Understanding chromatin-remodeling during transcriptional regulation in single cells

Background and Rationale: The regulation of gene expression is essential for cell fate decisions and long-term responses to alterations in the extracellular environment. It is now well recognized that transcription is a stochastic process that results in cellular heterogeneity in the number of RNA and proteins in genetically identical cells[1]. However, both the sources and biological consequences of this heterogeneity remain poorly understood. Cell-to-cell variability in chromatin state provides a potential mechanism for gene expression heterogeneity. Chromatin-remodeling complexes use the energy of ATP-hydrolysis to alter the positioning of nucleosomes which may contribute to cell-to-cell variability or noise in gene expression. Pioneering single-cell studies suggest that chromatin remodeling complexes impact the fraction of cells that induce gene expression[2]. However, these studies only focused on gene expression at a few time points limiting our understanding of how dynamic chromatin regulation impact transcription regulation. This state of affairs leaves open the questions of how and to what extent chromatin remodelers affect cell-to-cell variability, expression dynamics, and memory in single-cell transcription. To study these questions, I will use the *S. cerevisiae* osmotic stress response as a model system. Upon changes in extracellular osmolarity (e.g., NaCl), measurements in population of cells have demonstrated that chromatin regulatory complexes, namely INOsitol requiring 80 (INO80) and SWItch/Sucrose Non-Fermentable (SWI/SNF), regulate inducible gene expression to protect cells from a high future osmotic stress[3-5]. In this system, INO80 represses[4] and SWI/SNF activates[5] expression of osmotic stress response genes. **I hypothesize that chromatin regulatory complexes differentially regulate specific transcription processes in single cells.**

Specific Goal 1. Determine how chromatin regulators differentially regulate transcription steps in single cells. This goal is important to fundamentally understand how chromatin regulators function in transcription using a systematic approach (Fig.1)[6,7]. Previous studies of chromatin regulators utilize catalytic subunit knockouts, that result in ambiguous conclusions due to indirect effects[8]. I will conditionally deplete the chromatin regulator catalytic subunits (ino80 and Snf2) using the auxin inducible degron system[9] to measure the direct effects of chromatin regulation on transcription of stress response genes (*STL1*, *CTT1*, and *HSP12)*. The spatial location of each RNA will be quantified with RNA-FISH in fixed yeast cells to differentiate between nascent and mature transcription regulation at a wide range of pre-specified timepoints during osmotic stress response. From this spatial data set, I will identify how *INO80* and *SNF2* affects transcription steps that include initiation, elongation, RNA export, mRNA processing and transcriptional noise[7]. Computational modeling will be implemented using the chemical master equation to build probabilistic models that describe these transcription steps over time during osmotic stress-response[6,7]. Integrating the spatial-temporal RNA measurement data with computational modeling will enable estimation of how chromatin regulator activity differentially regulates these transcription steps. An alternative or complementary approach to this method is using single cell CUT & Tag[10] to identify how modulating the chromatin state via *SNF2* and *INO80* affects chromatin accessibility and the potential for stress response induced genome-wide transcription in single cells. By combining transcriptional readout with mathematical modeling, I will quantify the extent to which the enzymatic activity of nucleosome-remodeling complexes differentially regulates specific steps in transcription.

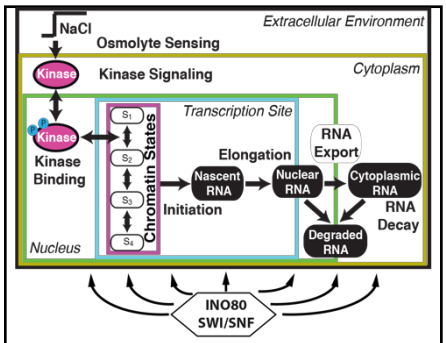

Fig.1 Our systematic identification of chromatin remodeler contribution to steps in transcription.

Specific Goal 2. Elucidate chromatin regulators contribution to single cell transcriptional memory. Previous studies show that populations of yeast cell are better adapted to stress if they are initially exposed to smaller stressor[11]. Our preliminary data shows that the timing between exposure to stress environments affects the amount of survival in a population of yeast cells, and that the timing between stresses affects the gene expression state of *STL1* in single cells (Fig. 2). Since it has been shown that gene-expression is required for future stress response[3], I hypothesize that regulation of gene expression state by chromatin-remodeling complexes may benefit individual cells by remembering previous stress response via activating the same stress response genes to survive. The idea being that it takes some amount of time for cells to transition from ON (primed) to OFF (naive) expression state, and that within this time cells can transcriptionally respond faster to secondary stress. Depletion of *SNF2* and *INO80* will be used to elucidate how nucleosome remodeling help to modulate memory in *STL1* expression state and stress resistance. Single cell live imaging upon pulses of NaCl stresses will be used to quantify *STL1* gene expression. These results will establish a relationship between the regulation of nucleosomes and how past *STL1* expression influences a cell's future *STL1* expression, and ultimately how *STL1* levels affect stress survival. Overall these studies will help me to shed light on how chromatin regulators affect the ability of cells to utilize transcriptional memory to survive in dynamic stress environments.

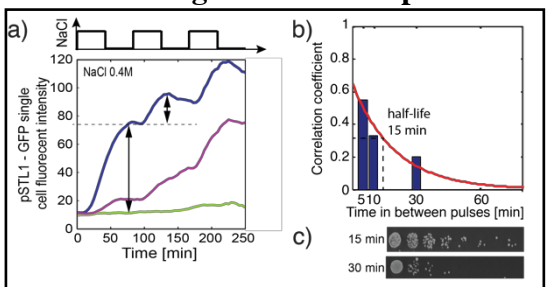

Fig. 2: Single Cells Can Maintain Specific Gene Expression States. a) *pSTL1-GFP* expression levels detected via microscopy in single cells (bottom) treated with the osmostress pulse-series (top). b) Plot of correlation coefficient vs. osmostress pulse gap used to determine gene expression state half-life. c) Spot formation assay of serially diluted cells that are treated in the 1st pulse with 0.7M NaCl and 15 or 30 minutes later with a 2nd pulse with 2M NaCl effectively reducing number of colonies.

Intellectual Merits: My coursework and research experiences so far in computational modeling have prepared me to implement the modeling approaches in my proposal. Our labs expertise in single-cell experiments with our capability to capture high resolution single cell data, single-cell tracking, and image analysis pipelines enable us to study gene-regulation at the single-cell level with high spatio-temporal resolution. This work will allow for a revolutionary understanding of how chromatin regulation affects noise in gene expression and how transcriptional memory affects biological fitness. For example, there could be an evolutionary advantage to temporarily maintaining the ON (primed) expression state if stress exposure is occurring frequently enough in cells surrounding environments to minimize resources spent. Mechanisms of chromatin regulation will then allow for cell-to-cell variability to be optimized to ensure that many cells in a population survive in many different environments.

Broader Impacts: Using this work, I plan to help more underrepresented students get interested in scientific research by linking the importance of heterogeneity in a cell population and how it is regulated to the importance of diversity in the STEM workforce. I will do this to show undergraduate students at Fisk University, a nearby historically black university, why they can pursue a career in science and why they should be involved in scientific research. What I will learn in gene regulation will aid in engineering gene expression of yeast cells for environmental clean-up by ensuring that all cells are optimally productive and robust to selective pressures that hinder their function. **References:**

[redacted]