Alabama State University
Boston University
Bryn Mawr College
New Jersey Institute of Technology
University of Pennsylvania
University of Texas at Austin
Washington University in St. Louis

End of Summer REU Symposium

Thursday, August 5, 2021, 10:00 am – 12:30 pm (CST) (REU student presentation setup with Ethan at 9:30 am)
In Person: Whitaker Hall, Room 100
or Virtual: Zoom Link (Meeting ID 933 5573 8212)

10:00-10:15 – Welcoming Remarks
Guy Genin, PhD, CEMB Co-Director and
Patricia Widder, CEMB Education Program Coordinator and Senior Lecturer in Biomedical Engineering

10:15 am - 10:30 am – Julia Behlmann, University of Tulsa
Elizabeth Haswell Lab
MS Ion Channel OSCA2A is Required for Normal Drought Response in Moss
10:35 am -10:50 am – Annika Avula, Centre College
Ram Dixit Lab
Developing Plant "Tissue-on-a-Chip" Technology to Study Mechanical Forces in Plant Cells
10:55 am - 11:10 am – Abdulaziz Said, Washington University in St. Louis
Amit Pathak Lab
Hypoxia Enhances Spreading and Migration of Cancerous Breast Epithelial Cells

11:10-11:20 – Break

11:20 am - 11:35 am – Osmar Torres Benavides, University of Texas at El Paso
Guy Genin/Ram Dixit Labs
Organ-level root skewing of the Arabidopsis thaliana right-handed mutant, spr1-3, switches handedness in response to increased shear force

11:40 am - 11:55 am – Miranda Copenhaver, Washington University in St. Louis
Marcus Foston Lab
UV Activation May Offer More Precise Control of Mechanical Properties of Cellulose Nanocomposites via Benzophenone Crosslinking
12:00 pm - 12:15 pm – Sorina Munteanu, University of California – Merced
Guy Genin Lab
The Role of Actomyosin Contractility in the Dynamics of Microtubule-Based Fibroblast Protrusion
12:15-12:30 – Closing Remarks
Guy Genin, PhD, CEMB Co-Director and Patricia Widder, CEMB Education Program Coordinator and Senior Lecturer in Biomedical Engineering

Boxed lunches and beverages will be available afterwards in the Whitaker Hall atrium.

MS Ion Channel *OSCA2A* is Required for Normal Drought Response in Moss

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Introduction: Mechanosensitive (MS) ion channels respond to physical forces in order for a cell to adapt to its environment. *OSCA*s are MS ion channels at the plasma membrane that are specific to Ca^{+2}, and they are implicated in drought response. In spreading earthmoss, *Physcomitrium patens*, 15 *OSCA*s are present across three clades (Yuan *et al.*, 2014). The goal of this research is to understand the function of *OSCA* proteins in the first two clades and help construct a library of MS channel mutants. Using reverse genetics, we aimed to generate new knock outs from clade 1 and phenotype two existing mutant lines in clade 2, *Δosca2a-45* and *Δosca2a-50*.

Materials and Methods: For clade 1, we decided to knock out three *OSCA*s, which we have called *OSCA1A, 1B,* and *1C*. To generate loss-of-function mutants, we opted to utilize 4- and 2-fragment multisite Gateway® Cloning. We identified CRISPR/Cas9 target sites in the genomic sequence that correspond to the final two transmembrane domains (Jojoa-Cruzetal, 2018). For phenotyping clade 2 mutants, we plated WT, *Δosca2a-45*, and *Δosca2a-50* moss lines on gravity plates (Repp *et al.*, 2004) as well as on media with Mannitol [0-0.4M], NaCl [0.1-0.2M], and KCl [0.1-0.2M]. We also used a desiccation assay (Stevenson *et al.*, 2016) wherein these same three moss lines were incubated overnight on media containing 0, 10, or 100 mM ABA, left to dry overnight on sterile plates, and recovered on BCDAT media for one week. Fiji allowed us to determine the percentage of survival using thresholding (Schindelin, J. *et al.,* 2012).

