Name: Tracy L. Kress

Email address: kress@tcnj.edu

Department or Program: Biology

Title of Proposal: **"Molecular Coupling: delineating the mechanisms by which multiple steps of gene expression are coordinated"**

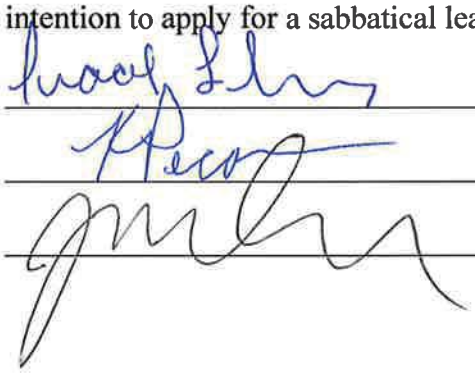
Date: October 10, 2016

Month and year of your first appointment at The College of New Jersey: August 2009

Dates of your previous sabbatical leaves: N/A

Current Application is for:
____Fall
_X_Spring
____Either Fall or Spring
____Full Academic Year

I have notified my dean and department chair (or program coordinator, as applicable) of my intention to apply for a sabbatical leave:



_____Applicant (signature)

_____Chairperson/Coordinator (signature)

_____Dean (signature)

2. Project Proposal:

A. Description of Scholarly Program: My research aims to understand a fundamental question in molecular biology relevant to all organisms: how do cells regulate the expression of their genes? Regulation of gene expression is critical for the generation of the different cell types, tissues, and organs in complex organisms, and also for cells within the body to be able to respond to changes in their environment. My work focuses on RNA, a molecule that serves as a "working copy" of the genetic instructions. RNA is synthesized using a template made of DNA (the molecule that stores all of the genetic information of an organism), and these instructions are then utilized to construct the various proteins that the cell needs to function and respond to internal and external environmental cues. Before it can be used, however, a newly synthesized RNA molecule must be chemically modified/processed in several ways. These processing steps must be executed with precision to prevent the production of abnormal proteins that are either non-functional or even harmful to the cell. Indeed, mutations in our genes that lead to imprecise RNA processing are known to underlie numerous human disorders, including cancer [1-5]. I investigate RNA processing through a combination of genetic, molecular cell biology and biochemical approaches that use yeast as a model organism. The molecules involved in RNA processing are highly similar between yeast and humans, so information learned from studying yeast can directly further our understanding of gene expression in humans.

Efficient and precise gene expression depends, in part, on effective coordination of RNA synthesis with the downstream steps in RNA processing to ensure that only functional RNA molecules (and later, proteins) are produced. This coordination is similar to that of a car assembly line where assembly steps can occur simultaneously in a coordinated manner; importantly, there are quality control checkpoints that allow the system to assess whether assembly is proceeding as expected and then shut it down if an error has occurred. The underlying mechanisms that orchestrate the coordination and quality control in the "gene expression assembly line" have remained elusive. Undoubtedly such coordination involves *specialized proteins* that mediate the interaction of RNA molecules with the cellular machines that perform the synthesis and processing steps. This is an exciting area of molecular cell biology that has been largely unexplored. My recent work has identified several candidate proteins that have potential to link the RNA synthesis step with an RNA processing step called RNA splicing [6-8]. During RNA splicing, discrete segments of an RNA molecule are pieced together in different ways to allow the production of different types of proteins. Using genetic techniques I have discovered that proteins that play important roles in RNA splicing have interactions with two other protein complexes (called NuA4 and Swr1) that regulate RNA synthesis, which strongly suggests that these proteins might work together in cells to coordinate RNA synthesis and RNA splicing (Figure 1). NuA4 and Swr1 have specific biochemical activities that allow them to help open the normally compacted DNA molecule to facilitate the movement of the RNA synthesis enzyme along the DNA template [9-15] (and hence help increase the rate of RNA synthesis). Thus, NuA4 and Swr1 are perfectly poised to coordinate RNA synthesis and RNA splicing.

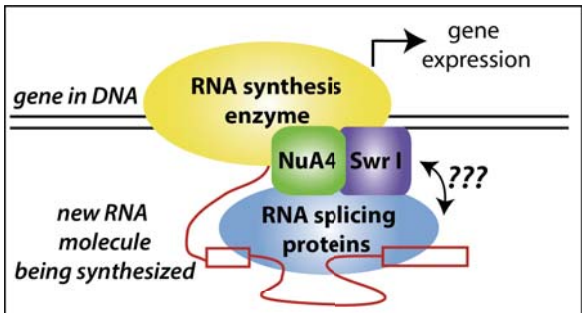


Figure 1: Model for the coordination of RNA synthesis and splicing via the NuA4 and Swr1 proteins.

