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September 10, 2005

Yves Brun
Systems Biology/Microbiology Faculty Search
Department of Biology
Indiana University
Jordan Hall 142
1001 E. 3rd St.
Bloomington, IN 47405-7005

Dear Members of the Search Committee,

I am writing to apply for the position of Assistant Professor in the Department of Biology at Indiana University. Currently, I am beginning the fourth year of a post-doctoral fellowship in the laboratory of Dr. Kevin Strange at Vanderbilt University. The research program that I have established during my postdoctoral fellowship exceptionally qualifies me for a position in your department.

As both a graduate student and postdoctoral fellow, I have utilized the nematode *C. elegans* as a model system. My graduate thesis focused on the developmental genetics of spermatogenesis, at the level of both sperm meiotic cell cycle control and sperm-oocyte interactions. During my postdoctoral fellowship, I have developed unique approaches to study the integrative physiology of animal stress signaling networks. The extraordinary experimental advantages of *C. elegans* are allowing me to explore signaling and gene regulatory mechanisms at the physiological, genetic, genomic, and systems levels. My studies have generated exciting new hypotheses, and I am eager to apply for external funding as an Assistant Professor to further pursue these newly emergent ideas. Additionally, the interdisciplinary nature of these studies provides ample opportunity for collaboration and expands upon the existing strengths of your department in the analysis of signal transduction pathways.

During my postdoctoral fellowship, I have taught graduate and undergraduate students in both the classroom and the laboratory. These teaching opportunities were scientifically invigorating, as questions from students constantly forced me to reexamine concepts from different perspectives. Additionally, my research program makes an outstanding platform for teaching students the principles and practices of genetic and genomics in the laboratory. I am strongly committed to the training of new young scholars, and, based on my previous teaching experiences, I will make an outstanding contribution to the training mission of your department in both the classroom and laboratory.

I am available to discuss this position with you in the upcoming weeks. I can be reached by phone at (615)-343-7383. I have enclosed my CV, statements of current and future research interests, statement of teaching philosophy, and two representative publications. Letters of recommendation will be arriving under separate cover. I look forward to hearing from you soon.

Sincerely,

Todd Lamitina

Todd Lamitina, Ph.D.

Research Interests

Current Research

A major unanswered question in metazoan physiology is how do cells sense and adapt to environmental stress? The difficulty in addressing this question in animals has mostly been due to the lack of a tractable model system in which to identify and manipulate the genes comprising stress signaling pathways. Elegant genetic and genomic approaches in yeast and bacteria have played important roles in our understanding of animal cell stress responses. However, similar approaches have not yet been applied to animals. As a model for understanding animal stress response mechanisms, I am studying the osmotic stress response of the nematode *C. elegans*. Using genetic, genomic, and bioinformatics approaches only possible in *C. elegans*, I am identifying the genes encoding osmotic stress sensors, their associated signal transduction pathways, and the physiological targets of these pathways.

C. elegans is an outstanding animal model in which to study osmoregulatory signaling. Nematodes normally live in the soil where they must adapt to extreme changes in water and solute levels. Identification of the genes and signaling pathways regulating the osmotic stress response in *C. elegans* is possible due to forward genetic tractability, the availability of a well annotated, complete genome sequence, and easy manipulation of gene function through transgenic and reverse genetic approaches. Additionally, results from functional genomic approaches, such as protein-protein interaction mapping, whole genome gene expression analysis, and high throughput RNA interference-mediated phenotypic screening, can be integrated to generate a systems level understanding of the osmotic stress response. The combination of these approaches is not currently possible using other animal model systems.

During my postdoctoral fellowship, I have defined the physiology of the hypertonic stress response in *C. elegans*. I have shown that both organic osmolyte accumulation and damage protection/repair mechanisms play critical roles in adaptation to hypertonic stress. My studies were the first to show that *C. elegans* adapts to hypertonic stress by synthesizing the organic osmolyte glycerol via increased mRNA expression of a glycerol biosynthetic enzyme (glycerol-3-phosphate dehydrogenase 1; *gpdh-1*). Based on this data, I developed a simple in *vivo* GFP based fluorescent assay to analyze osmosensitive gene expression. Using this assay, I performed a genome-wide RNAi screen to identify genes that function in osmotically regulated signaling pathways controlling *gpdh-1* expression. Out of ~16,000 individual gene inactivations tested, I identified 106 that caused inappropriate activation of *gpdh-1* expression under isotonic conditions. Many of these genes normally function to prevent the accumulation of damaged or denatured proteins, which is a well known consequence of hypertonic stress. My studies suggest the intriguing and novel hypothesis that increased levels of damaged or denatured proteins activate osmoregulatory signaling pathways. Currently, I am using genetic and transgenic approaches to test this hypothesis.

