Title- Epigenetic regulation of cell fate decisions

Introduction

This application is requesting one course release for the Fall 2021 semester to prepare manuscripts for publication. The resulting manuscripts will include Stockton University undergraduate students as coauthors and are the continuation of ongoing active research projects in my laboratory.

Project background and aims

Research in my laboratory is focused on understanding how cells respond to their environment to make the correct decisions about their ultimate fate. Cell fate decisions rely upon faithfully integrating external signaling cues with internal cellular states. In this manner, cells that contain identical genetic blueprints (DNA) can develop into a wide range of tissues and organ systems. Mistakes in signal interpretation can lead to severe consequences including developmental defects or cancer.

If all cells contain the same DNA, then can they manifest different physical appearances? The answer to this question lies in our current understanding of how genes interact with the environment. While DNA provides the genetic code to construct all of the parts of an organism, not all of the DNA blueprints are read by every single cell. One process that directs which parts of DNA will be used in each cell type is termed epigenetics ('epi-'upon or above). Epigenetics is analogous to the software on your computer. While two computers may be identical in make and model (same DNA), they may run distinct software packages (different epigenetics). The long-term goal of my research is to understand how epigenetics can be programmed and reprogrammed to influence cell fate decisions.

To understand the complexities of epigenetics and cell fate decisions, my laboratory uses the budding yeast *Saccharomyces cerevisiae* as an experimental model. While budding yeast are commonly used in baking and brewing, they have been seminal in forwarding our understanding of epigenetics and cell fate decisions. Importantly, yeast provide a facile experimental tool that is ideally suited for immersive research projects with undergraduate students.

Recent work in my laboratory spearheaded by my undergraduate research team has uncovered new functions for an epigenetic regulator termed COMPASS. COMPASS is a multi-subunit complex that plays well-described and evolutionarily-conserved functions in epigenetic modifications (BRIGGS *et al.* 2001; MILLER *et al.* 2001; KROGAN *et al.* 2002; BOA *et al.* 2003; SCHNEIDER *et al.* 2005; DEHE AND GELI 2006). Using a systematic approach, we have found new functions for individual COMPASS subunits in controlling cell fate decisions in yeast. This proposal aims to disseminate these research findings by submitting two manuscripts for publication in peer-reviewed scholarly research journals:

Aim 1 Complete remaining revisions for a manuscript titled "Distinct roles for COMPASS subunits Swd1 and Swd3 during meiosis in *Saccharomyces cerevisiae*" that will be initially submitted to the Genetics Society of America-sponsored journal *Genetics* in April 2021.

Aim 2 Prepare a second manuscript, tentatively titled "Genetic and metabolic analyses of filamentous growth in *Saccharomyces cerevisiae*" for publication. Once completed, this manuscript will be submitted to the Genetics Society of America-sponsored journal *G3: Genes, Genomes, Genetics* or the Federation of European Microbiological Society-sponsored journal *FEMS Yeast Research*.

Background work already accomplished

We have collected all of the data and prepared a draft manuscript geared towards completing the first proposed Aim. This draft manuscript includes former Stockton University student Miranda Czymek as a coauthor. The manuscript is currently in its final revision stages and I anticipate submitting the completed version to the Genetics Society of America journal *Genetics* in April 2021. This project describes COMPASS function during yeast meiosis and is compelling to a broad range of experts in the field. In support of this, the results described in this manuscript were the focus of an abstract that was accepted for an oral presentation at the premiere international genetics conference ("The Allied Genetics Conference"), which was cancelled due to COVID-19. Therefore, I am quite confident that the manuscript will garner editorial consideration and a rigorous peer review process. While peer reviews are highly likely, there are no standards for how long it will take to receive the reviews and prepare an adequate response to allow an accepted publication. The journal *Genetics* states on their website that the average length of time from initial submission to review is 35 days. However, based upon my previous experience and the ongoing COVID-19 pandemic, this process can take 4-6 months. The course release will provide time to address reviewers' concerns, perform any additional experimental work, and resubmit the edited manuscript for publication. If this manuscript is accepted and in print prior to the Fall 2021 semester, then I will focus all of my attention on completing Aim 2 of this proposal.

We have gathered most of the data and are in initial manuscript preparation stages for the second proposed Aim. The final version of this manuscript will include current and former Stockton undergraduates Kai Nguyen, Diana Sanchez-Zevallos, and Dominic Bates as coauthors. We are currently organizing large datasets and preparing figures for this manuscript. I anticipate completing this process by the end of May 2021 at which point I will address any experiments that need to be added to the study during the Summer 2021 and anticipate manuscript preparation to initiate in September 2021.

