Final Report

The Arabidopsis Wave Complex: Mechanisms of Localized Actin Polymerization and Growth

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Executive summary

Plant cells are complex energy storage systems that convert solar radiation to reduced carbon. The chemical form of the storage molecule varies depending on the species and cell type, but sucrose, fructans, starch, or lipids are common examples. The compartmentation of metabolic activities within specific organelles and the intracellular transport and fusion of specialized compartments are key components to energy storage. In the context of cellular engineering, it is critical to gain a deep understanding of how metabolic activities are integrated with the organelles and inter-organelle transport. In plant cells, actin filaments are highly dynamic structural elements that define the overall architecture of the cytoplasm and organelle transport activities in the cell. Stable actin bundles are critical architectural elements that position organelles and serve as tracks for motor-dependent organelle trafficking. Subsets of dynamic actin do work by generating membrane tension or by driving organelle fusion, and organelle motility. However, in plants we know very little about when and how particular actin filament arrays are generated and their cellular function. The lack of knowledge impedes efforts to use protein assemblies to dictate the trafficking and growth behaviors of cells. The objective of this project was to discover the protein complexes and control mechanisms that determine the location of actin filament roadways in plant cells. Our work provided the first molecular description of protein complexes that are converted from inactive complexes to active actin filament nucleators in the cell. These discoveries provided a conceptual framework to control the roadways in plant cells that determine the location and delivery of plant metabolites and storage molecules that are relevant to the bioenergy economy.

Project Goals, Accomplishments, and Summary of Project Activities

2001-2004

Goal 1: To identify the precise cellular defects of *dis3* trichomes. Our working hypothesis is that long-distance actin-based transport is altered during trichome branch elongation in *dis3* trichomes.

DISTORTED3 (DIS3) proved to be difficult to clone because there were multiple linked mutations in our original mutant line. In the meantime we cloned 3 other genes using DOE support. These encoded ACTIN-RELATED PROTEIN 2/3 complex subunits ARP2, ARP3, and ARPC2. The phenotype of these mutants was similar to *dis3*, but more severe. In 2005, we cloned *DIS3*, and showed in our 2005 Plant Cell paper, that *DIS3* corresponded to the Scar/WAVE-like gene *SCAR2*. We showed that *SCAR2* is a direct positive regulator of ARP2/3 and converts it into a potent actin filament nucleator. The weaker *scar2* phenotype compared to other *arp2/3* and *wave* mutants suggested that

additional SCAR genes functioned redundantly, and this was addressed in later research objectives.

Goal 2: To determine the function of DIS3 during polarized trichome expansion. Our working hypothesis is that DIS3 is a scaffolding protein that modulates long-distance Golgi stack transport.

Our analyses of distorted mutants focused on the control of the actin network assembly in trichomes because that phenotype was most directly linked to the identity of the DIS3/SCAR2 and ARP2/3 complex gene products. We developed a series of novel methods to quantitate actin architecture as a function of cell shape. This allowed us to conclude that the ARP2/3 pathway is used to organize actin bundle networks at whole-cell spatial scales.

Goal 3: To test for the requirement for DIS3 function in additional cell types.

In a series of publications we demonstrated that DIS3 and ARP2/3 genes are important throughout the plant. Specifically, these proteins control epidermal pavement cell growth, the ability of epidermal cells to adhere to each other normally, and the elongation of the hypocotyl.

2005-2009

Goal 1: To determine if Arabidopsis WAVE-like proteins function redundantly in an ARP2/3-dependent growth pathway.

The weaker *scar2* phenotype compared to other *arp2/3* and *wave/scar complex* mutants suggested that additional SCAR genes functioned redundantly. In this project we determined that 3 other SCAR genes functioned within the ARP2/3 activation pathway. We are able to demonstrate that the relative importance of the different SCAR proteins was correlated with the efficiency with which they activated ARP2/3 and actin filament nucleation in vivo and their mRNA expression levels. Molecular genetic data proved that the proteins could function interchangeably when overexpressed. This was important because it clearly showed that SCAR proteins do not have isoform specific activity that generates specialized SCAR complexes.

Goal 2: To determine if a five subunit Arabidopsis WAVE complex assembles and regulates polarized growth.

