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10 October, 2005

Dr. Yves Brun,
Systems Biology/Microbiology Faculty search
Dept. Biology, Indiana University,
Jordan Hall 142, 1001 E 3rd St.
Bloomington IN 47405-7005 USA

Dear Dr. Brun,

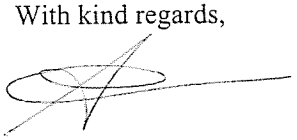
I am submitting the enclosed documents in support of my application for the assistant professor position advertised in The Department of Biology and the Biocomplexity Institute, Indiana University. I finished my Ph.D. in October 2003 (Simon Fraser University (SFU), British Columbia, Canada), and am presently working as a post-doctoral scholar at the University of Iowa, USA. Included with this covering letter, please accept (1) my current C.V., (2) an overview of my current and future research and teaching plans, and (3) three reprints of recent papers. Four letters of reference from Drs. Barry Honda (SFU), Lori Wallrath, Marc Wold and Bryant McAllister (University of Iowa, Iowa City, USA) are being forwarded under a separate cover.

I am a molecular biologist with extensive training as a geneticist. My model organism of choice is the fruit fly *Drosophila melanogaster*. My primary area of research as a **post-doctoral scholar** is the role nuclear architecture plays in regulating chromatin structure and differential gene expression. Mutations in nuclear envelope proteins lead to a variety of tissue-specific diseases in humans known as laminopathies, and I have been using a combination of transgenic, genetic and molecular approaches to develop Drosophila as a model to study the etiology of these diseases. I am also highly interested in the evolution of the nuclear envelope and its components, some of which are conserved across taxa, while others vary considerably in sequence (though not necessarily function). As a **Ph.D. student**, I studied genes resident within the transcriptionally repressive compartment of heterochromatin, which is present in most eukaryotic organisms. It consists largely of repetitive sequences, and is located in telomeric and pericentromeric regions of the chromosomes. It is also often closely apposed to the nuclear envelope in interphase cells. During the course of my Ph.D. studies, I determined that heterochromatic genes paradoxically appear to require an inactivating environment in order to function. I also studied their evolutionary history by examining their locations in a number of related Drosophila species. I showed that the same gene could evolve to function in contrasting chromatin environments.

As a principal investigator, I plan to divide my laboratory's efforts into two main projects. While working at the University of Iowa I have focused on studying the nuclear envelope in Dr. Lori Wallrath's laboratory, and this shall continue to be the main focus of my work. But I have also established collaboration here at the University of Iowa with Dr. Bryant McAllister during the course of preparing a manuscript for the second half of my Ph.D. thesis, which relates to the evolutionary history of heterochromatic genes in Drosophila. Dr. McAllister is an evolutionary biologist who specializes in Drosophila chromosome evolution, and I plan to maintain my work with both him and Dr. Barry Honda in order to continue research in this area. It is possible that splitting my interest between both my Ph.D. and post-doctoral studies might be considered unusual. However I have published on each of these topics, and feel both subjects fall within the interrelated areas of nuclear architecture and gene expression. I like to think of them as two potentially fruitful new M.Sc. or Ph.D. projects for prospective students.

Finally I should like to make clear that I am a post-doctoral scholar who deliberately seeks out teaching experience. I consider every aspect of my proposed research in terms of training potential, and prefer a career in which teaching is considered on equal par with research. I trust that my application is complete, and look forward to your reply. Please do not hesitate to contact me should you require anything further.

With kind regards,



Sandra Schulze, Ph.D.

SANDRA SCHULZE RESEARCH SUMMARY AND TEACHING INTERESTS

INTRODUCTION:

For this job application, I have elected to outline two areas of research, which I envision as distinct yet related graduate thesis projects. The first and principal project focuses on the role nuclear envelope proteins play in regulating nuclear architecture, differentiation and development, using Drosophila as a model. The second project concentrates on genomic aspects of chromosome evolution, by examining the location and structural changes of heterochromatic genes in a well-defined set of Dipteran species.

BACKGROUND: NUCLEAR ENVELOPE PROJECT:

The inner nuclear envelope is lined with a meshwork of proteins known as lamins that belong to the intermediate filament family[1]. They provide structural support for the nucleus, in addition to making contacts with nuclear components such as chromatin, DNA, and transcriptional regulators[2]. Nuclear lamins (or lamin-like molecules) appear to be confined to multi-cellular animals and plants. One explanation for restriction of lamin molecules to metazoan phylogenetic lineages is that they are required in organisms that exhibit complex differentiation and development.

