

AtAGP18, a lysine-rich arabinogalactan protein in *Arabidopsis thaliana*, functions in plant growth and development as a putative co-receptor for signal transduction

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Abbreviations: AGPs, arabinogalactan-proteins; Caspr, contactin-associated protein; FGF, fibroblast growth factor; GFP, green fluorescent protein; GPI, glycosylphosphatidylinositol; Hpt, histidine phosphotransfer protein

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Arabinogalactan-proteins (AGPs) are a class of hyperglycosylated, hydroxyproline-rich glycoproteins that are widely distributed in the plant kingdom. *AtAGP17*, *18* and *19* are homologous genes encoding three classical lysine-rich AGPs in *Arabidopsis*. We observed subcellular localization of AtAGP18 at the plasma membrane by expressing a translational fusion gene construction of AtAGP18 attached to a green fluorescent protein (GFP) tag in *Arabidopsis* plants. We also overexpressed AtAGP18 without the GFP tag in *Arabidopsis* plants, and the resulting transgenic plants had a short, bushy phenotype. Here we discuss putative roles of AtAGP18 as a glycosylphosphatidylinositol (GPI)-anchored protein involved in a signal transduction pathway regulating plant growth and development.

Arabinogalactan-proteins (AGPs) are plant cell surface glycoproteins or proteoglycans which are thought to play important roles in various aspects of plant growth and development, such as somatic embryogenesis, cell proliferation and elongation, pattern formation and hormone signaling.¹ The lysine-rich classical AGP subfamily in *Arabidopsis* contains three members: AtAGP17, 18 and 19. The subcellular localization of AtAGP17 and AtAGP18 was previously studied in our laboratory by expressing GFP-AtAGP17/18 fusion proteins in tobacco cell cultures.^{2,3} In a recent report, we used *Arabidopsis* plants to overexpress GFP-AtAGP17/18/19 fusion proteins to observe subcellular localization of

the lysine-rich AGPs in planta, in contrast to our previous plant cell culture work.⁴ Moreover, the lysine-rich AGPs alone (i.e., AtAGP17/18/19 without the GFP tag) were overexpressed in *Arabidopsis* plants, and only AtAGP18 overexpressors had a distinctive phenotype. This phenotype included shorter stems, more branches and less seeds, indicating a role for AtAGP18 in plant growth and development.⁴ In this addendum, we further discuss the putative biological role of AtAGP18 on a molecular level and its possible mode of action in cellular signaling.

Classical AGPs are frequently predicted to have a glycosylphosphatidylinositol (GPI) anchor, which would allow for the localization of such AGPs to the outer surface of the plasma membrane. Biochemical analyses were carried out to support this hypothesis in tobacco, pear,⁵ rose⁶ and *Arabidopsis*.⁷ The lysine-rich classical AGPs, AtAGP17 and 18, were predicted to have a GPI anchor.⁸ To test this idea, tobacco cell cultures expressing GFP-AtAGP17/18 fusion proteins were plasmolyzed and GFP fluorescence was observed on the plasma membrane.^{2,3} To corroborate this finding in planta, GFP-AtAGP17/18 were expressed in *Arabidopsis* plants and leaf trichome cells were plasmolyzed. Enhanced GFP fluorescence was observed at the plasma membrane of these transgenic trichome cells, indicating the presence of GFP-AtAGP17/18 at the plasma membrane.⁴ The localization of these lysine-rich classical AGPs at the plasma membrane suggests possible biological roles in sensing