Methodology

Scientific publication requires that you document discoveries that your research has made and identify how your discoveries fit into the bigger picture biological question. When preparing a manuscript, my goal is to submit a clearly described and error-free document that reflects the care and attention that I dedicate to laboratory experiments. This approach also facilitates the review process, thus allowing the reviewers to focus on the content rather than carelessness. To accomplish this lofty goal, I dedicate a tremendous amount of focus and time that is typically not available when I am teaching a full course load.

To prepare a manuscript, I first need to analyze both quantitative and qualitative observations that were generated in the laboratory. Following data analysis, I construct figures and data tables which are the backbone of any scientific manuscript. After extensive review of the current literature, I then begin writing the Methods and Results sections of the manuscript. The Discussion section allows me to construct a model that integrates the discoveries that are described in the Results with current knowledge on the topic. Finally, the Introduction and Abstract sections are prepared based upon the 'story' that the manuscript is telling. Once the draft is completed, I perform rigorous and careful editing and revising. Following my review, I send the manuscript to colleagues that are willing to provide feedback. Typically, I will send the draft document to 2 or 3 individuals in a sequential manner. This allows the manuscript to get the close attention that it needs for submission.

The undergraduate students make contributions to each step of this process. They are most immersed in data analysis and figure preparation and are often surprised when they experience how much effort is put into preparing a professional-quality scientific manuscript. Despite the added time and effort that this takes, ensuring that undergraduates make contributions to manuscript preparation provides them with invaluable experience and understanding of what it means to work as a professional scientist. When I can dedicate my full attention to this process (for example during Summer months) I can initiate and submit a manuscript in a 4-6 week timeline. During the semester, the process is much longer due to the high demands of a full teaching load. This course release would therefore afford me the time that is needed to get this manuscript submitted for review.

Project Outcomes

This project will result in dissemination of research findings in high profile, peer-reviewed, journals that are sponsored by professional research societies. During previous academic terms, I have maintained full teaching loads, developed two upper-level biology courses and a general studies course, remained active in university and professional service, and mentored 22 undergraduate students engaged in independent research projects. I anticipated preparing and submitting the manuscript described in Aim 1 of this proposal during the Summer of 2020. This plan, and all face-to-face undergraduate research opportunities in my laboratory, were interrupted by the COVID-19 pandemic. Therefore, getting the undergraduate research projects back on track while completing these manuscripts will take a significant time dedication. This course release would allow me to continue to provide high-quality educational opportunities for undergraduate students both in the classroom and laboratory while bolstering my scholarly activity.

Literature Cited

Boa, S., C. Coert and H. G. Patterton, 2003 Saccharomyces cerevisiae Set1p is a methyltransferase specific for lysine 4 of histone H3 and is required for efficient gene expression. Yeast 20**:** 827-835.

Briggs, S. D., M. Bryk, B. D. Strahl, W. L. Cheung, J. K. Davie *et al.*, 2001 Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and rDNA silencing in Saccharomyces cerevisiae. Genes Dev 15**:** 3286-3295.

Dehe, P. M., and V. Geli, 2006 The multiple faces of Set1. Biochem Cell Biol 84**:** 536-548.

Krogan, N. J., J. Dover, S. Khorrami, J. F. Greenblatt, J. Schneider *et al.*, 2002 COMPASS, a histone H3 (Lysine 4) methyltransferase required for telomeric silencing of gene expression. J Biol Chem 277**:** 10753-10755.

Miller, T., N. J. Krogan, J. Dover, H. Erdjument-Bromage, P. Tempst *et al.*, 2001 COMPASS: a complex of proteins associated with a trithorax-related SET domain protein. Proc Natl Acad Sci U S A 98**:** 12902-12907.

Schneider, J., A. Wood, J. S. Lee, R. Schuster, J. Dueker *et al.*, 2005 Molecular regulation of histone H3 trimethylation by COMPASS and the regulation of gene expression. Mol Cell 19**:** 849-856.

CURRICULUM VITAE- Michael J. Law

Education

• Stockton University	B. S.	Biology	2000
• University of Southern California	Ph. D.	Biochemistry & Mol. Biol.	2006
• UMDNJ-Stratford	postdoc	Molecular Biology	2010

Professional positions

- Assistant Professor, Biology program, Stockton University, 2017-date
- Assistant Professor, Dept. of Molecular Biology, Rowan University, 2015-17
- Instructor, Dept. of Molecular Biology, UMDNJ/Rowan University, 2010-15
- Adjunct Professor, Biology program, Stockton University, 2007-09

Selected peer reviewed publications

Law, M. J. and M. A. Finger. 2017 The *Saccharomyces cerevisiae* Cdk8 mediator represses *AQY1* transcription by inhibiting Set1p-dependent histone methylation. *G3* (Bethesda) Mar 10;7(3)1001-10.

Lardenois A., E. Becker, T. Walther, **M. J. Law**, B. Xie, P. Demougin, R. Strich, and M. Primig. 2015 Global alterations of the transcriptional landscape during yeast growth and development in the absence of Ume6-dependent chromatin modification. *Mol Genet Genomics*. Oct; 290(5):2031-46.

