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Systems Biology/Microbiology Faculty Search
Department of Biology
Indiana University

Dear Dr. Brun:

I would like to apply for the faculty position you recently advertised in *naturejobs*. I am a post-doctoral fellow at Harvard Medical School working under the direction of Dr. Steve Buratowski. I believe that my teaching and tutoring experience, combined with my research background, makes me a strong candidate for the position.

The regulation of gene expression must occur in a precise manner to allow survival. In eukaryotes, synthesis of mRNA is synchronized with a series of modifications that give rise to a mature mRNA. Using *Saccharomyces cerevisiae,* I study the mechanism of transcription termination and 3'end mRNA formation. Specifically, I have found that the SR (serine/arginine) protein, Npl3 acts as an anti-terminator antagonizing an HRP factor (Hrp1) in 3'-end formation and termination (Bucheli and Buratowski, EMBO 2005). The model I proposed highlights the importance of RNA in the termination and 3'end processing of transcripts. Future studies will address in more detail subsequent steps in the termination mechanism, plus the role of other RNA binding proteins in transcription termination and mRNA processing.

Throughout my scientific career I have taken advantage of opportunities to teach and mentor students. In addition, I tutored introductory biology courses to students at a neighboring institution (Simmons College), and I was a teaching fellow for the core biology course "Genetics and Genomics" at Harvard University. These experiences have been instrumental in shaping my interests. Teaching has become a main component of my career goals. In addition to classroom teaching, I look forward to continue my research program as a way to involve students in hands-on learning. Yeast provides an excellent, and rather inexpensive, experimental system that I can use to teach students about the beauty of science.

I am currently in the process of submitting a K01 Career Development Grant to the National Cancer Institute – National Institutes of Health. This grant has the full support of my mentor, Dr. Steve Buratowski and a co-mentor, Dr. Kevin Struhl. If granted, this award will provide a laboratory start-up package, which will allow me to transition comfortably into a new faculty position.

Enclosed in this application I have included my *curriculum vitae,* teaching statement, research proposal and letters of recommendation. I have asked Dr. Steve Buratowski (post-doctoral advisor), Dr. Kevin Sweder (thesis advisor), and Dr. Kevin Struhl to write letters of recommendation. Thank you for your consideration and I look forward to hearing from you.

Most Sincerely,

Miriam Bucheli

Miriam E. Bucheli, Ph.D.

RESEARCH PROPOSAL

The regulation of gene expression must occur in a precise manner for the proper growth and development of any organism. In most cases known, this regulation occurs at the level of production of mRNA, or transcription. Many human diseases, including some cancers, are caused by mutations that impair normal transcription. In eukaryotes ranging from yeast to humans, the synthesis of mRNA by RNA polymerase II (RNApII) is synchronized with a series of modifications that give rise to a mature mRNA. Upon transcription initiation, the 5'-ends of nascent transcripts are capped and as transcription proceeds through elongation, the mRNA is packaged into ribo-nucleoprotein particles (RNPs) for transport out of the nucleus. As RNApII elongates, splicing of intron containing mRNAs and ligation of exons also occurs[1]. Past the 3'end of a gene, mRNA cleavage occurs at a specific site, and is followed by the addition of a tail of adenosines. It is becoming evident that the production (transcription) and processing (capping, cleavage and polyadenylation) of an mRNA are coupled events. Thus, mRNAs are co-transcriptionally processed before transcription is terminated. My research is focused on the last step of transcription, or termination, and the corresponding mRNA 3'-end processing event, and how these two events are coupled.