Humans have two types of lamin proteins, A and B types, that differ in their structural and biochemical properties. Mutations in A-type lamins lead to a variety of predominantly tissue-specific diseases that overlap symptomatically. These diseases are collectively known as laminopathies, and include muscular dystrophies, lipodystrophies, neuropathies and premature aging syndromes[3]. It is not understood how a ubiquitously expressed mutant lamin protein can have such tissue-restricted effects. In general, mutations in lamins could weaken the nuclear envelope, which would manifest more readily in a tissue under mechanical stress (muscular dystrophy). Alternatively, mutations in lamins could disrupt interactions with nuclear factors that regulate tissue-specific developmental genes (lipodystrophies, aging syndromes). The existence of these diseases underscores the notion that it is the A-type lamins that are specifically involved in regulating differential gene expression.

I have been developing Drosophila as a model to study lamin biology in order to test these (and other) hypotheses. Drosophila is an ideal model for this work for several reasons. The genome has been fully sequenced and annotated; there are lesions in almost every vital gene; experimental tools allowing for tissue and stage specific expression abound, and since this animal has been a favorite genetic model for more than a century, there is an enormous wealth of biological, genetic and genomic resources freely available to the community. Two other features establish Drosophila as a valuable model for studying laminopathies. Firstly, Drosophila is the only tractable invertebrate model that possesses both A and B type lamins. Secondly, the A-type lamin in Drosophila encodes an essential function, and when mutant exhibits nuclear defects remarkably similar to those observed in human laminopathic cell cultures[4]. As an independent investigator, I plan to dissect lamin function in Drosophila, using a combination of genetic, transgenic and biochemical approaches.

EXPERIMENTAL AIM 1: *In vivo* expression of domain-specific A-type lamin mutations in Drosophila.

The published literature correlating lamin protein domains with specific functions is conflicting. A broad-scale *in vivo* biological study in a tractable model organism is therefore desirable. I am generating transgenic flies that express deletions in specific domains of the protein that are conserved between flies and humans, and within which common laminopathic mutations occur (Figure 1). These include entire N and C terminal deletions, in addition to smaller scale deletions within these domains and the rod domain, that in humans have been confirmed *in vitro* to interact with specific nuclear factors, including chromatin proteins, transcriptional regulators and other components of the nuclear envelope.

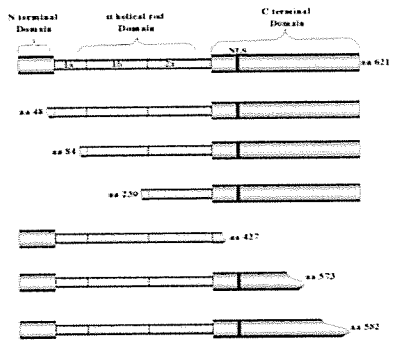


Figure 1: Drosophila A-type lamin protein structure, indicating principle domains and proposed truncations for transgenic constructs

EXPERIMENTAL AIM 2: Isolation of Lamin protein interaction partners

Lamins interact with a variety of nuclear components[2]. I plan to discover analogous and novel interactions in Drosophila by employing a yeast two hybrid screen with the Drosophila A-type lamin. I shall employ a general library screen, in addition to a candidate protein approach. I also plan to use the domain-deletion constructs generated in the first specific aim to narrow down which protein domains are responsible for the

interaction. After biochemical confirmation (GST pull-downs, and co-immunoprecipitation from nuclear extracts) I will make use of the unique tools Drosophila offers to study those interactions *in vivo*, using both transgenics and genetics.

EXPERIMENTAL AIM 3: Evolutionary comparison of insect and human lamin proteins: I am using a transgenic approach to study conservation of function between human and insect envelope proteins, by expressing human lamins in flies, and testing their ability to rescue mutations in Drosophila lamin genes. My work so far has demonstrated that human lamins are stably expressed in flies, and incorporate into the fly lamina. These studies raise interesting questions about the evolution of lamina function, which is highly conserved in animals, in addition to supporting the use of Drosophila as a model to study human disease.

BACKGROUND: HETEROCHROMATIC GENE EVOLUTION PROJECT:

Heterochromatin exists in nearly all eukaryotes, forms mainly around the centromeres and telomeres of chromosomes, and is normally transcriptionally repressive. As such, it is gene poor, but I cloned and characterized two genes (*RpL15* and *Dbp80*) located next to each other within this region, and demonstrated that they require this repressive environment in order to function. Paradoxically, both genes exhibit transcriptional dependence on chromatin proteins normally associated with transcriptional silencing[5]. I also cloned both genes from another Drosophila species, where I discovered that one of the genes was euchromatic, proving that genes can evolve to function in contrasting chromatin environments. This is an on-going project[6], principally involving two laboratories (Honda Lab, Simon Fraser University Canada; McAllister Lab, University of Iowa, USA) which we plan to extend to a larger set of heterochromatic genes.

