel shift assays suggested that AGCTGT/G are at least partially responsible for the interaction between VIP1 and the *CYP707A1/3* promoters. The expression level of *CYP707A1* was lowest in *vip1* and highest in VIP1-GFP-expressing plants, but no clear difference was observed in the expression level of *CYP707A3* among the genotypes studied, raising the possibility that VIP1 and its close homologs are functionally redundant.

2.2. VIP1 interacts with AGB1

The interaction between AGB1 and VIP1 was further examined by Y2H assays, an in vitro GST pull-down assay, and bimolecular fluorescence complementation (BiFC). Because VIP1 was suggested to play a role in osmosensory signaling, effects of extracellular osmolarities on the AGB1-VIP1 complex were examined by a BiFC assay using onion epidermal cells. VIP1-GFP was expressed in *agb1-1* to examine the effects of AGB1 on the functions of VIP1.

In the Y2H assays and the GST pull-down assay, both full-length VIP1 and the C terminal region (amino acids 165-341) of VIP1 interacted with AGB1. BiFC signals were speckled when transformed cells were incubated in distilled water, but not when cells were incubated in 0.5 M mannitol, suggesting that extracellular osmolarities regulate the subcellular localization and/or formation of the AGB1-VIP1 complex. However, no clear difference was observed between WT and *agb1-1* in either the time course of the nuclear-cytoplasmic shuttling of VIP1 or the expression levels of *CYP707A1/3* and another VIP1 target gene, *MYB44*. Expression of VIP1-GFP did not significantly affect the ABA hypersensitivity of

agb1-1, either. Thus, although AGB1 and VIP1 physically interact, the physiological relevance of the interaction between AGB1 and VIP1 is still unclear.

2.3. Further studies on VIP1

Further studies on VIP1 were performed to test the possibilities that (1) rehydration triggers changes in the modification pattern and/or the conformation of VIP1, and that (2) VIP1 and its close homologs are functionally redundant.

Because phosphorylation is a possible modification of VIP1, the phosphorylation state of VIP1 during rehydration was examined using Phos-tag SDS-PAGE. This experiment suggested that VIP1 is dephosphorylated when its nuclear localization is enhanced upon rehydration. Okadaic acid, an inhibitor of type 1 and type 2A protein phosphatases (PP1 and PP2A, respectively), inhibited the rehydration-induced enhancement of the nuclear localization of VIP1. Components of Arabidopsis PP1 and PP2A were subjected to a Y2H analyses, and ATB'κ, one of the B' subunits of PP2A, was found to interact with VIP1. These results strongly support the possibility (1), namely the idea that phosphorylation is involved in regulating the subcellular localization of VIP1. It is also possible that factors other than phosphorylation regulate the subcellular localization of VIP1.

Quantitative RT-PCR showed that some of the genes encoding close homologs of VIP1 (plant group I bZIP proteins) are expressed as highly as *VIP1*. Two of the group I bZIP proteins, PosF21 and bZIP29, were expressed as GFP-fused proteins in Arabidopsis, and both showed the rehydration-responsive nuclear-cytoplasmic shuttling as VIP1 showed. Y2H and BiFC experiments suggested that VIP1 can form a homodimer and heterodimers with other bZIP proteins. Together, these results support the possibility (2).

Here the relation between AGB1 and BR signaling has been addressed in more details, and VIP1 has been identified as a novel regulator of osmosensory signaling. However, many points remain unclear. Further studies are required to elucidate the molecular mechanisms underlying the AGB1-mediated signaling pathway and the VIP1-mediated signaling pathway, and to determine how the two signaling pathways interact with each other.