Over the last few years, I have completed experiments to test the hypothesis that NuA4 and Swr1 work with splicing proteins to coordinate RNA synthesis and splicing. For example, I completed experiments to show that deletion of the NuA4 or Swr1 proteins block RNA splicing. In addition, I gathered preliminary data to show that mutations that block RNA splicing reduce the association of NuA4 with DNA, which could lead to changes in the rate of RNA synthesis. **My current objective is to investigate the mechanistic details of this coordination. Specifically, I plan to (1) to determine the mechanism(s) by which NuA4 and Swr1 regulate RNA splicing and (2) determine the extent to which RNA splicing can promote NuA4 and/or Swr1 association with DNA and/or enzymatic activity to modulate RNA synthesis.**

I was recently awarded a highly competitive National Institutes of Health AREA (R15) grant to help fund these studies. To achieve the goals of this grant and thus maximize the chances of getting refunded in my next application cycle, I need an extended period to time to dedicate to my scholarly work. Importantly, my sabbatical will allow me time to travel to work with collaborators, as critical aspects of my research must be carried out in their labs (*see attached letters of support*). First, I will carry out genome-wide splicing analyses at Cornell University; these are technically sophisticated experiments that I cannot carry out at TCNJ. Second, I will travel to Dr. Jacques Cote's laboratory at Université Laval (Quebec City, Canada) to learn a new suite of techniques that I can then establish in my lab at TCNJ. Together, my work complete at TCNJ and in my collaborators' labs will enable me to achieve the goals of my grant and to be competitive for my next application.

B. Methodology/Approach:

Project #1: To determine the mechanism by which NuA4 and Swr1 regulate RNA splicing.
NuA4 and Swr1 might coordinate RNA splicing with RNA synthesis by recruiting the splicing proteins to an RNA molecule as it is being synthesized (Figure 1). This would serve to increase the efficiency of the production of the RNA molecule, as it would allow its synthesis and splicing to occur simultaneously. Our previous studies showed that deletion of either NuA4 or Swr1 caused a block in RNA splicing. While these studies were informative, they are limited in two ways. First, deletion mutations are a blunt way to remove an entire complex but it does not allow for detailed studies of enzymatic activity of the complex. Second, we only analyzed the splicing of a handful of candidate RNAs. Thus, I plan to test whether the *enzymatic activity* of the NuA4 and Swr1 complexes, which decompact DNA in a specific manner, are required for RNA splicing. I will complete genome-wide splicing analyses (to simultaneously monitor the splicing of ~300 RNA molecules) in yeast strains that harbor mutations in NuA4 and Swr1 that abolish enzymatic activity. These studies will be completed in Jeffrey Pleiss' lab at Cornell University and I will carry out additional, distinct experiments at TCNJ to confirm our results.

In spring 2017, I will begin to utilize biochemical techniques at TCNJ to determine if NuA4 and/or Swr1 can physically interact with specific splicing proteins in collaboration with John Allison in the TCNJ Chemistry Department. These experiments will help me to identify specific splicing proteins to include in subsequent experiments. I will test whether mutations that abolish NuA4 and Swr1 enzymatic activity reduce the recruitment of specific splicing proteins to an RNA molecule that is being actively synthesized. I predict that the recruitment of the specific splicing protein will be reduced in the NuA4 and/or Swr1 mutant yeast, which would ultimately

slow or stop RNA splicing. I have constructed specialized yeast strains that contain mutations in either NuA4 or Swr1 and also include splicing proteins that have a special chemical tag (HA-tag) that allows us to biochemically purify the splicing protein and test whether it is associated with RNA and with NuA4 or Swr1.

