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Regulation of Cell Wall Assembly: Myosin and Exocyst Involvement in Cellulose Synthase Delivery to the Plasma Membrane
Period of Performance; 05/15/2004-08/14/2021

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Overall research goals:

Cellulose is the most abundant biopolymer on the planet and is produced in the primary and secondary cell wall of terrestrial plants by a plasma membrane (PM)-localized, multimeric protein complex. The catalytic enzyme, or cellulose synthase, belongs to a multigene family known as CESA. It is generally accepted that the behavior and trajectories of cellulose synthase complexes are oriented by the position of cortical microtubules, but how exactly these complexes are delivered and recycled from the PM remains poorly understood. Limited evidence suggests that microtubules determine the site for delivery of new complexes; however, abolishing microtubules with the inhibitor oryzalin has absolutely no effect on rates of delivery. Thus, there is a pressing need to explore the contribution of another component of the cortical cytoskeleton, actin filaments and the associated motor protein myosin, to the delivery and dynamics of CESA at the PM.

In the current proposal, we tested the ***central hypothesis*** that myosin XI and the exocyst complex cooperate to tether CESA-containing secretory vesicles at the PM and facilitate vesicle fusion. The ***specific aims*** include:

1) testing whether actin filament organization and individual myosin XI isoforms are necessary for CESA vesicle tethering and fusion, using a powerful combination of genetics and quantitative live-cell imaging; and

2) determining whether myosin–exocyst interactions are necessary for vesicle tethering and fusion during the delivery of CESA complex to the PM.

In general, the Staiger laboratory aims to understand dynamic control of the cortical actin cytoskeleton and how it is used to deliver materials to and from the PM and cell wall. Although it is commonly accepted that actin filaments serve as tracks for exocytosis, there is little direct evidence that secreted cargo traffics along single filaments or bundles. Moreover, which dynamic properties of the cortical cytoskeleton regulate vesicle trafficking remain poorly understood. We recently demonstrated that class XI myosins from *Arabidopsis* participate in cellulose deposition during primary cell wall formation. Capitalizing on the identification of a new small molecule inhibitor, pentabromopseudilin or PBP, we implicated myosin in trafficking of CSCs and CESA dynamics. We will pursue further analysis of myosin activity and its role in cell wall assembly by combining high spatiotemporal live-cell imaging with molecular genetic analysis of class XI myosins.

Significant achievements (2019-2021):

- Through chemical and genetic inhibition of plant myosin XI, demonstrated a role for this molecular motor in cellulose deposition via the delivery of cellulose synthase complex (CESA) to the plasma membrane.
- Demonstrated a role for myosins XI in tethering and fusion of CESA-containing vesicles at the plasma membrane, for the first time implicating actomyosin in the late stages of secretory vesicle trafficking in plants.
- Identified Myosin XIK isoform as the major isoform involved in CSC vesicle trafficking.
- Provided *in vitro* and *in vivo* evidence for myosin XI–exocyst complex interactions, specifically between the globular tail domain (GTD) of myosin XIK and the Sec5B subunit of exocyst.
- Using chemical and genetic inhibition, demonstrated the dependence of exocyst dynamics and lifetime on myosin activity.
- Demonstrate that myosin XIK and exocyst subunits transiently colocalize with CESA at the PM during vesicle tethering.
- Reported that the plant phytohormone auxin signals to actin cytoskeleton remodeling in root epidermal cells via the auxin transporter AUX1.
- In collaboration with Chunhua Zhang (Purdue) identified a new chemical inhibitor of cellulose synthase, endosidin 20 or ES20, that inhibits CESA catalytic activity and perturbs vesicle trafficking.
- Developed quantitative imaging analysis tools to reveal single particle behavior of CSCs during the late stages of secretion. A novel tethering assay revealed that cortical microtubules play a minor role in vesicle capture at the PM.
- Generated preliminary evidence using genetics and chemical biology to support a role for the ARP2/3 complex in secretory vesicle tethering.

Publications supported by this project (2019-2021):

Zhang, W., C. Cai, and **C.J. Staiger**. 2019. Myosins XI are involved in exocytosis of cellulose synthase complexes. *Plant Physiology* 179: 1537-1555, doi:10.1104/pp.19.00018

Arieti, R., and **C.J. Staiger**. 2020. Auxin-induced actin cytoskeleton rearrangements require AUX1. *New Phytologist.* 226: 441-459, doi.10.1111/nph.16382

Huang, L., X. Li, W. Zhang, N. Ung, N. Liu, X.Yin, Y. Li, R.E. Mcewan, B. Dilkes, M. Dai, G.R. Hicks, N.V. Raikhel, **C.J. Staiger**, and C. Zhang. 2020. Endosidin20 targets the cellulose synthase catalytic domain to inhibit cellulose biosynthesis. *Plant Cell* 32: 2141-2157, doi:10.1105/tpc.20.00202

Zhang, W., L. Huang, C. Zhang, and **C.J. Staiger**. 2021 Arabidopsis myosin XIK interacts with the exocyst complex to facilitate vesicle tethering during exocytosis. *Plant Cell* 33: 2454-2478, doi:10.1093/plcell/koab116

Zhang, W., and **C.J. Staiger**. 2022. Revising the role of cortical cytoskeleton during secretion: Actin and myosin XI function in vesicle tethering. *Int. J. Mol. Sci.* 23: 317, doi:10.3390/ijms23010317