Results and Discussion: We observed that *Δosca2a-45* and *Δosca2a-50* lines had reduced survival after desiccation compared to the WT: 19.8% in the WT verses only 11.3% in *Δosca2a*-45 and 8.99% in *Δosca2a-50* (Figure 1A, B). Only tissue that received 10 mM ABA recovered; all lines on 0 and 100mM ABA plates were completely white (dead). *Δosca2a-45* and *Δosca2a-50* lines appeared denser under the microscope than the WT. In addition, *Δosca2a-45* and *Δosca2a-50* lines appeared to have slightly increased survival compared to the WT on 0.2M KCl assays based on their darker green color. We observed no difference in gravity, Mannitol, 0.1M KCl, and NaCl assays. For clade 1, the 2-fragment recombination mutant line *Δosca1c* is too young to phenotype; nonetheless, this work will contribute to building a library of MS channel mutants. The 4-fragment recombination destination vector for *Δosca1a* and *1b* is still in preparation.

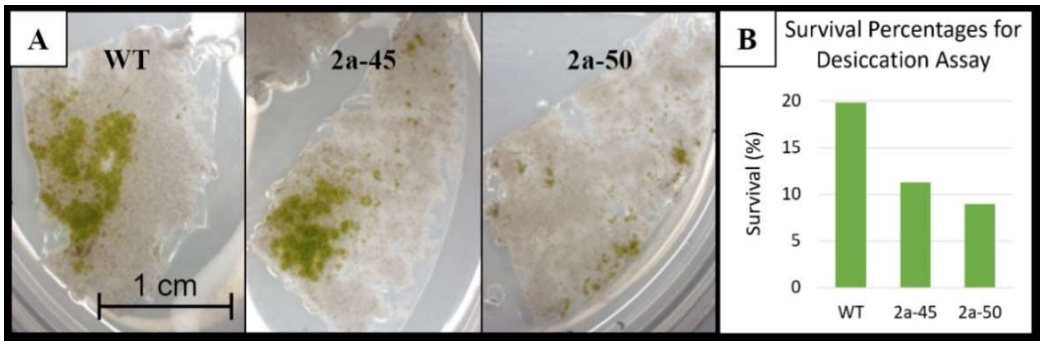

Figure 1.
(A) Desiccation assay recovery images from 10mM ABA plates.
(B) Graph of survival percentages that were calculated from images in (A) using Fiji. WT moss recovered approximately twice as well as *Δosca2a-45* and *Δosca2a-50* lines.

Conclusions: The desiccation assay we conducted suggests that the *OSCA2A* channel plays an important part in drought survival. With the slight difference in phenotype on 0.2M KCl, *OSCA2A* seems to be involved in salt stress. We made progress towards creating *Δosca1c* lines; once this is complete, the assays described above can be repeated. Eventually, we intend to generate double and triple *OSCA* clade 1 mutants. We will also compare phenotypes between clade 1 and 2 *OSCA* mutants to determine any differences within *OSCA* genes. The experiments from this research establish the utility of moss as a model system for studying MS ion channels.

Acknowledgements: I would like to thank all of the members of the Haswell lab for their help, the Bezanilla lab for the majority of the protocols used in this research, and Sorina Munteanu for her assistance with Fiji. Equally, I would like to thank the Center for Engineering Mechanobiology for funding this research. I would like to thank BMES for providing me this opportunity to share my research.

Developing Plant "Tissue-on-a-Chip" Technology to Study Mechanical Forces in Plant Cells

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In plants and animals, mechanical forces are critical for cell growth and development. While this has been studied extensively in mammalian cells, little is known about how mechanical forces affect plant cells on the molecular level. In addition, current methods of studying mechanical forces in plants are invasive and not physiologically relevant. To overcome these shortcomings, we are using BY2 cells derived from *Nicotiana tobacum* to develop a microfluidics device as an *in vitro* model system in which the impact of mechanical forces on plant cells can be studied under control conditions. This device relies on a matrix made of PVDF-TrFE, which has been previously shown to display properties that mimic the plant cell wall, and to which plant cells adhere. To investigate the specific responses to externally applied force, we are developing genetic marker lines to measure known fast responses to mechanical forces, such as pH and calcium intake, and known slow responses, such as microtubule reorientation and cell polarity. In addition, we are generating cell cultures from *Arabidopsis thaliana* plants which will allow us to take advantage of various Arabidopsis cell wall mutants to study the contribution of specific cell wall components to the adhesion of plant cells to PVDF-TrFE scaffolds. While our previous enzymatic digestion studies indicated that pectin is important for cell adhesion, the contribution of hemicellulose to cell-scaffold adhesion was not addressed. Here, we will use cell cultures generated from *Arabidopsis xxt1*/*xxt2* and *qua1* mutants to investigate the contribution of xyloglucan (the dominant hemicellulose in Arabidopsis) and pectin, respectively, to cell-scaffold adhesion. The development of a microfluidics device will enable us to create a biomimetic environment *in vitro* to allow manipulation of mechanical and biochemical stimuli and study how cell-cell communication affects plant responses to these forces. Understanding responses to mechanical force has a multitude of applications and the creation of a plant "tissue-on-a-chip" is long overdue for the field of plant mechanobiology.