The purpose of this aim is to directly test for the presence of a SCAR2-containing WAVE complex in vivo and to determine which WAVE complex proteins regulate the stability of SCAR2. We have generated an antibody against SCAR2 that allows us determine that BRK1, but not the WAVE complex proteins SRA1 and NAP1, are required for SCAR2 protein stability (Le et al., 2006). We also have successfully used the yeast 2-hybrid assay and purified SCAR/WAVE complex proteins to detect direct interactions among protein complex subunits and to show that complex assembly mechanisms in plants were similar to those of vertebrates. We published results showing that SHD domain of SCAR2

interacts with a plant protein ABIL1, an interaction that may link SCAR2 to the WAVE complex (Basu et al., 2005). Further biochemical characterization of WAVE complex biochemistry has been difficult. Both the SHD domain of SCAR2 and its putative binding partner ABIL1 have proved difficult to express as recombinant proteins. It has also proved difficult to solubilize the endogenous WAVE complex using mild detergents. Therefore we were not able to directly assay the size of the endogenous WAVE complex using size exclusion chromatography. We also discovered the plant NAP1 and SRA1 subunits of the SCAR regulatory complex that couple small GTPase signaling to ARP2/3 activation.

Goal 3: To determine if ATWAVEL1, 2, 3, and 4 are direct, positive regulators of ARP2/3.

We were able to show that ATWAVEL1-4, which were renamed to AtSCAR1-4 are functional ARP2/3 activators using both genetic criteria and reconstitution of ARP2/3 activatoin in vitro. We showed that AtSCAR5 is a pseudogene. These important analyses clearly demonstrated the activity of SCAR proteins in terms of ARP2/3 activation, and showed the SCAR is the lone pathway for ARP2/3 activation. In yeasts and vertebrates there are at least 5 different types of ARP2/3 activators.

Publications

Peer-reviewed research articles:

- Basu, D., El-Assal, S. E., Le, J., Mallery, E. L. and Szymanski, D. B. (2004) 'Interchangeable functions of Arabidopsis PIROGI and the human WAVE complex subunit SRA1 during leaf epidermal development', *Development* 131: 4345-4355.
- Basu, D., Le, J., El-Assal, S. E., Huang, S., Zhang, C., Mallery, E. L., Koliantz, G., Staiger, C. J. and Szymanski, D. B. (2005) 'DISTORTED3/SCAR2 is a putative Arabidopsis WAVE complex subunit that activates the Arp2/3 complex and is required for epidermal morphogenesis', *Plant Cell* 17: 502-524.
- El-Assal, S. E., Le, J., Basu, D., Mallery, E. L. and Szymanski, D. B. (2004a) 'Arabidopsis *GNARLED* encodes a NAP125 homologue that positively regulates ARP2/3', *Curr. Biol.* 14: 1405-1409.
- El-Assal, S. E., Le, J., Basu, D., Mallery, E. L. and Szymanski, D. B. (2004b) '*DISTORTED2* encodes an ARPC2 subunit of the putative Arabidopsis ARP2/3 complex', *Plant J.* 38: 526-538.
- Le, J., El-Assal, S. E., Basu, D., Saad, M. E. and Szymanski, D. B. (2003) 'Requirements for Arabidopsis *ATARP2* and *ATARP3* during epidermal development', *Curr. Biol.* 13: 1341-1347.
- Le, J., Mallery, E. L., Zhang, C., Brankle, S. and Szymanski, D. B. (2006) 'Arabidopsis BRICK1/HSPC300 is an essential WAVE-complex subunit that selectively stabilizes the Arp2/3 activator SCAR2', *Curr. Biol.* 16: 895-901.

Peer reviewed review articles:

- Szymanski, D. B. (2005) 'Breaking the WAVE complex: the point of Arabidopsis trichomes', *Curr. Opin. Plant Biol.* 8: 103-112.
- Szymanski, D. B. (2009) 'Plant cells taking shape: new insights into cytoplasmic control', *Curr. Opin. Plant Biol.* 12: 735-744.
- Szymanski, D. B. and Cosgrove, D. J. (2009) 'Dynamic coordination of cytoskeletal and cell wall systems during plant cell morphogenesis', *Curr. Biol.* 19: R800-R811.
- Zhang, C., Mallery, E. L., Schlueter, J., Huang, S., Fan, Y., Brankle, S., Staiger, C. J. and Szymanski, D. B. (2008) 'Arabidopsis SCARs function interchangeably to meet actin-related protein 2/3 activation thresholds during morphogenesis', *Plant Cell* 20: 995-1011.