EXPERIMENTAL AIM 1: *In situ* localization of *Dbp80* and *RpL15* in other *Drosophila* species: During (and since) the course of my Ph.D. studies in Barry Honda's lab at Simon Fraser University, I have mapped these genes in seven species of Drosophila, spanning approximately 70 million years of evolution. I have confirmed chromosomal positions for three species (by a combination of chromosomal in situ and genetic analyses) which has revealed a high degree of inter-chromosomal movement for both genes. This was a surprising finding, because although *intra*-chromosomal rearrangement of genetic elements over time is not unusual, *inter*-chromosomal movement is considered rare[7]. This analysis has confirmed and corrected data obtained for these genes by purely computational methods[8], which depends on the assumption that genes remain confined to chromosomal elements over evolutionary time. Direct chromosomal *in situ* analysis is necessary to confirm or correct these assumptions. My analysis has also revealed specific rearrangements that normally would be missed by bioinformatics. I plan to generate genomic probes to physically map both *Dbp80* and *RpL15* in the remaining 4 intervening Drosophila species from our study.

EXPERIMENTAL AIM 2: Map a set of 3rd chromosome heterochromatic genes in *D. melanogaster* to six additional species: This is an extension and expansion of the above study, employing the same combined molecular, cytological and bioinformatic procedures. I will concentrate on genes located in the heterochromatin of the third chromosome, a region I am highly familiar with from my PhD work. Applying the same methods to a larger subset of genes will create a more biologically informative picture of chromosome and genome evolution. At the same time, this research will provide a valuable resource to the Drosophila community, which relies on individual laboratories to confirm and validate freely available sequence assembly data. My proposal is to maintain collaboration with both Dr. Barry Honda (Simon Fraser University) and Dr. Bryant McAllister (University of Iowa) in order to continue this molecular genetic, bioinformatic and cytological analysis, which has both confirmed and corrected existing genome assemblies, and provided novel insights into chromosome evolution.

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TRAINING POTENTIAL AND TEACHING INTERESTS:

Collaborative and Technical Potential of Proposed Research:

The main focus of research in my lab would be the study of the nuclear envelope. Most of my independent training has come from this work, and I have generated substantial material and resources in this area. My experimental approaches described above are designed to produce definitive biological information concerning the role A-type lamins play in development and disease. I envision significant opportunities for collaboration with developmental biologists, computational and structural biologists, and protein biochemists. The practical advantages of using a fly model include a rapid generation time, ease of generating transgenic animals, and access to an enormous wealth of genetic and genomic resources.

The second research project I have proposed results from continuing collaboration with my Ph.D. supervisor (Dr. Barry Honda), and from a recent and highly fruitful collaboration with Dr. Bryant McAllister, a specialist in Drosophila chromosome evolution at the University of Iowa. This project depends substantially on the bioinformatic analysis of data resulting from the Drosophila Comparative Genome Sequencing Project (http://www.genome.gov/11008080). It also presents an opportunity to develop cytological tools to analyze chromosome banding patterns in less well characterized Drosophilids, essential for confirming or correcting *in silico* assembly techniques. The Fly community is depending on the development of precisely these kinds of skills to provide verification for the Comparative Genome Project.

While these two projects may appear rather different in scope and focus, my feeling is that they overlap at a fundamental level, connecting nuclear architecture, chromosome mechanics, and the regulation of gene expression.

Training Potential of Proposed Research:

I have chosen my experiments mindful of the training potential each set of experiments will allow. For undergraduates and gifted high school students, these experiments include basic cloning techniques and bioinformatic skills that may inspire them to continue in this field. For senior undergraduates and junior graduate students, more detailed work will be required for their projects, including Southern and western analysis of transgenic lines, *in situ* chromosomal techniques, imaging and microscopy, genetic analysis and possibly microarray analysis. For senior graduate students, the generation and maintenance of transgenic animals, genetic dissection with endogenous mutants and biochemical strategies for downstream analysis will constitute the larger part of their thesis work. In general, my main educational objective is to provide an opportunity to generate new findings from molecular, genetic, bioinformatic and evolutionary approaches that connect nuclear organization with gene expression.

Teaching experience and interests:

My teaching experience to date has included working as a teaching assistant for both hands-on laboratory courses, and more lecture-based theoretical courses (please see C.V.). As a post-doctoral scholar I have participated in the design and teaching of a summer course ("Chromatin Effects on Replication and Transcription" undergraduate series – please see C.V.). I have also had the pleasure of training a number of undergraduates who have gone on to continue either in an academic or medical school context. Since I have been spear-heading the lamin project in the Wallrath lab, I have also been required to work with talented technicians, and have both benefited from and added to their repertoire of expertise. But the most rewarding teaching experience I have had has been in introducing talented high school students to laboratory life. I have found that the tenacity and talent required of science is not determined by age or circumstance, and it is an enormous pleasure to witness the growth of interest in the scientific process in someone who would not otherwise have any access to a real-life laboratory environment. Therefore I plan to coordinate with local high schools in recruiting interested students for short term projects in my lab.