Title- Dissecting COMPASS, a molecular machine that controls cell fates

Introduction

This application requests $8299 for molecular biology supplies and services for gene sequencing for a project that will take an entire academic year. This specific project is focused on understanding how cells make important decisions about their fates. It is one of the main focuses of research in my lab.

Project background and aims

Every cell in our body contains identical blueprints (DNA), but does not have the same appearance. This is because as cells mature, they can change their appearance while they are making important decisions about their ultimate fate. Cell fate decisions involve complex processes that require cells to first sense their environment and then make a decision based on their interpretation of the environmental signals. The environmental interpretation is limited by the cell's identity, thus restricting the possible decisions in their repertoire. For example, a major decision that cells must make during their lifetimes is whether to divide (replicate), making an exact replica of themselves, or to differentiate and become a new cell type. Mistakes in this critical decision can lead to developmental defects or cancers.

In order to understand the complexities of the cell fate decision-making process, my laboratory relies on the budding yeast *S. cerevisiae* as an experimental model. While yeast are simple cells that you can use to make bread or beer, they share relationships with human cells that have furthered our understanding of many diseases. Importantly, research using yeast has allowed scientists to understand cell fate decisions. Due to their simplicity, yeast are ideally suited for research involving undergraduates as they are easy to grow and to manipulate.

When cells have made the decision to transition from replication to differentiation, they must remodel their interior to accommodate new tasks. If you think about cells as manufacturing plants, then they must take the existing machines in their assembly line and retool them to make new products. Cells can do this by changing how their molecular machines function during replication to accomplish new jobs for differentiation. By remodeling their molecular machines, cells avoid the need to invest valuable resources into making an entirely new factory. By asking questions about which parts of the molecular machines are essential to the remodeling process, my laboratory has uncovered new functions for a molecular machine called COMPASS.

The COMPASS protein complex is a well-characterized molecular machine that is critically important for the correct interpretation of environmental signals. Molecular machines are built out of proteins that are regulated based on cell signaling. COMPASS is composed of seven proteins, with a protein called Set1 responsible for all machine activity. Most studies of COMPASS have focused on its importance for replication. However, my research has started to investigate how COMPASS proteins must change to accommodate the decision to differentiate.

This research project will work on characterizing COMPASS using yeast differentiation as an experimental system. We will examine COMPASS activity by focusing on two Aims:

1. Identify how COMPASS is remodeled during yeast differentiation.
2. Determine if COMPASS protein destruction is part of its remodeling.

Background work accomplished

My expertise includes analysis of protein behavior during time course experiments of yeast differentiation (see CV). My previous research has found new requirements for specific members of COMPASS during yeast differentiation. These findings were the focus of a previous National Institutes of Health grant submission and a poster presentation at the 2018 Yeast Genetics Meeting. After careful consideration of the feedback from these experiences, I have

identified follow-up experiments that will clarify our molecular understanding of COMPASS behavior.

My lab has identified which parts of the COMPASS complex are reorganized during yeast differentiation. To do this, we have used an approach that eliminates specific proteins of COMPASS using genetic deletions. We then look to see if the mutant yeast is capable of completing the differentiation process. If it is unable to complete the process, the protein deleted is most likely an essential part of this process. This simple experimental approach has been executed by undergraduate research students and has led to some surprising discoveries for COMPASS protein function. I, along with my undergraduate researchers, wish to perform follow-up analyses on these results by specifically measuring COMPASS re-organization during the differentiation process (Aim 1). To accomplish this Aim, I will leverage my previous successes using highly sensitive molecular measurements.

In addition to identifying new roles for COMPASS proteins during differentiation, I have also observed destruction of the critical COMPASS protein Set1. Cells will frequently destroy proteins during differentiation to ensure that there is no backtracking after meeting important milestones. Our results suggest that destroying Set1 is a critical part of executing the differentiation program efficiently. I wish to assess if this is true by arresting cell differentiation at various milestones and then measuring if Set1 is still destroyed in the same way (Aim 2).

