[Plant Signaling & Behavior 2:1, e1-e3, EPUB Ahead of Print: http://www.landesbioscience.com/journals/psb/abstract.php?id=3599; January/February 2007]; ©2007 Landes Bioscience

Addendum

A Cellular Networking Model Involving Interactions Among Glycosyl-phosphatidylinositol (GPI)-Anchored Plasma Membrane Arabinogalactan Proteins (AGPs), Microtubules and F-actin in Tobacco BY-2 Cells

Harjinder Singh Sardar

Allan M. Showalter*

Department of Environmental and Plant Biology and Molecular and Cellular Biology Program; Ohio University; Athens, Ohio USA

*Correspondence to: Allan M. Showalter; Department of Environmental and Plant Biology; Ohio University; Athens, Ohio 45701-2979 USA; Tel.: 740.593.1135; Fax: 740.593.1130; Email: showalte@ohio.edu

Original manuscript submitted: 11/14/06
Manuscript accepted: 11/14/06

This manuscript has been published online, prior to printing for Plant Signaling & Behavior, Volume 2, Issue 1. Definitive page numbers have not been assigned. The current citation is: Plant Signaling & Behavior 2007; 2(1):
http://www.landesbioscience.com/journals/psb/abstract.php?id=3599
Once the issue is complete and page numbers have been assigned, the citation will change accordingly.

KEY WORDS

Glycosylphosphatidylinositol (GPI), Arabinogalactan proteins (AGP), β-Yariv reagent, amiprophosmethyl (APM), cytochalasin-D, GFP-MBD (microtubule binding domain), GFP-LeAGP-1 (*Lycopersicum esculentum*), rhodamine phalloidin, Hechtian strands, terminal cell bulging

Addendum to:

Molecular Interactions of Arabinogalactan-proteins (AGPs) with Cortical Microtubules and F-actin in Bright Yellow-2 (BY-2) Tobacco Cultured Cells

Harjinder Singh Sardar, Jie Yang, and Allan M. Showalter

Plant Physiol 2006; 142:1469-1479.

ABSTRACT

Arabinogalactan-proteins (AGPs) are perhaps the most abundantly expressed set of proteins at the plant cell surface and play probable roles in cellular architecture and signaling. Although considerable progress has been made to understand the role of AGPs in plant growth and development, their exact functional roles and the molecular mechanisms underlying their interactions with either intra- or extra-cellular molecules are unknown. These unknown interactions were addressed in a recent research article in Plant Physiology. This study reported molecular interactions between AGPs and the cytoskeleton [microtubules, (MTs) and F-actin] in tobacco BY-2 cells. Here in this addendum, a summary of this recent publication and additional perspectives are presented. As reported, perturbation studies were conducted in tobacco BY-2 cells to analyze the effects of an AGP inhibitor (β-Yariv reagent) on the organization of microtubules [labeled by GFP-MBD (green fluorescent protein-microtubule binding domain)] and F-actin (labeled by rhodamine-phalloidin) and conversely to analyze the effects of a microtubule inhibitor (amiprophosmethyl) and an F-actin inhibitor (cytochalasin-D) on the localization of GPI-anchored GFP-LeAGP-1. These studies implicate a role for GPI-anchored LeAGP-1 in mediating a cell wall-plasma membrane-cytoskeleton connection.

Despite the immense progress in understanding plant cell architecture, it remains unclear which molecules/structures/components connect the cell exterior to the interior.[1-6] A recent report in Plant Physiology is a step forward in understanding these connections at the molecular level and more specifically the role of a GPI-anchored tomato AGP (LeAGP-1) as a linker connecting the cell wall and the cytoskeleton (microtubules and F-actin).[7] These interactions were investigated in tobacco BY-2 cells by employing fluorescent probes such as GFP-MBD, rhodamine phalloidin and GFP-LeAGP-1. Initially, the localization of GFP-LeAGP-1 was determined in untreated BY-2 cells and its localization was compared after treatment with microtubule and F-actin inhibitors amiprophosmethyl (APM) and cytochalasin-D, respectively.[7] These inhibitors disrupt the uniform distribution of GFP-LeAGP-1 on the cell wall, plasma membrane and Hechtian strands.[7] Conversely, β-Yariv reagent treatment effectively disrupts the distribution and organization of cortical microtubules and F-actin.[7] β-Yariv treatment also results in defects in cell morphology such as terminal cell bulging,[7] and this phenotype partially phenocopies a previously reported *Arabidopsis* reb-1 (root epidermal bulger) mutant that shows a reduction in the total amount of AGPs.[8] Based on these results, a hypothetical cell surface networking model was presented involving direct or indirect interactions among GPI-anchored AGPs, cortical microtubules and F-actin.