Project #2: Determine the extent to which RNA splicing can promote NuA4 and/or Swr1 enzymatic activity or association with DNA to modulate RNA synthesis. A model in which NuA4 and Swr1 interact with splicing proteins provides an opportunity for a quality control checkpoint in the "gene expression assembly line". If my proposed model is correct, then an error in RNA splicing should lead to a shutdown of RNA synthesis. Indeed I have completed preliminary experiments to show that perturbing splicing lowers the binding of NuA4 to the DNA molecule, which likely leads to reduced RNA synthesis. To test this directly, at TCNJ I will perturb RNA splicing by making mutations in the splicing proteins and use biochemical techniques to test whether RNA synthesis is reduced. This is determined by measuring the precise amounts of particular RNA molecules in yeast that harbor splicing protein mutations and comparing the amounts to those in yeast that do not contain any mutations.

If I find that perturbation of splicing can reduce RNA synthesis, it will be important to test whether this is due to an effect on the NuA4 or Swr1 proteins. A reduction in RNA synthesis could be due to two, not mutually exclusive, possibilities. First, it is possible that NuA4 and Swr1 recruitment to the DNA during RNA synthesis could be reduced when splicing is perturbed. In this scenario a splicing protein could help to stimulate recruitment of NuA4 or Swr1 to DNA or stabilize the NuA4 or Swr1 interaction with the DNA molecule. Our early preliminary studies revealed that NuA4 binding is impacted when splicing is inhibited. I plan to make additional mutations in splicing proteins to perturb splicing and biochemical techniques to (1) confirm that NuA4 binding to DNA is impaired and (2) test whether Swr1 binding to DNA is impacted. If my model is correct I should observe reduced binding.

An alternative model could be that NuA4 and Swr1 bind perfectly well to DNA but instead these enzymes have reduced activity in opening the DNA and stimulating RNA synthesis. In this scenario, a splicing protein could interact with NuA4 or Swr1 to stimulate its biochemical activity. To test this possibility, I will utilize the yeast strains that harbor mutations in splicing proteins to test for specific biochemical activities of NuA4 and Swr1. For example, I can measure the level of modification and compaction of the DNA. I predict that the activity of NuA4 or Swr1 would be reduced when splicing is perturbed. This is a new line of experimentation that I am eager to establish in my own research laboratory, as the ability to bring these approaches to my lab at TCNJ will vastly improve my competitiveness for future funding. Thus, I will travel to Dr. Jacques Cote's laboratory at *Université Laval* (Quebec City, Canada) where I will train with his team and also complete an initial set of experiments that I can build upon once I return to TCNJ.

Significance: My proposed research on NuA4, Swr1 and RNA splicing proteins provides an exciting avenue to explore the molecular mechanisms that coordinate RNA splicing and RNA synthesis. Very little is known about these mechanisms, and even less is known about the proteins that are involved in the coordination process. Therefore my findings will significantly advance our knowledge in this new, exciting field of molecular biology. I was awarded a NIH

AREA (R15) grant to complete these studies and the sabbatical award period will afford me the time to meet the goals of my grant which will help me to be to be competitive for future funding.

C. Schedule: In order for me to meet the goal outlines above, I need an extended period of time to dedicate 100% of my efforts to my scholarly work, thus I am requesting a single semester sabbatical. This length of time, combined with the winter and summer breaks (total of 6-7 months), will be sufficient for me to complete proposed experiments at TNCJ, to travel to complete experiments and analyze data collected in my collaborators labs, and to set-up new lines of experimentation in my research lab at TCNJ. In addition, it will allow me time to write a manuscript on Project #1 and to begin my NIH AREA (R15) grant renewal application.

Time Period	**Plan**
Spring/Summer 2017	Initiate experiments in collaboration with John Allison in support of Project #1.
Winter Break 2017/18	Create any additional yeast strains required for analyses (based on work completed in 2017); Begin experiments in support of Project #1 and #2
February 2018	Travel to collaborators labs at Cornell University (Project #1)
March 2018	Travel to *Université Laval* (Project #2) to complete experiments and learn new techniques
April- May 2018	Establish new line of experiments learned in Cote lab in my own research lab at TCNJ
Summer 2018	Write manuscript on Project #1; Continue experiments in support of project #2
Academic Year 2018/19	Continue experiments for project #2 and begin writing my NIH AREA (R15) renewal application (to be submitted in spring/summer 2019).