Statement of Procedures/Methodology

Aim 1- Identify how COMPASS is re-organized during differentiation

In this Aim, I will couple my expertise in yeast differentiation with a technique that I have previously published called chromatin immunoprecipitation (ChIP). In general, there are two ways that you can measure proteins; you can either measure protein abundance or measure protein location. While both approaches have their advantages, ChIP is appropriate to determine protein location along each chromosome. My lab has successfully performed ChIP analyses for Set1 (Law and Finger, 2017) and will extend these published results by determining how Set1 is moved during differentiation.

To analyze ChIP experiments you typically need to have some idea of where the protein may be located. Since the goal of our experiment is to identify how Set1 moves to *new, previously unknown* locations during differentiation, I need to use an unbiased analysis approach. Therefore, I will merge our ChIP experiments with next-generation sequencing (ChIP-seq). When coupled to ChIP, next-generation sequencing allows you to measure protein abundance along every chromosome in the cell simultaneously. I have recently published ChIP-seq analyses and therefore do not anticipate any significant issues in this approach (Law and Finger, 2017). In this Aim, students will be involved in data analyses and interpretation, as it requires a minimal understanding of genetics and computational approaches for successful completion.

Aim 2- Determine if COMPASS protein destruction is part its remodeling

I have observed that Set1 is destroyed as cells commit to complete differentiation. This is somewhat of a paradox since yeast that do not have any Set1 fail to differentiate. My interpretation of these incongruent results is that Set1 is important for cells to meet early milestones in differentiation. Once it has done its job, Set1 can then be destroyed. In this Aim, student researchers will measure Set1 protein abundance using a technique called Western blot. Set1 protein will be measured after yeast are induced to differentiate. Students will arrest yeast differentiation at various milestones using chemical inhibitors and determine if arresting differentiation prevents Set1 destruction. The results of this Aim will tell us if Set1 destruction is integrated into the differentiation program as a marker for specific milestones.

Timeline

Fall 2019- Perform ChIP for Set1 at three time points during yeast differentiation. Send ChIP samples to an outside facility (such as Genewiz) for next-generation sequencing

Spring 2020- Perform Western blot analysis of Set1 for yeast induced to differentiate and treated with chemical inhibitors of differentiation

Computational analysis of next-generation sequencing data

Present research findings at NAMS symposium and The Allied Genetics Conference

Summer 2020- Prepare NIH grant re-submission

Project Importance

The COMPASS complex is one of the most well-studied of all protein complexes in the cell. Its functional conservation and importance in many cellular processes are of significant interest to the scientific community. The proposed work will provide insight into how COMPASS behavior is regulated during differentiation, which may translate into other organisms such as humans. Our results will provide a definitive model for how COMPASS rearrangement and protein degradation are integrated into the differentiation program. Regardless of our results, we will learn more about this important protein complex thus guiding future work in the laboratory.

Project Outcomes

There are several significant outcomes from this work that will contribute to my research and professional development:

1. Dissemination of research findings at venues such as the NAMS research symposium and The Allied Genetics Conference in Washington D. C., April 2020.
2. Data will be central in the re-submission of a grant proposal to the National Institutes of Health.
3. Data will appear as part of a manuscript with student coauthors focused on COMPASS during differentiation to be submitted to a journal such as *Genetics*.
4. Involvement of undergraduate research students in all facets of the work including experimental design, execution, interpretation, and dissemination of findings.
5. Significant contributions will be made to specific aspects of my faculty plan. I will accomplish goals set out in my scholarship section (*Goal 1*. Apply for internal and external funding, *Goal 3*. Present research findings in symposia, scientific meetings, and as a guest speaker, and *Goal 4*. Involve undergraduates in research).

Dissemination of the results

Undergraduate students will present our results at the NAMS research symposium. In addition, I will submit an abstract with student coauthors to present results at The Allied Genetics Conference in April 2020. The coauthors will have the opportunity to present their findings at this international research meeting that attracts scientists who utilize different model organisms to understand fundamental biological processes. Given the general interest in COMPASS behavior in all of these models, I anticipate many positive interactions will colleagues from diverse scientific backgrounds.

In the longer term, I anticipate that results from this study will appear in a scientific manuscript. Since the experiments described in this proposal are a natural outgrowth of current research in the lab, they will add important data to this ongoing research which will be critically important for manuscript completion.