The coupling of transcription termination by RNApII to 3'-end processing is conserved throughout eukaryotes. While significant progress has been made in understanding this essential aspect of gene expression, many fundamental aspects remain to be discovered. Cleavage and processing of 3' ends requires the recognition and binding of cleavage factors I and II (CF I and CF II) to specific sequence elements on the nascent mRNA, followed by endonucleolytic cleavage at this site. Subsequently, the pre-mRNA is polyadenylated by three factors: CF I, polyadenylation factor I (PF I) and polyA polymerase (PAP)[2,3]. In contrast to this level of understanding about 3' processing and polyadenylation, less is known about the mechanism of transcription termination, the event that occurs downstream of the 3' processing site. There are currently two models that explain how transcription termination might occur. The anti-termination model postulates that upon the emergence of signals (polyA) on the nascent RNA, an elongation or RNA binding factor (the "anti-terminator") dissociates from RNApII[4]. This would allow for recruitment of polyA/termination factors, which would act to destabilize RNApII. In the Torpedo model, cleavage of the nascent mRNA generates a 3'-end product with an exposed uncapped 5'-end. This 5'RNA is recognized by an exonuclease (Rat1/Rai1) that acts as a "torpedo" degrading the remaining 3'-end product and leading to release of RNApII[5]. It is not clear whether these two models reflect termination mechanisms acting at different loci, or whether a combination of the mechanisms postulated actually leads to termination[6]. Neither of the current models recognizes the role of RNA or the RNA/DNA hybrid in the termination reaction, and only recently, a model that acknowledges a possible contribution of the carboxy terminal domain (CTD) of RNApII has emerged. Thus, it is very likely that a number of factors involved in termination are yet unidentified.

Post-doctoral Studies.

Until recently, most studies of transcription termination in eukaryotes have been approached by biochemical methods. During my post-doctoral training, I initiated a genetic approach to study eukaryotic transcription termination in the yeast *Saccharomyces cerevisiae*. By the isolation and analysis of mutants defective for termination, I hoped to identify previously unknown factors involved in this process. The selection for mutants, diagrammed in Figure 1, made use of the well studied *GAL* genes of yeast. This approach relied in the suppression of a read-through transcript generated from a mutated *GAL10* terminator and expression of the downstream *GAL7* gene (see Figure 1). Although the *GAL10/GAL7* genes are closely spaced in their chromosome (726bp apart), —

normally, the expression of *GAL10* does not interfere with expression of *GAL7*. A partial deletion of 56bp of the polyadenylation signal of *GAL10* created a cryptic termination sequence that is not efficiently utilized. As a result, read-through transcription impedes binding of basal transcription factors at the *GAL7* promoter, which leads to the inability of a strain with this deletion to grow in galactose (Gal$^-$) as the sole carbon source. Thus, expression of *GAL7* and growth on selective galactose media, for the *gal10/ΔpolyA* strain is dependent on restoring termination and processing of *GAL10*.

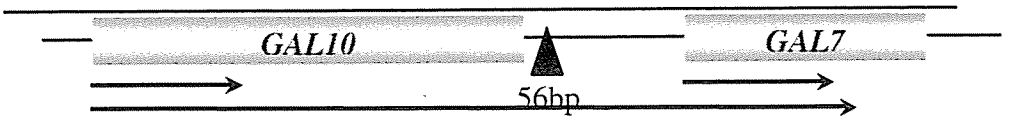

Figure 1. The *GAL10/GAL7* locus.

The results of my genetic analysis revealed that the Gal$^-$ phenotype of the *gal10/ΔpolyA* strain could be suppressed by elongation factors Spt6 and Spt4, the mRNA transport protein Npl3 and a yet unidentified factor. The identification of elongation factors adds validity to my genetic approach, since less processive RNApIIs are likely to abort transcription early and allow some expression of the downstream *GAL7*. I focused more specifically on Npl3 since this was a factor novel to termination. Previous work on Npl3 gave us hints linking Npl3 to transcription and 3'-end processing. Biochemically, Npl3 was shown to crosslink to the *GAL10* open reading frame, and genetically, temperature sensitive mutations of some *npl3* alleles were suppressed by mutations in genes with demonstrated functions in cleavage/polyadenylation. My hypothesis was that Npl3 was acting as an anti-terminator at the 3'-end of genes. My results from *in vitro* and *in vivo* experiments showed that strains with a mutated *npl3* had a remarkable improvement in termination. By using chromatin immunoprecipitation (ChIP) experiments I confirmed that in the *npl3* mutant, association of polyA/termination factors (Hrp1 and Rna15) at the 3'-end of *GAL10* was restored despite the polyA deletion. I also observed a reduced association of mutant Npl3 at *GAL10* suggesting a weaker interaction of Npl3 with the RNA, which likely allows the recognition of the cryptic termination signal and accounts for the suppression in *npl3/gal10/ΔpolyA*. Based on these results I proposed a model where the normal function of Npl3 is to act as an anti-terminator, antagonizing polyA/termination factors in 3'-end formation and termination. This function of Npl3 would prevent the recognition of cryptic termination sequences and "only" the appearance of functional polyA signals would cause Npl3 to be out-competed by the polyA/termination machinery. The role I have proposed for Npl3 in termination strongly supports the anti-termination model with the caveat that my model underlines the importance of the RNA in mediating the narrowly localized dissociation of the anti-terminator.

