Proposal for TCNJ Mentored Undergraduate Summer Experience (MUSE)
Summer 2010

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Number of Years at TCNJ:	0 years, 7 months
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Title:	**Investigating the coordination of multiple steps in gene expression**
Number of Student Collaborators:	2

Project and Learning Plan:

I. Intellectual Merit:

The major goal of my research is to understand how the various cells in an organism regulate gene expression to adapt to different environments or situations. Regulation of gene expression is critical to a cell's function and includes a step known as RNA processing. An RNA molecule is initially synthesized from DNA (which stores the genetic information of an organism) through a process known as RNA synthesis. Once made, RNA serves as a "working copy" of the genetic instructions, which are utilized to construct all of the proteins a cell needs to function. Before it can be used, however, a newly synthesized RNA molecule must be modified (processed) in several steps. These processing steps, which I liken to an "RNA assembly line", must occur precisely to prevent the production of abnormal proteins that are either non-functional or even harmful. Indeed, mutations in our genes that lead to imprecise RNA processing underlie numerous human disorders, including cancer. My work involves a combination of genetic, molecular cell biology and biochemical approaches that use yeast as a model organism.

The accuracy of gene expression depends, in part, on effective coordination of the various steps in RNA processing with each other and with initial RNA synthesis. However, the underlying mechanisms that orchestrate these steps are poorly defined. This coordination undoubtedly involves specialized proteins that mediate the interaction of RNA molecules with larger protein complexes that modify the RNA. My recent work has identified two likely candidates, the Npl3 and Snu66 proteins. Both Npl3 and Snu66 play an important role in an RNA processing step called RNA splicing, during which discrete segments of an RNA molecule are pieced together in different ways to encode different types of functional proteins. In addition, recent work suggests that Npl3 and Snu66 may coordinate the synthesis and splicing of an RNA molecule. This is an exciting area of molecular cell biology that has been largely unexplored. The overall goals of this MUSE summer research are to:

(1) Further define the mechanism of Npl3 in coordinating RNA splicing with RNA synthesis (to be carried out by Daniel Mitchell)

(2) Begin to characterize the role of Snu66 in the coordination of RNA splicing with RNA synthesis (to be carried out by Christine Scaduto)

Project #1: Define the mechanism of Npl3 in coordinating RNA synthesis and RNA processing. To further characterize Npl3's role in this process we will determine the specific portions of Npl3 that interact with the RNA splicing machinery and those that interact with the RNA synthesis protein Bre1. To do this, Daniel will finish constructing a set of Npl3 proteins that contain chemical alterations ("mutations") in different portions of the protein and then test, using biochemical and molecular biological assays, (1) whether these mutations abolish RNA synthesis or RNA splicing and (2) whether these modification alter the interactions of Npl3 with the cellular proteins that mediate RNA splicing or RNA synthesis. By the end of MUSE, Daniel will have collected preliminary data identifying which regions of the Npl3 protein are important for its function in RNA splicing and which regions contact the RNA synthesis machinery.

Project #2: Characterize the role of Snu66 in coordinating RNA splicing with RNA synthesis. Christine recently demonstrated that Bre1 interacts genetically with Snu66, the RNA synthesis protein that interacts with Npl3. We now plan to further investigate how Snu66, Npl3 and Bre1 might function together to coordinate RNA processing with RNA synthesis. Christine will use molecular biological techniques to (1) test whether mutation of the *NPL3*, *SNU66*, and

BRE1 genes affects the splicing of a similar subset of RNAs, (2) test whether combining two mutations, for example by creating mutations of both *SNU66* and *BRE1* in the same yeast strain, will causes a more severe splicing defect than that seen in strains carrying either single gene mutation as predicted, and (3) test using biochemical experiments whether Snu66 can bind Npl3 and/or Bre1. By the end of the summer, I expect that Christine will have determined whether Npl3, Bre1 and Snu66 target the same RNAs and will have collected preliminary data on the interactions between Npl3, Snu66 and Bre1.

Most importantly, by the end of the summer Christine and Daniel will have made significant headway on the projects that they plan to continue during the 2010/2011 academic year as they complete Independent Research in my laboratory.

II. Role of the student(s) and mentor:

I have chosen Christine and Daniel to work with me in the MUSE program for the following reasons: 1) to make significant progress on this research I will need the help of two students as this research is labor intensive; 2) both of these projects will employ a similar approach so the students will be able to hold constructive discussions about their projects; 3) Christine is currently completing independent research in my laboratory, therefore she will be able to serve as a mentor to Daniel. She is remarkably independent and by summer she will be well versed in both the scientific literature and the techniques required to complete these projects; 4) Daniel has completed a full year of Genetics/Advanced Genetics laboratory and has experience in techniques that are similar to those that we employ in my laboratory.

Each student will be actively engaged in all aspects of his/her project under my direct supervision and guidance. In addition to research in the lab, I plan to meet with the students independently twice each week to discuss their results and to plan future experiments, and to hold weekly group meetings where Daniel and Christine will discuss their findings with each other. In addition, Dr. Killian (who is also applying for MUSE) and I plan to hold joint group meetings every two weeks so that our students may present their work in a more formal setting. Finally, the students will read and evaluate papers from the secondary and primary literature that are related to their individual projects in a "journal club" format. All of these planned activities will allow the students to learn to keep an organized laboratory notebook and develop the critical thinking and communication skills that are essential for a career in science.

III. Broader impacts:

I am a new pre-tenure faculty member at TCNJ, therefore having summer students supported by the MUSE program would provide me the opportunity to make significant progress on my research program. This research topic has been relatively unexplored, therefore I expect that the data generated by Christine and Daniel will be sufficient to apply for Federal funding next fall. In addition, their continued work during the 2010/2011 academic year should lead to a manuscript (s) that I will submit to a peer-reviewed journal within the next year.

Both Christine and Daniel plan to pursue careers in biological research, and MUSE will provide them the opportunity to initiate long-term research projects in my lab. This experience will give them a taste for what an exciting career in research science entails. I plan to have them present their findings at the Cold Spring Harbor RNA Processing meeting in 2011. This summer experience will not only prepare them for the year of Independent Research but will be beneficial for their future studies both here at TNCJ and in their post-graduate education experiences.