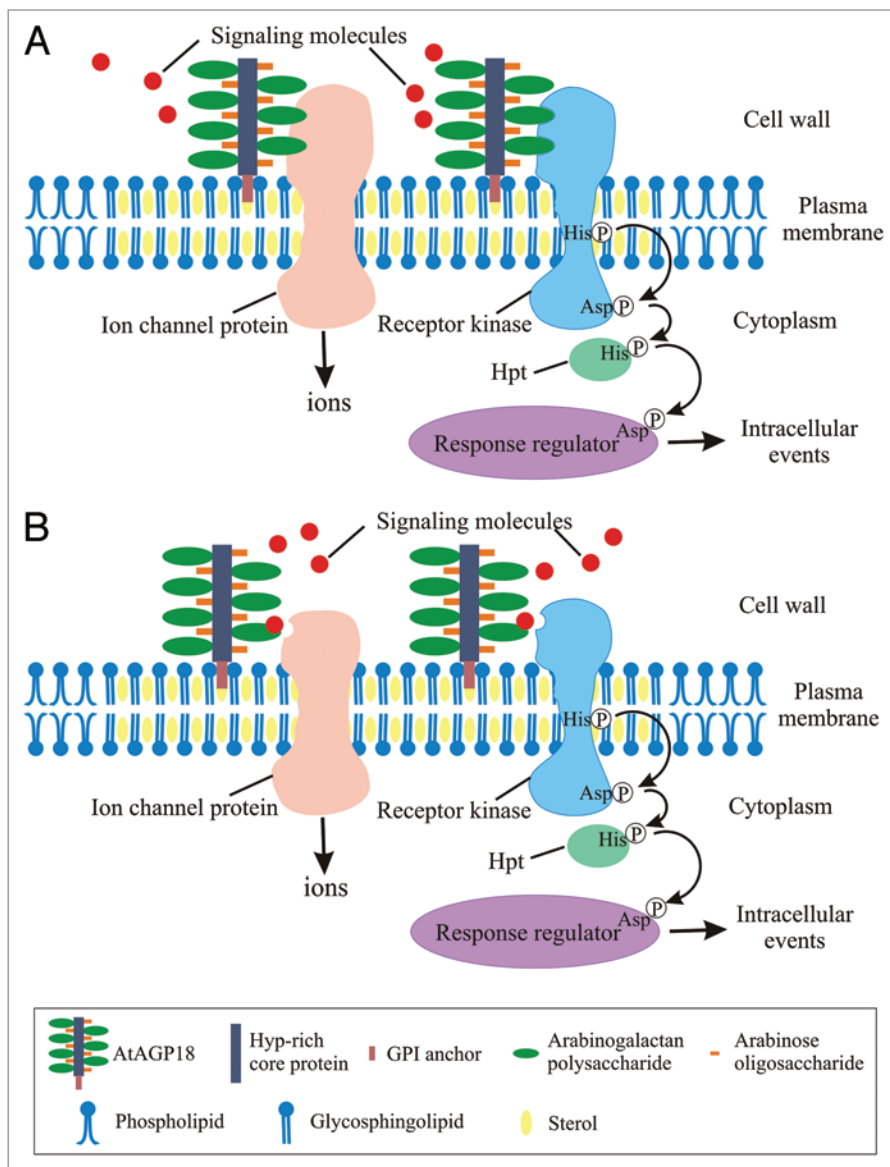


Figure 1. Model for AtAGP18 functioning in cellular signaling to control plant growth and development. In this model, lipid rafts are enriched in glycosphingolipids, sterols, transmembrane proteins (such as receptors, receptor kinases and ion channel proteins) and GPI-anchored proteins including AtAGP18. (A) AtAGP18 acts as a co-receptor by binding to signaling molecules and directly interacting with transmembrane proteins in the lipid rafts. (B) AtAGP18 acts as a co-receptor by binding to signaling molecules and bringing the signaling molecules to transmembrane proteins in the lipid rafts. Upon activation by the extracellular signals, the transmembrane proteins initiate signaling and lead to various intracellular events (e.g., phosphorylation similar to the two-component signaling system, influx of calcium ions). The different components of the AtAGP18 molecule and the various lipid components of lipid rafts and plasma membrane are shown in the boxed inset. Hpt, histidine phosphotransfer protein.