In this model, a few possible modes of interactions between the cell surface AGPs and the cytoskeleton were suggested including: (1) direct interactions via a transmembrane protein, (2) indirect interactions via the lipid rafts/detergent resistant membranes, and (3) a mass action involving β-Yariv-induced disruption of the distribution of cell surface AGPs and various other wall/membrane components.[7] As reported earlier, a number of potential candidates such as wall associated kinases (WAKs), formins, cellulose synthases, endo-1-4-β-D-glucanases, proline-rich extensin-like receptor kinases (PERKs), phospholipase-D, and lectin receptor kinases (LecRKs)[2,5,6,9,10] may directly interact with AGPs. Formins include an extracellular extensin-like domain and actin binding domains making them one of the most suitable candidates for association with cell surface AGPs.[11-13] Apart from transmembrane proteins discussed in the recent manuscript, other proteins such

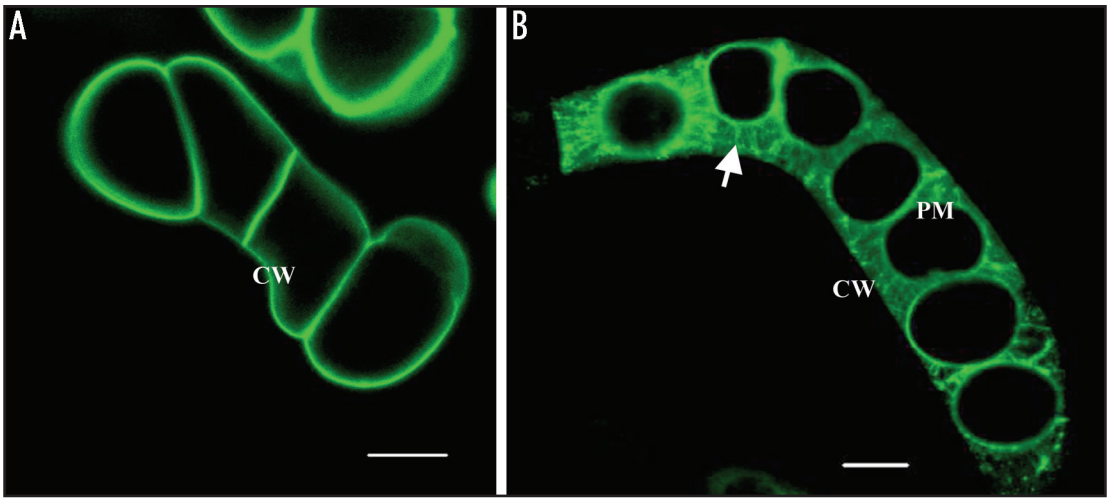

Figure 1. Confocal laser scanning microscope (CLSM) images showing transgenic tobacco BY-2 cells with uniform distribution of GFP-LeAGP-1 on the cell surface, plasma membrane and Hechtian strands. (A) Transgenic BY-2 cells expressing GFP-LeAGP-1 on cell wall (CW). (B) Transgenic BY-2 cells were salt plasmolysed (4% NaCl) for 15 min to demonstrate the relocalization of cell surface GPI-anchored LeAGP-1 on plasma membrane (PM) and Hechtian strands (arrow). (Scale bars = 20 μm).

as callose synthases and class VIII myosin can also potentiate these direct interactions.[5] GPI-anchored AGPs may structurally interact with transmembrane callose synthases that are found at sites of active callose deposition. Previous studies suggest that myosin VIII linked to F-actin at the plasma membrane interface is enriched at sites of callose deposition.[5,14,15] Thus any changes in the distribution or structure of AGPs can produce downstream effects in the F-actin organization via the callose synthase-myosin VIII connections. Another emerging link between the extracellular AGPs and the cytoskeleton is via the lipid rafts/detergent resistant membranes. The link is interesting because it relates to the fact that lipid rafts can act as centers of signaling cascades. In animals, various studies have implicated roles of lipid rafts in signaling processes.[16-19] In both animals and plants, GPI-anchored proteins have emerged as efficient markers for lipid rafts because of their high affinity for the lipid microdomains.[20-23] These lipid microdomains/detergent resistant membranes in plants are enriched with sterols such as stigmasterol, campesterol, and β-sitosterol and also include a GPI-anchored AGP, namely AtAGP4.[23] It remains to be demonstrated whether all GPI-anchored AGPs are localized to these lipid microdomains and play significant roles in signaling. Nevertheless, these rafts can potentially transduce signals via the GPI-anchored AGPs to the cytoskeleton, thereby controlling its organization. Recently, an animal lipid raft associated protein, CLIPR-59, was shown to affect microtubule dynamics by reducing the elongation of microtubules.[24] Also, other proteins such as RhoA and endostatin have been shown to associate with lipid rafts and play a role in the regulation of the actin cytoskeleton.[25-27] These studies indicate that in plants, lipid rafts may potentially perceive signals via some cell surface receptors (GPI-anchored AGPs) and transduce them downstream to the cytoskeleton via various signaling molecules.