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Hypoxia Enhances Spreading and Migration of Cancerous Breast Epithelial Cells

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The processes and factors leading to metastatic cancer is an important area of research, with the potential to lead to therapies for such diseases. It has been shown that changes in the tumor microenvironment can alter cells' ability to migrate. Rho kinase and the RhoA G-protein family is involved in a signaling pathway acting to facilitate cytoskeleton remodeling, to ultimately cause tumor cells to become malleable for cell migration. Over cancer progression, tumor microenvironment can vary in terms of its mechanics, chemokine factors, and oxygen conditions. In epithelial breast cancer, the oxygen content of the tumor microenvironment is hypothesized to impact the expression of the Rho family of proteins. Here we conducted a phenotypic and migration study to test the hypothesis that hypoxia positively impacts migration behavior of breast epithelial cells. A comparison was done between normal and cancerous epithelial cells primed in a normoxic or hypoxic oxygen environment. It was shown that breast cancer epithelial cells exhibited a larger cell area under hypoxic conditions as compared to those in normoxic environment, with over three-fold increase in area. However, healthy cells were negatively impacted by the lack of oxygen. Normal epithelial cells showed a decrease of 60% in cell area in hypoxic conditions than in the normoxic environment. These results suggest that hypoxia plays an important role in enhancing the migratory and mechano-responsive capabilities of cancer breast epithelial cells. Thus, hypoxia may enhance cancer cell mechanosensing, which may be a future direction of research.

Organ-level root skewing of the *Arabidopsis thaliana* right-handed mutant, *spr1-3*, switches handedness in response to increased shear force

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Plant roots direct their growth to obtain nutrients or water and avoid obstacles. Their directional root growth is determined by the orientation of the microtubule cytoskeleton. Mutations that affect microtubules often leads to aberrant or skewed cell growth. For example, the Arabidopsis thaliana spiral1-3 (spr1-3) mutant, which lacks a microtubule plus-end binding protein, displays strong right-handed skewed roots and cell files compared to wild-type Col-0. Previous work in the lab showed that root skewing of spr1-3 plants switched handedness in response to increased shear force caused by increasing agar concentration in the growth medium. To test if handedness also switches at the cell file-level, Col-0 and spr1-3 plants were again grown on 1%, 3%, and 5% agar media, and then imaged at the cell level using a brightfield dissecting microscope. ImageJ analysis of cell file angles revealed that while organ-level skewing of spr1-3 roots straightened on 3% agar and switched handedness on 5% agar, cell file skewing was unaffected by increased shear force. These findings suggest that root direction is also influenced by mechanobiological forces.

UV Activation May Offer More Precise Control of Mechanical Properties of Cellulose Nanocomposites via Benzophenone Crosslinking

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Cellulose Nanocrystals (CNC's) can be combined with soy proteins to create nanocomposites, offering a wide range of applications such as biodegradable food packaging and tissue repair. A new method of synthesizing nanocomposites utilizes a UV activated compound, known as benzophenone, to create covalent bonds between CNC's and soy proteins, which ensures a more precise control of crosslinking within nanomaterials. Preliminary experiments were completed to confirm the presence of crosslinks between soy proteins and benzophenone-coated CNC's (BP-CNC's) in this novel method. Results were confirmed with water absorption testing. Nanocomposite films containing benzophenone that were exposed to UV radiation showed significantly less water absorption capabilities than their counterparts that did not receive UV and/or benzophenone treatment. This phenomenon is explained by the covalent bonds between BP-CNC's and soy proteins, making the nanocomposite films less permeable to water and further confirming the viability of the new crosslinking method. This new method offers the possibility of both greater control over crosslinking and greater strength in nanomaterials. The increased control over crosslinking stems from the ability to manipulate intensity and location of UV exposure. Methods such as varied distance and photomasks revealed the covalent bonding between BP-CNC's and soy proteins offer an increased mechanical strength compared to other nanocomposites that are held together by intermolecular interactions.