Future Research.

A. Tests of the role of Npl3 in transcription anti-termination. Specific experiments will be performed to test a prediction from the anti-termination model that Npl3 is out-competed from the RNA by polyA/termination factors. Two factors that will be specifically tested are Hrp1 and Rna15, components of CF I that bind to specific RNA sequences. This binding specificity may underly a possible competition with Npl3. To test this idea, I am using gel mobility shift assays with RNA templates and recombinant Hrp1, Rna15 and Npl3. In addition, using genetics and RNA footprinting, I am mapping the binding sites for Hrp1, Rna15 and Npl3 on the RNA. In another approach to study the binding specificities for the (RNA recognition motif) RRM domain of Npl3, we are using nuclear magnetic resonance (NMR). Npl3 binds single-stranded RNA through a putative RNA-binding domain (RRM), a motif found in various RNA binding proteins. Through a collaboration I have with—

Gabriel Varani (University of Washington), we will determine the residues in Npl3 involved in binding the RNA. Site-directed mutagenesis of *NPL3* will then allow me to demonstrate *in vivo,* the importance of the residues identified in the structural analysis. Therefore, we will be able to compare the binding specificity of Npl3 to that of Hrp1 and Rna15. Results from these experiments will help to elucidate what happens as polyA signals emerge during transcription, and whether the competition for RNA polyA signals by polyA/termination factors and Npl3 is in fact, driving an initial step leading to termination.

Another prediction from my model is that competition between Npl3 and polyA/termination factors also prevents aberrant termination. Therefore, can we expect cryptic sites to be recognized in *npl3*? This will require a genome-wide analysis of yeast. As I showed previously, in *npl3,* crosslinking of the polyA/termination factor Rna15 is increased at the 3'end of some genes and at the *gal10* cryptic termination site. Therefore, DNA from chromatin immunoprecipitated with anti-Rna15 will be hybridized to RNA in microarrays. Results from these experiments will show whether alternative termination sites are being used in *npl3*. This is suggestive of the protection from cryptic termination that Npl3 may confer.

B. Genetic analysis of known polyA/termination factors. Understanding the mechanism of transcription termination and 3'end formation requires determination of each step of the reaction. What happens when the anti-terminator/Npl3 is competed by polyA/termination factors? What polyA/termination factors are required to terminate transcription? My previous work demonstrated that the *gal10/ΔpolyA* system is a good tool for studying termination. As I have demonstrated, by understanding the role of a mutant in the suppression of *gal10/ΔpolyA*, I can extrapolate the role that factor may have in termination. I have started a "genetic dissection" to identify factors required for termination at the *GAL10* site. I will genetically characterized polyA/termination within the context of *gal10* termination using alleles of known components required for termination (*i.e., PCF11, HRP1, RNA15, RNA14, RAT1, RAI1*). An additional bonus of using the *GAL* locus is that this is a hallmark for studies of gene expression. Therefore, by determining how termination of *GAL10* is regulated, my work will contribute important knowledge to those using this system.

C. Screen for additional RNA binding proteins with a role in termination. Npl3 is a good example of an RNA binding protein that acts in termination. But are there other RRM containing proteins able to work as anti-terminators? In yeast, there is a family of sixteen genes that contain an RRM motif. I am proposing to use alleles of RRM containing genes in a genetic screen using some of the reporter assays that I have used previously to measure transcription termination. The results from this screen will give me an initial lead into other RRM containing genes that may have defects in termination and possibly uncover novel factors with a predictable function in termination. My hypothesis is that termination requires a core of polyA/termination factors, but in addition, there are gene-specific proteins for different loci. The clear evidence for gene specific regulation in transcription initiation and elongation supports this idea. In addition, yeast termination sequences are fairly degenerate, with no clear consensus sequence as in higher eukaryotes, which is also strongly suggestive of gene-specific regulation. Npl3 and other RNA binding proteins may constitute such gene-specific factors.