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Future Research Directions

The long term goal of my research program is to create a systems level understanding of animal stress responses at the molecular, cellular, and organismal level using *C. elegans* genetics and genomics. As mentioned above, I have identified >100 genes that regulate osmosensitive gene expression using a reverse genetic screen. In the future, I plan to continue characterizing the genes and mechanisms identified in this screen to understand how they regulate osmosensitive signal transduction. Additionally, I will develop similar high-throughput reverse genetic approaches for identification of genes regulating other components of the osmotic stress response, such as ion transport pathways and damage repair mechanisms. These studies will provide unprecedented molecular insights into how these distinct components of the osmotic stress response are integrated with each other to generate a coordinated organismal response.

I also plan to exploit the tremendous forward genetic tractability of *C. elegans* to gain structural and functional insights into genes that participate in osmoregulatory signaling pathways. I have already performed a forward genetic screen and identified >30 mutants that ***a***ctivate ***gpdh-1*** expression (*agpd* mutants) under isotonic conditions. An essential component of my future research plan is the genetic and molecular characterization of the *agpd* mutants using well-defined *C. elegans* genetic and positional cloning approaches. Once these genes are molecularly identified, I will use live animal fluorescent imaging to determine where these genes are expressed, how osmotic stress modulates their subcellular localization in *vivo*, and how specific mutations perturb these processes. My studies will provide important details about the molecular mechanisms of osmoregulatory signaling in animals and will provide a framework for testing the evolutionary conservation of these pathways in other metazoans.

Another part of my future research plan is to identify signaling pathways that positively regulate osmosensitive gene expression. While mitogen-activated protein kinase (MAPK) signaling pathways clearly serve this role in yeast, the role of MAPK signaling pathways in animal osmotic stress responses is less well defined. The *gpdh-1* GFP transgene provides an extraordinarily powerful genetic screening method for the unbiased identification of genes required for osmosensitive gene expression. Mutation or RNAi inhibition of such genes should produce animals that are ***d***efective in osmotically induced ***gpd*h-1** activation (*dgpd* mutants). Such mutants can easily be identified by their inability to express the osmosensitive GFP reporter when exposed to hypertonic stress. Additionally, the opposing phenotypes of the *apgd* and *dgpd* mutants (GFP 'ON'/'OFF') provide an outstanding opportunity to define the global organization of these signaling pathways using traditional genetic epistasis analysis.

Finally, my proposed studies lay the foundation for a systems biological analysis of animal stress signaling. Each of the studies described above will contribute to a continuously evolving model of osmotic stress signaling networks that are founded upon the results of whole genome microarray measurements, genome-wide RNAi phenotypic screens, forward genetic analysis, and large scale protein-protein mapping. As models become increasingly detailed, predictions will be rapidly tested using genetic and transgenic approaches. Overall, these studies will provide unparalled insights into the architecture of stress signaling systems in animals. Ultimately, I plan to integrate osmotic stress signaling networks with other stress response networks to understand how stress specific responses are generated in animals.

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Teaching Philosophy

Effective teaching in the biological sciences poses two unique challenges. First, teachers must effectively communicate the basic biological information necessary to understand cell and organismal function. Second, teachers must demonstrate how this information is obtained through hypothesis formation, experimentation in the lab, and critical evaluation of scientific findings. Based on my own educational experiences, I recognize that the skills fostered by both of these approaches are paramount in developing critical thinkers and effective scientists.

In the classroom, I believe in actively engaging students in learning by encouraging free discussion and student-led teaching. In my experiences, students are initially hesitant to participate in such a classroom environment. However, I have found that determination and patience with these methods wins over most students and leads to lively discussions in class and better student performance. For example, in a graduate school class in developmental biology, I was assigned to lead the class discussion on cell cycle control. During the three hour class, I continually asked questions of each student in the class. This was quite different from previous presentations, so enlisting participation proved difficult at first. However, my persistence eventually paid off and within 30 minutes, the entire class was participating in the discussion. Later, the course director indicated that mine had been one of the most successful classes of the year.

In the laboratory, research projects should emphasize and expand upon principles learned in the classroom. In many cases, hands-on work in the laboratory can clarify difficult classroom concepts for students. For example, I struggled with many of the concepts in my undergraduate genetics class. While choosing rotations for graduate school, I decided to give genetics another try and rotated through a *C. elegans* genetics lab. With first-hand experience in the lab, the principles that seemed so difficult to grasp in the classroom, such as recombination and genetic mapping, became perfectly clear once put into practice. This success inspired me and led to a successful Ph.D. thesis that was heavily based in genetics. Without my success in the lab, genetics would have simply become an obscure class that was required for graduation.

My proposed research directions provide outstanding opportunities for such hands-on teaching and training of students in the laboratory. My future studies of *C. elegans* stress response mechanisms encompass straightforward research projects suitable for short term undergraduate projects or graduate student rotations, such as genetic mapping or phenotypic characterization of specific osmotic stress signaling mutants. Additionally, my studies offer the potential for longer-term thesis projects, such as cloning and characterization of genes involved in *C. elegans* stress responses. Whether students are engaged in short or long term projects, I take great pride in training young scholars. I will continue to encourage and challenge students to meet their potential in hopes of developing excited new future scientists.