

DOE Final Report (DE-FG02-08ER15975)

Title: Powdery Mildew Disease Resistance

PI: Shauna Somerville

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The overall goal of this project was to characterize the PMR5 protein, a member of the DUF231/TBR family, and to determine its role in plant cell wall biogenesis. Since the *pmr5* mutants are also resistant to the fungal powdery mildew pathogen, we wished to determine what specific cell wall changes are associated with disease resistance and why. The graduate student working on this project made mutations in the putative active site of PMR5, assuming it is a member of the SGNH/GDSL esterase superfamily (Anantharaman and Aravind, 2010, *Biology Direct* 5, 1). These mutants were inactive in planta suggesting that PMR5 is a functional enzyme and not a binding protein or chaperone. In addition, she determined that cell wall preparations from the *pmr5* mutant exhibited a modest reduction (13%) in total acetyl groups. To pursue characterization further, the graduate student expressed the PMR5 protein in a heterologous *E. coli* system. She could purify PMR5 using a two step protocol based on tags added to the N and C terminus of the protein. She was able to show the PMR5 protein bound to pectins, including homogalacturonan, but not to other cell wall components (e.g., xyloglucans, arabinans). Based on these observations, a postdoctoral fellow is currently developing an enzyme assay for PMR5 based on the idea that it may be acetylating the homogalacturonic acid pectin fraction.

Our initial experiments to localize PMR5 subcellularly suggested that it occurred in the endoplasmic reticulum. However, since the various pectins are believed to be synthesized in the Golgi apparatus, we felt it necessary to repeat our results using a native promoter expression system. Within the past year, we have demonstrated conclusively that PMR5 is localized to the endoplasmic reticulum, a location that sets it apart from most cell wall biogenesis and modification enzymes.

The graduate student contributed to the characterization of two suppressor mutants, which were selected as restoring powdery mildew susceptibility in the *pmr5* mutant background. These suppressor loci, *TBR* and *RWA2*, both appear to influence the degree of acetylation of cell wall polysaccharides, although in different ways (unpublished, and publication 4 below). Once the specific cell wall change is identified in *pmr5* cell walls, we will be able to characterize this cell wall fragment in *tbr*, *rwa2* and *pmr5 tbr* and *pmr5 rwa2* mutants for insights into what kinds of cell wall changes impact disease resistance. The smaller stature of the *pmr5* mutants is reversed in the *pmr5 tbr* and *pmr5 rwa2* double mutants, suggesting that analyses of these lines will also provide insights into cell expansion and growth.

We collaborated with H. Scheller (DOE – Joint Bioenergy Institute, Emeryville, CA) on the *rwa2* suppressors of *pmr5* and the role of *RWA2* in disease resistance (see publication 4). We also collaborated with Berkeley colleagues Marcus Pauly and Chris Somerville, who work on other members of the 45-member DUF231/TBR family, Mary Wildermuth (University of California, Berkeley, CA) and Mike Hahn (Complex Carbohydrate Research Center, Athens, GA). Publications 1 and 3 result from work funded in part by a previous DOE grant.

Publications

1. Sánchez-Rodríguez, C., Estévez, J.M., Llorente, F., Hernández-Blanco, C., Jordá, L., Pagán, I., Berrocal, M., Marco, Y., Somerville, S., Molina, A. (2009) The ERECTA Receptor-like kinase regulates cell wall-mediated resistance to pathogens in *Arabidopsis thaliana*. *Molecular Plant-Microbe Interactions* 22, 953-963; doi:10.1094/MPMI-22-8-0953.
2. Hématy, K., Cherk, C., Somerville, S.C. (2009). Host-pathogen warfare at the plant cell wall. *Current Opinion in Plant Biology* 12, 406-413; DOI 10.1016/j.pbi.2009.06.007.

3. Voigt, C.A., Somerville, S.C. (2009) Chapter 4.4.5: Callose in biotic stress (pathogenesis). Biology, biochemistry and molecular biology of callose in plant defense: callose deposition and turnover in plant-pathogen interactions. In: *Chemistry, Biochemistry and Biology of (1-3)- β -Glucans and Related Polysaccharides* (A. Bacic, G. Fincher, B. Stone, eds.), Elsevier Publishing, New York. Pp. 533-570.
4. Manabe, Y., Nafisi, M., Verhertbruggen, Y., Orfila, C., Gille, S., Rautengarten, C., Cherk, C., Marcus, S., Somerville, S., Pauly, M., Knox, J.P., Sakuragi, Y., Scheller, H.V. (2011) Loss-of-Function mutation of *REDUCED WALL ACETYLATION 2* in Arabidopsis leads to reduced cell wall acetylation and increased resistance to *Botrytis cinerea*. *Plant Physiology* 155, 1068-1078 (First published on January 6, 2011) 10.1104/pp.110.168989

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