TITLE: **Investigation of the functional role of CSLD proteins in plant cell wall deposition**

GRANT NUMBER: DE-FG02-07ER15887

FUNDING PERIOD: 08/01/2015-07/31/2016

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Funding Opportunity Announcement: DE-FOA-0001204

DOE Program Office: Basic Energy Sciences – Physical Biosciences

DOE Program Manager Contact: Robert Stack or Gail McLean

FINAL TECHNICAL REPORT: GRANT NO. DE-FG02-07ER15887

SUMMARY:
The overall goal of this research proposal was to characterize the molecular machinery responsible for polarized secretion of cell wall components in *Arabidopsis thaliana*. We have used the polarized expansion that occurs during root hair cell growth to identify membrane trafficking pathways involved in polarized secretion of cell wall components to the expanding tips of these cells, and we have recently shown that CSLD3 is preferentially targeted to the apical plasma membranes in root hair cells, where it plays essential roles during cell wall deposition in these cells. The specific aims of the project are designed to answer the following objectives:

OBJECTIVES:

(1) **Identification of the cell wall polysaccharide class that CSLD proteins synthesize.**

BUDGET AND SPENDING:
Funds from this award were used to provide salary support for one Postdoctoral researcher (Gwangbae Bak), and one PhD student (Jiyuan Yang), as well as for experimental materials and supplies related to the research aim. All funds were expended by the end of the grant period.

RESEARCH OUTCOMES:

Reconstitution of catalytically active CSLD3 proteoliposomes. In order to understand the function of CSLD synthases during plant growth and development their enzymatic activity must be established. We successfully developed methods to express, detergent-solubilize, and purify His-tagged CSLD3, CESA6, and CSLA9 proteins (Fig1A). When reconstituted into proteoliposomes and provided UDP-Glucose (UDP-Glc), saturable UDP-forming activities were observed for both CSLD3 and CESA6 proteins, but not for CSLA9 which utilizes GDP-Mannose (Fig1B). The apparent K_m values of CSLD3 and CESA6 for UDP-Glc were 65μM and 73μM, consistent with values recently reported for reconstituted PpCESA8 (~30μM; {Purushotham, 2016 #2090}), and significantly lower than values determined for the bacterial cellulose synthase, BcsA (~500μM; {Omadjela, 2013 #1904}). ***These results provide a unique and powerful foundation for the successful future characterization and comparison of CSLD and CESA biosynthetic activities.***

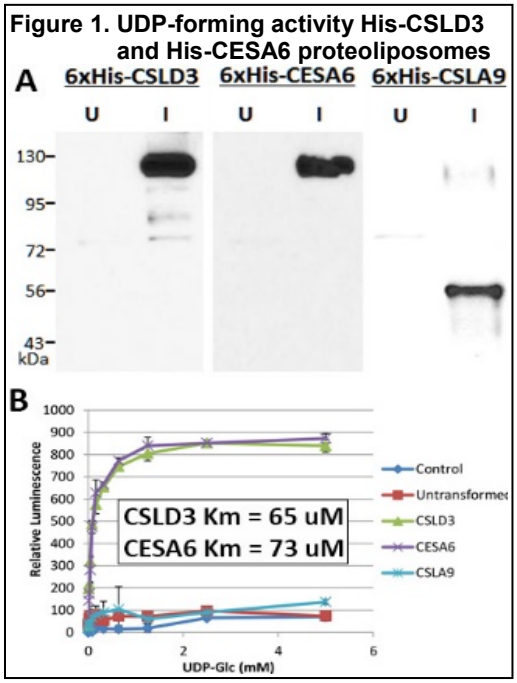

Figure 1. UDP-forming activity His-CSLD3 and His-CESA6 proteoliposomes

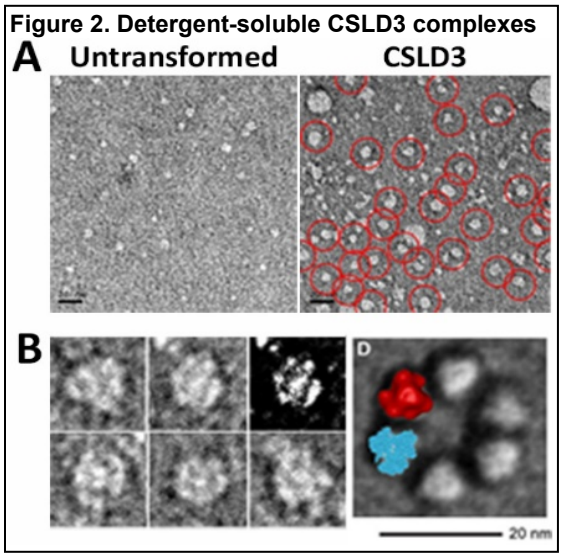

Figure 2. Detergent-soluble CSLD3 complexes

Structural characterization of detergent-solubilized CSLD3 complexes. The ability to detect significant amounts UDP-forming activity upon reconstitution of detergent-solubilized CSLD3 complexes into proteoliposomes suggested that these protein complexes maintain appropriate conformation during their isolation and purification. Size-exclusion chromatography demonstrated that both CSLD3 and CESA6 complexes migrated as large molecular-weight complexes (~0.8-1MDa). Based on their large apparent molecular sizes, we attempted to visualize these purified, detergent-solubilized CSLD3 and CESA6 fractions. When detergent-solubilized membrane fractions from both untransformed yeast, and yeast expressing His-CSLD3 were affinity purified, placed on EM grids, negatively stained, and imaged at 50,000x magnification, clear enrichment of ~10-12nm particles in the CSLD3 fraction was observed (Fig2A; red circles). When examined at higher magnification these particles often displayed structural detail reminiscent of a "wagon-wheel," with a bright central mass often surrounded by three pairs of smaller circular structures oriented at roughly 120-degree intervals around the central mass (Fig2B). Five representative CSLD3 particles are presented, plus one reproduced with high contrast to highlight the central mass and six surrounding structures (Fig2B; top right panel). ***These CSLD3 particles showed a striking resemblance to a computer-generated surface representation of the transmembrane domain region of putative trimeric CESA complexes*** (Fig2B; red structure at right, adapted from {Nixon, 2016 #2038}).

PUBLICATIONS (2015-2016):

1).Gu, F., Bringmann, M., Combs, J., Yang, J., Bergmann, D.C., and **Nielsen, E.** (2016) The *Arabidopsis* CSLD5 functions in cell plate formation in a cell cycle dependent manner**.** *Plant Cell, **28***, 1722-37.

MANUSCRIPTS IN PREP:

1).Kim, D-W., Gu, F., Park, S., Bahk, J.D., and **Nielsen, E.** (2017) Functional Characterization of *ltl2,* a Novel Temperature-Sensitive FERONIA Mutant Allele That Alters Root Hair Growth. *Plant Journal, **in revision***.

2).Bak, G., Yang, J., Combs, J., Mayes, H., Peña, M., Urbanowicz, B., and Nielsen, E. (2018) CSLD cell wall synthases represent a new class of plasma membrane-localized beta-1,4-glucan synthases and share significant functional and structural similarities with plant cellulose synthases. In prep., to be submitted sometime in 2018.