D. Outcomes and Value: This sabbatical period is essential for me to achieve goals that I have set forth in my NIH AREA (R15) grant. The time afforded will provide me the opportunity to 1) complete experiments that cannot be completed at TCNJ due to the need for specialized equipment and 2) to learn new techniques that I will establish in my own lab at TCNJ that will allow me to explore a new research avenue. I will travel to my collaborator Jeffery Pleiss' lab at Cornel University to complete genome-wide analysis of splicing using the technically sophisticated RNA sequencing technique to analyze RNA splicing patterns in a panel of yeast mutant strains. **These experiments are crucial for me to write a manuscript on project #1 that I plan to submit in summer 2018.** In addition, I will travel to Jacques Cote's lab at Université Laval to learn new techniques that will allow me to monitor the enzymatic activity of the NuA4 and Swr1 complexes to support project #2. **Finally, the goals proposed for the sabbatical period are critical in order for me to be competitive for my NIH AREA (R15) renewal application in spring/summer 2019.**

While visiting and completing experiments in my collaborators labs I will participate in laboratory group meetings where I will engage deeply in discussion of my work and I plan to deliver formal seminars at both Universities. I also plan to attend two national scientific meetings (the American Society for Biochemistry and Molecular Biology in April 2018 and the Cold Spring Harbor pre-mRNA processing Meeting in August 2018) to present my findings.

References:

1. Zink LM, Hake SB. Histone variants: Nuclear function and disease. Curr Opin Genet Dev 2016; 37:82-89.

2. Zhang J, Manley JL. Misregulation of pre-mRNA alternative splicing in cancer. Cancer Discov 2013; 3:1228-1237.

3. Judes G, Rifai K, Ngollo M, Daures M, Bignon YJ, Penault-Llorca F, Bernard-Gallon D. A bivalent role of TIP60 histone acetyl transferase in human cancer. Epigenomics 2015; 7:1351-1363.

4. Farria A, Li W, Dent SY. KATs in cancer: Functions and therapies. Oncogene 2015; 34:4901-4913.

5. Chen J, Weiss WA. Alternative splicing in cancer: Implications for biology and therapy. Oncogene 2015; 34:1-14.

6. Kress TL, Krogan NJ, Guthrie C. A single SR-like protein, Npl3, promotes pre-mRNA splicing in budding yeast. Mol Cell 2008; 32:727-734.

7. Moehle EA, Ryan CJ, Krogan NJ, Kress TL, Guthrie C. The yeast SR-like protein Npl3 links chromatin modification to mRNA processing. PLoS Genet 2012; 8:e1003101.

8. Sorenson MR, Jha DK, Ucles SA, Flood DM, Strahl BD, Stevens SW, Kress TL. Histone H3K36 methylation regulates pre-mRNA splicing in saccharomyces cerevisiae. RNA Biol 2016:0.

9. Allard S, Utley RT, Savard J, Clarke A, Grant P, Brandl CJ, Pillus L, Workman JL, Cote J. NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. EMBO J 1999; 18:5108-5119.

10. Altaf M, Auger A, Monnet-Saksouk J, Brodeur J, Piquet S, Cramet M, Bouchard N, Lacoste N, Utley RT, Gaudreau L, Cote J. NuA4-dependent acetylation of nucleosomal histones H4 and H2A directly stimulates incorporation of H2A.Z by the SWR1 complex. J Biol Chem 2010; 285:15966-15977.

11. Auger A, Galarneau L, Altaf M, Nourani A, Doyon Y, Utley RT, Cronier D, Allard S, Cote J. Eaf1 is the platform for NuA4 molecular assembly that evolutionarily links chromatin acetylation to ATP-dependent exchange of histone H2A variants. Mol Cell Biol 2008; 28:2257-2270.

12. Cheng X, Auger A, Altaf M, Drouin S, Paquet E, Utley RT, Robert F, Cote J. Eaf1 links the NuA4 histone acetyltransferase complex to Htz1 incorporation and regulation of purine biosynthesis. Eukaryot Cell 2015; 14:535-544.

13. Kobor MS, Venkatasubrahmanyam S, Meneghini MD, Gin JW, Jennings JL, Link AJ, Madhani HD, Rine J. A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. PLoS Biol 2004; 2:E131.

14. Krogan NJ, Keogh MC, Datta N, Sawa C, Ryan OW, Ding H, Haw RA, Pootoolal J, Tong A, Canadien V, Richards DP, Wu X, Emili A, Hughes TR, Buratowski S, Greenblatt JF. A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. Mol Cell 2003; 12:1565-1576.

15. Mizuguchi G, Shen X, Landry J, Wu WH, Sen S, Wu C. ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science 2004; 303:343-348.