THE ROLE OF ACTOMYOSIN CONTRACTILITY IN THE DYNAMICS OF MICROTUBULE-BASED FIBROBLAST PROTRUSIONS

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Introduction: Mechanical stresses on and within the actin cytoskeleton are critical determinants of cellular function and morphology. These stresses can arise from actomyosin contraction and from interactions between the cell and the extracellular microenvironment. Stresses that resist microtubule growth have been shown recently to mediate the degree to which cells can extend the protrusions needed to remodel extracellular matrix (ECM) proteins. However, the relationship between actomyosin contractility and protrusion dynamics has been difficult to quantify because of challenges in separating out the effects of ECM proteins in three-dimensional (3D) culture, and in separating out effects of substrate interactions in 2D culture. These effects are critical to understanding how cells manipulate ECM in wound healing and fibrosis. We therefore studied cells in adhesion to reveal how actomyosin contractility and microtubules work in concert to form cell protrusions. We tested the hypothesis that microtubule extension and actomyosin contractility compete to govern the rates and extents of protrusion growth.

Materials and Methods: NIH/3T3 cells (immortalized mouse embryonic fibroblasts) were transfected using CellLight Tubulin-GFP BacMam 2.0 (Invitrogen, Waltham, MA) in 24-well plates for live cell imaging of microtubule dynamics. After incubation overnight in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), cells were trypsinized and either adhered in DMEM plus FBS with 1.00 µL nocodazole (a microtubule inhibitor), blebbistatin (a myosin II inhibitor), or Y27632 (a rho kinase inhibitor). Microtubule behavior and cellular protrusion dynamics were observed via confocal fluorescence microscopy, and quantified using ImageJ.

Results: After optimizing cell staining and culture protocols to enable visualization of suspended cells, we tested our hypothesis by quantifying how actomyosin contractility affected the dynamics of microtubules and protrusion growth. Indeed, after culturing, transfecting, and treating fibroblasts with the various drugs, it was discovered that there is protrusion growth. In untreated cells, protrusions of 4.82 ± 1.62 µm in length were evident, with 40.0 ± 0.00 per cell (Figure 1). Nocodazole treatment repressed growth of protrusions, with 0.00 ± 0.00 per cell and rounded cells unable to develop polarity. Conversely, treatment with the rho kinase inhibitor Y27632 resulted in cells with fewer (3.50 ± 0.707 per cell) and longer protrusions (20.87 ± 8.36 µm), with protrusions branching (8.00 ± 2.83 branches per cell process, whereas none were observed in other cases) and cells becoming polarized. Treatment with blebbistatin resulted in still longer (17.99 ± 13.59 µm) protrusions that were less branched (4.40 ± 2.42 branches per cell process), and, like Y27632, resulted in cells adopting polarity.

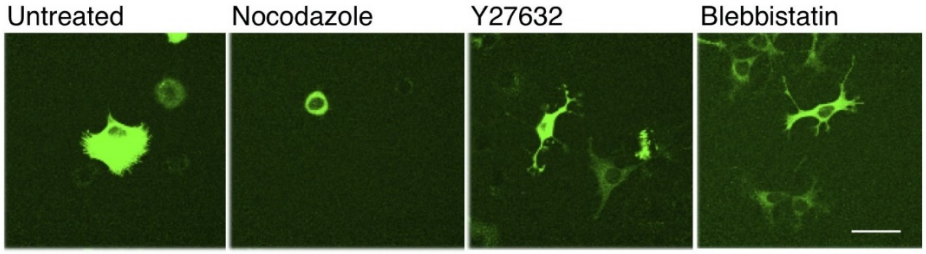

Figure 1. Fluorescence microscopy of microtubule activity in attached cells and its dependence upon actomyosin contractility. Cells treated with the microtubule inhibitor nocodazole remained rounded, while those treated with actomyosin inhibitors Y27632 or blebbistatin developed polarity and long, branched protrusions. Scale bar: 20 µm.

Discussion and Conclusions: Cell shape and protrusion dynamics arose from a competition between actomyosin contractility and microtubule growth. With actomyosin contractility inhibited, protrusion growth and cell polarization were accentuated, suggesting that contractility restricts microtubule growth. With microtubule growth attenuated, cells remained rounded with no protrusions, suggesting that actomyosin contractility alone is inadequate to drive cellular protrusions. Results provide baseline parameters for the fitting of mathematical models of cell behavior, and suggest a role for competition between microtubules and actomyosin contractility in wound healing and fibrosis.