extracellular signals. They are likely associated with lipid rafts involved in cell signaling for the following reasons. In plants as well as animals, there are sterol-enriched, detergent-resistant plasma membrane microdomains called lipid rafts. Lipid rafts are known to be involved in signal transduction and are enriched

in transmembrane receptors and GPI-anchored proteins, including AGPs.⁹⁻¹¹ The accumulation of these proteins in such microdomains may allow for interactions between these proteins in sensing extracellular signals which lead to various intracellular events. Interestingly, a recent study shows that lipid rafts from hybrid

aspens cells contain callose synthase and cellulose synthase, and these enzymes are active since in vitro polysaccharide synthesis by the isolated detergent-resistant membranes was observed. These results demonstrate that lipid rafts are involved in cell wall polysaccharide biosynthesis.¹² In addition, an Arabidopsis *pnt* mutant study shows GPI-anchored proteins are required in cell wall synthesis and morphogenesis.¹³ These observations, coupled with previous observations that cellulose synthases as well as AGPs interact with microtubules, suggest that AGPs in lipid rafts may have a role in signal events, including those regulating cellulose and/or callose biosynthesis or deposition.^{14,15}

To examine the role of LeAGP-1, a lysine-rich AGP in tomato, transgenic tomato plants were produced which expressed GFP-LeAGP-1 under the control of the cauliflower mosaic virus 35S promoter.¹⁶ The tomato LeAGP-1 overexpressors and Arabidopsis AtAGP18 overexpressors both have a bushy phenotype similar to transgenic tobacco plants overproducing cytokinins.^{4,16,17} Cytokinins are an important class of plant hormones involved in many plant growth and development processes, such as cell growth and division, differentiation and other physiological processes.¹⁸ Therefore, Sun et al. proposed that LeAGP-1 might function in concert with the cytokinin signal transduction pathway.¹⁶ Since the overexpression phenotypes of AtAGP18 are similar to those of LeAGP-1, AtAGP18 is also likely associated with the cytokinin signal transduction pathway. The prevailing model for cytokinin signaling in Arabidopsis is similar to the two-component system in bacteria and yeast. In this model, the cytokinin receptor contains an extracellular domain, a kinase domain and a receiver domain. When the cytokinin receptor senses cytokinin signals, it auto-phosphorylates at a His residue in the kinase domain. The phosphoryl group is then transferred to an Asp residue in the receiver domain. Subsequently, the phosphoryl group is transferred to a His residue in the histidine phosphotransfer protein (Hpt) and the Hpt translocates to the nucleus and transfers the phosphoryl group to an Asp residue in a downstream response regulator to activate it.¹⁹ This model is consistent

with our hypothesis since the cytokinin receptor in this model is a receptor kinase located in the plasma membrane with an extra-cellular domain that can potentially interact with AtAGP18. AtAGP18 may function as a co-receptor that first binds to cytokinins, then either directly interacts with cytokinin receptors or brings the cytokinins to cytokinin receptors in the plasma membrane. The first scenario is analogous to the interaction of contactin and contactin-associated protein (Caspr) in neurons. In this model, contactin is a GPI-anchored protein on the cell surface that binds to signal molecules and interacts with the transmembrane receptor Caspr to transmit signals to the cell interior.²⁰ The second scenario is analogous to fibroblast growth factor (FGF) signal activation in which heparan sulfate proteoglycans bind to FGF molecules and bring them to the FGF receptor.²¹

Based on all the above observations and findings, a hypothetical model for AtAGP18 function is proposed in **Figure 1**. The model shows AtAGP18 located on the outer surface of the plasma membrane in lipid rafts where it could act as a co-receptor to sense extracellular signals (such as cytokinin) and interact with transmembrane proteins, possibly receptor kinases or ion channels, in the lipid rafts to initiate signaling by triggering various intracellular events. Interestingly, receptor tyrosine kinases and ion channels are known to be present in lipid rafts.^{9,22} Moreover, AGPs are likely associated with ion channels since addition of the AGP-binding reagent Yariv phenylglycoside resulted in elevated cytoplasmic calcium concentrations in tobacco cells and lily pollen tubes.^{15,23,24}

Clearly, additional work will be required to verify such a model, and to better understand how AtAGP18 might sense extracellular signals and interact with the transmembrane proteins in the lipid rafts.

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