Stuparevic I., E. Becker, **M. J. Law**, and M. Primig. 2015 The histone deacetylase Rpd3/Sin3/Ume6 compex repress an acetate-inducible isoform of *VTH2* in fermenting budding cells. *FEBS Letters* Apr 2;589(8):924-32.

Liu Y., I. Stuparevic, B. Xie, E. Becker, **M. J. Law**, and M. Primig. 2015 The conserved histone deacetylase Rpd3 and the DNA binding regulator Ume6 repress *BOI1's* meiotic transcript isoform during vegetative growth in *Saccharomyces cerevisiae*. *Mol Microbiol* May;96(4):861-74.

Becker E., Y. Liu, A. Lardenois, T. Walther, J. Horecka, I. Stuparevic, **M. J. Law**, R. Lavigne, B. Evrard, P. Demougin, M. Riffle, R. Strich, R. W. Davis, and M. Primig. 2015 Integrated RNA- and protein profiling of fermentation and respiration in diploid budding yeast provides insight into nutrient control of cell growth and development. *J. Proteomics* Apr 24;119:30-44.

Law M. J. and K. Ciccaglione. 2015 Fine-tuning of Histone H3 Lys4 Methylation During Pseudohyphal Differentiation by the CDK Submodule of RNA Polymerase II. *Genetics* Feb: **199(2)**: 631-42.

Selected peer reviewed abstracts

Trainor, B. M., K. Ciccaglione, M. Czymek and **M. J. Law** 2020
"Using COMPASS to navigate through meiosis"
Platform presentation, *The Allied Genetics Conference* Washington, D. C.

Cunha, B. K. Ciccaglione, D. Sanchez-Zevallos, K. Nguyen, and **M. J. Law** 2020
"Genetic and metabolic analyses of cell fate decisions"
Poster, *The Allied Genetics Conference* Washington, D. C.

Romanowksi, J., M. Elko, D. Stoyko, and **M. J. Law** 2020

"Analyses of double-stranded DNA break repair in space"
The Allied Genetics Conference Washington, D. C.

Trainor, B. M., K. Ciccaglione, M. Czymek and **M. J. Law.** 2018
"Distinct roles for COMPASS subunits Swd1 and Swd3 during yeast meiosis".
Poster, *Yeast Genetics Meeting* Stanford University, CA

Law, M. J. and M. A. Finger. "Controlling methylation during cell fate determination" 2016
Poster, *The Allied Genetics Conference* Orlando, FL

Selected extramural Grant funding
Pending
National Institutes of Health Academic Research Enhancement Award (R15)- *Principal Investigator* "Control of Histone Methylation During Differentiation" 3 years- $300,00 direct costs
Impact score: 32 (fundable impact score; final budget decision pending)

National Science Foundation Integrative Research in Biology- *Co-Principal Investigator* "Mechanisms and consequences of the urban ant feeding syndrome: a multi-scaled approach"
4 years- $2,000,000 direct costs

Selected service
Service to the Biology program
Biology program assessment committee 2020-date
Working group member 2019-date
Genetics; Cells and Molecules
Search committee-tenure track Microbiology 2019 and 2020
Search committee-sabbatical replacement 2018

Service to Stockton University
Health Professions committee member 2020-date
Undergraduate Research Council member 2018-date
Vice-Chair, Academic Programs and Planning 2019-date
Faculty senate member at-large 2018-date

Service to the Scientific Community
Steering committee member 2020-date
BREWMOR- Bridging Research and Education with Model Organisms
Scientific Advisory Board member 2013-18
Saccharomyces genome database; www.yeastgenome.org
Ad Hoc Reviewer
Database 2016
BMC Genomics 2016
PLoS One 2013

Service to the community
Judge, *Jersey Shore Science Fair* 2013-date
Stockton University
Instructor, *Boy Scout Merit Badge Academy* 2017-date
Stockton University

Internal Funding Application – Dean's Approval

Faculty Name: Michael Law

Type of Funding: Course Release

Amount Requested: _____

Title of Project: Epigenetic regulation of cell fate decisions

◉ **R&PD:** By signing this proposal, I indicate my support for the requested release time.

○ **Sabbatical:** By signing this proposal, I acknowledge the request for Sabbatical Leave.

○ **Provost Faculty Opportunities Fund & Adjunct Faculty Opportunity Fund:** This project encourages cost share with the applicant's School, if possible. Whether or not the proposal includes a commitment of funds from the School, the applicant's Dean must indicate support for the request. By signing this proposal, I indicate my support for the request and identify my commitment to a financial contribution to this project in the amount of $_____.

○ **General Acknowledgement:** By signing this proposal, I acknowledge the above request for funding.

Dean's Signature: _____

Date: 3/11/21