References

1. Wyatt SE, Carpita NC. The plant cytoskeleton-cell-wall continuum. Trends Cell Biol 1993; 3:413-7.
2. Kohorn BD. Plasma membrane-cell wall contacts. Plant Physiol 2000; 124:31-8.
3. Darley CP, Forrester AM, McQueen-Mason SJ. The molecular basis of plant cell wall extension. Plant Mol Biol 2001; 47:179-95.
4. Martin C, Bhatt K, Baumann K. Shaping in plant cells. Curr Opin Plant Biol 2001; 4:540-9.
5. Baluska F, Samaj J, Wojtaszek P, Volkmann D, Menzel D. Cytoskeleton-plasma membrane-cell wall continuum in plants. Emerging links revisited. Plant Physiol 2003; 133:482-91.
6. Gouget A, Senchou V, Govers F, Sanson A, Barre A, Rougé P, Pont-Lezica R, Canut H. Lectin receptor kinases participate in protein-protein interactions to mediate plasma membrane-cell wall adhesions in *Arabidopsis*. Plant Physiol 2006; 140:81-90.
7. Sardar HS, Yang J, Showalter AM. Molecular interactions of arabinogalactan-proteins (AGPs) with cortical microtubules and F-actin in bright yellow-2 (BY-2) tobacco cultured cells. Plant Physiol 2006; 142:1469--79.
8. Andème-Onzighi C, Sivaguru M, Judy-March J, Baskin TI, Driouich A. The *reb-1* mutation of *Arabidopsis* alters the morphology of trichoblast. The expression of arabinogalactan-proteins and the organization of cortical microtubules. Planta 2002; 215:949-58.
9. Kohorn BD. WAKs; cell wall associated kinases. Curr Opin Cell Biol 2001; 13:529-33.
10. Nakhamchik A, Zhao Z, Provart NJ, Shiu SH, Keatley SK, Cameron RK, Goring DR. A comprehensive expression analysis of the *Arabidopsis* proline-rich extensin-like receptor kinase gene family using bioinformatic and experimental approaches. Plant Cell Physiol 2004; 45:1875-81.
11. Keller B. Structural cell wall proteins. Plant Physiol 1993; 101:1127-30.
12. Cvrckova F. Are plant formins integral membrane proteins? Genome Biol 2000; 1:001.1-001.7.
13. Deeks MJ, Hussey PJ, Davies B. Formins: Intermediates in signal transduction cascades that affect cytoskeletal reorganization. Trends Plant Sci 2002; 7:492-8.
14. Baluska F, Barlow PW, Volkmann D. Actin and myosin VIII in developing root cells. In: Staiger CJ, Baluska F, Volkmann D, Barlow PW, eds. Actin: A Dynamic Framework for Multiple Plant Cell Functions. Dordrecht, The Netherlands: Kluwer Academic Publishers, 2000:457-76.
15. Reichelt S, Knight AE, Hodge TP, Baluska F, Samaj J, Volkmann D, Kendrick-Jones J. Characterization of the unconventional myosin VIII in plant cells and its localization at the post-cytokinetic cell wall. Plant J 1999; 19:555-69.
16. Ikonen E. Roles of lipid rafts in membrane transport. Curr Opin Cell Biol 2001; 13:470-7.
17. Pike LJ. Lipid rafts: Heterogeneity on the high seas. Biochem J 2004; 378:281-92.
18. Simons K, Toomre D. Lipid rafts and signal transduction. Nat Rev Mol Cell Biol 2000; 1:31-9.
19. Zajchowski LD, Robbins SM. Lipid rafts and little caves: Compartmentalized signalling in membrane microdomains. Eur J Biochem 2002; 269:737-52.
20. De Angelis DA, Miesenbock G, Zemelman BV, Rothman JE. **PRIM: Proximity imaging of** green fluorescent protein-tagged polypeptides. Proc Natl Acad Sci USA 1998; 95:12312-6.
21. Mayor S, Sabharanjak S, Maxfield FR. Cholesterol-dependent retention of GPI-anchored proteins in endosomes. EMBO J 1998; 17:4626-38.
22. Nichols BJ, Kenworthy AK, Polishchuk RS, Lodge R, Roberts TH, Hirschberg K, Phair RD, Lippincott-Schwartz J. Rapid cycling of lipid raft markers between the cell surface and Golgi complex. J Cell Biol 2001; 153:529-41.
23. Borner GHH, Sherrier DJ, Weimar T, Michaelson LV, Hawkins ND, MacAskill A, Napier JA, Beale MH, Lilley KS, Dupree P. Analysis of detergent-resistant membranes in *Arabidopsis*. Evidence for plasma membrane lipid rafts. Plant Physiol 2005; 137:104-16.
24. Lallemand-Breitenbach V, Quesnoit M, Braun V, El Marjou A, Poüs C, Goud B, Perez F. CLIPR-59 is a lipid raft-associated protein containing a cytoskeleton-associated protein glycine-rich domain (CAP-Gly) that perturbs microtubule dynamics. J Biol Chem 2004; 279:41168-78.
25. Nobes CD, Hall A. Rho, Rac and Cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia and filopodia. Cell 1995; 81:53-62.

26. Giancotti FG, Ruoslahti E. Integrin signaling. Science 1999; 285:1028-32.
27. Wickström SA, Alitalo K, Keski-Oja J. Endostatin associates with lipid rafts and induces reorganization of the actin cytoskeleton via down-regulation of RhoA activity. J Biol Chem 2003; 278:37895-901.