In summary, my initial work in the identification of factors involved in transcription termination has blossomed into a wealth of information and ideas not only into the actual mechanism of transcription termination, but also into whether these mechanisms are acting in a gene specific fashion throughout the genome. I am very excited about the knowledge gained and I feel very confident that I will be able to capitalize in the more important information that is still to come.

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STATEMENT OF TEACHING PHILOSOPHY

I have a personal commitment to creating a learning environment that gives all students a place to freely explore and expand their imagination. My past teaching experience at Harvard taught me how, as an instructor, that I can influence the learning environment of my students. As a teaching fellow for the Genetics and Genomics core course, I was responsible for educating a group of students in concepts covered in class as well as stimulating their thinking with discussion and problem solving. Because this course had a 400-student body, part of my role was to establish a more direct and personalized communication with the students. In addition, I supervised the students as they carried their projects in the laboratory portion of the course. The experiments we covered were aimed at introducing students to topics in genetics. The effectiveness of my teaching methods were reflected by the students' evaluations, which included comments such as "Miriam was exceptional at guiding as through the procedure", the "presentations were well done" and the "explanation of concepts and the problems helped quite a lot".

Teaching introductory and advanced classes presents different sets of challenges. Biology is such an important part of everyday life and being literate in basic biological areas is of benefit for all students, majors and non-majors alike. Therefore, introductory classes serve the purpose to provide a general education to students and also have the potential to attract undecided students to the subject. One challenge inherent to introductory courses is to be able to keep students with a more extensive biology background engaged in the class. I believe that presenting the information in a manner that reaches all students and at the same time inspires their thinking is absolutely critical for both novice and more experienced students. In general, I believe that for lectures the use of clear, uncluttered slides, combined with the use of a blackboard, is very important for keeping the students interested in the material being presented. When appropriate, I will use more sophisticated technological devices such as videos, live demonstrations or any other visual aid that can enhance the presentation of the subject. Finally, I will encourage students to form study groups in and out of the classroom. Student groups can be effective in the completion of larger projects and the input of different problem solving strategies. In groups, students learn how to effectively teach themselves through the teaching of others. Learning a difficult concept through a fellow classmate in a smaller group can be less intimidating for some students than approaching a professor. An additional advantage of grouping students in small study groups is that it emphasizes the importance of teamwork early on in the students' careers. Ultimately, I hope my students will have a sense that they have been active participants in their learning experience.

Advanced courses present a number of different challenges. One strategy that I will use to engage students in the subject matter will be to design the laboratory component of these courses in a way that will actively engage them in semester-long projects. Such projects are much more stimulating to students. The hands-on-experience that students get in the lab can help them visualize concepts learned in the classroom. In addition to the lab, I will introduce students to current literature. I will encourage students to present papers in class. These presentations will be followed by discussion periods, where the presenter, other students, and myself will engage in the active analyses of papers. Finally, I plan on occasionally inviting other scientists to talk to students about their research as a way to give students a sense of the applicability of the material they will be studying in the courses.

In order to accommodate different learning styles, I will use a variety of assignments, such as written essays, oral presentations, posters and different examination methodologies (*i.e.,* short essays, multiple choice questions, etc). This will ensure that my students have various avenues to express themselves and receive credit. I also consider it my responsibility to shape my students in the ethical conduct of science. It is imperative for me to instruct my students early on in their careers on how to conduct themselves appropriately and respectfully towards others.

Finally, I extend my commitment to equality in teaching, especially concerning gender, race and cultural ethnicity. As a Hispanic woman who has succeeded at the highest levels of academic research, I am particularly sensitive to the plea to increase the number of women and underrepresented minority individuals in science. Throughout my scientific career I have sought to alleviate this problem for others and myself. In graduate school, I organized a support group for graduate students, and we organized seminars and scientific activities to help keep us motivated. I am currently a member of the governing board of the Harvard Medical School Post-doctoral Association. In this position, I have organized career development seminars for fellow post-docs. These seminars are aimed at improving the training of post-docs. In addition, I hold a second research appointment at the Bok Derek Center for Excellence in Teaching at Harvard University. The purpose of my research at the Bok Center is to investigate how to better train science instructors an awareness to gender, race and ethnicity. As a faculty member, I look forward to mentoring students, especially women and minorities. I benefited from excellent mentoring as an undergraduate and belief it was key in my career.