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UNIVERSITY of LOUISVILLE

September 21st, 2005

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

Dear Dr. Brun::

I have known Dr. Louis-Bruno Ruest since 1996 when he joined my laboratory as a graduate student (Ph.D) at the Bloomfield Center for Research in Aging, Lady Davis Institute for Medical Research, Sir Mortimer B. Davis Jewish General Hospital, McGill University (Montréal, Canada), and have since interacted with him at various levels.

In my laboratory at the Lady Davis Institute then at the University of Louisville, Dr. Ruest worked on the characterization of peptide elongation factors (EF1A-1/EF-1 α and EF1A-2/S1) and caspase proteins in skeletal muscle, particularly in events associated with skeletal muscle cell death. Skeletal muscle cell death is one of the pathological event causing the loss of muscle mass associated with aging, muscle wasting or following injury, rendering affected people more frail and subject to further injuries. EF1A-2/S1 is homologous to EF1A-1/EF-1 α and is expressed in terminally differentiated neurons, cardiomyocytes and skeletal muscle fibers. Both elongation factors have similar activity during protein synthesis; however the biological reasons for the developmental switch from EF1A-1/EF-1 α to EF1A-2/S1 remains mostly unknown. Bruno's work brought the first evidence that this switching was important for these tissues. Bruno demonstrated that EF1A-2/S1 protects differentiated muscle from apoptosis while EF1A-1/EF-1 α promotes cell death. His work has been cited in several peer-reviewed publications. Bruno demonstrated a superb ability to absorb concepts of molecular and cellular biology and utilized them in a sophisticated manner during his studies. He also demonstrated great organizational skills on several occasions, including organization of the Bloomfield Center's Aging Journal Club. In 2002 after obtaining his Ph.D., Bruno left my laboratory to pursue research in the field of developmental biology (embryology).

I believe Dr. Ruest is a great young scientist capable of competitive and original research as proven by his publication records and his ability to secure competitive external funding at all levels. He was awarded a Research Student Fellowship from the Fond de

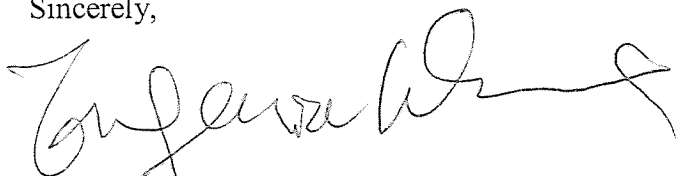
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Recherche en Santé du Québec (FRSQ) during his undergraduate studies, a Doctoral Research Award from Medical Research Council of Canada (now Canadian Institutes of Health Research) during his graduate studies and a Research Fellowship from the Heart and Stroke Foundation of Canada for his postdoctoral studies. All of these awards are extremely competitive and prestigious.

Being an accomplished scientist, Bruno possesses a unique combination of skills that have served him well throughout the years. He has a profound knowledge of his field of research. Bruno is a talented individual with a unique ability to develop new concepts and ideas and see them through. He is motivated, goal driven and has a clear vision of his field of research. I have no doubt that Dr. Ruest will be capable of sustaining an independently funded research program and will be a great asset to your program. I would have no hesitations in hiring Dr. Ruest as a faculty member.

Should you have any questions or need more information please do not hesitate to contact me by phone or email.

Sincerely,

A handwritten signature in black ink, appearing to read 'Eugenia Wang', with a long horizontal flourish extending to the right.

Eugenia Wang, Ph.D.
Gheens Endowed Chair
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BIRTH DEFECTS CENTER

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Yves Brun
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Dear Dr. Brun,

It is my pleasure to write a letter of reference for Dr. Louis-Bruno Ruest, who is seeking a tenure-track faculty position in your department. Bruno came to the University of Louisville from McGill University with his Ph.D. mentor, Dr. Eugina Wang, in 2000. As his wife was a year behind him in Dr. Wang's lab, Bruno had no choice but to stay in Louisville after he finished his Ph.D. This was quite fortunate for me, as I was looking for my first post-doc. I firmly believe that Bruno's decision to come to my lab was a significant factor in my success. My lab focuses on understanding the role of endothelin-A receptor (Ednra) signaling during craniofacial and cardiovascular development. Ednra and Edn1 mutant mice are born with severe craniofacial and cardiovascular birth defects resulting from defects in neural crest cell (NCC) development. Our primary goal is to understand the hierarchical signaling pathways and patterning mechanisms that are induced by Ednra signaling within cephalic and cardiac NCCs and surrounding cell types.

Bruno arrived in my lab with a rigorous work ethic but little experience in developmental biology. Over the first year, he worked tirelessly in learning a new area of science as well as the techniques and approaches used by the modern developmental biologist. By the end of year one, he had assumed responsibility for my mouse colony and was simultaneously handling three projects, all of which required complicated breeding protocols. The first was the analysis of two transgenic mouse lines I had made before coming to Louisville. In these strains (*Hand2-Cre* and *Dlx5/6-Cre*), Cre recombinase is expressed in a subset of cephalic and cardiac NCCs. By crossing these mice with mice from the *R26R* strain, in which *Cre* expression results in permanent β -galactosidase expression, Bruno was able to define the timing of induction of *Hand2* and *Dlx5/6* in NCCs and to show the long term fate of *Hand2* and *Dlx5/6* daughter cells. In the *Hand2-Cre* project, Bruno's results helped establish multiple putative functions for *Hand2* and to illustrate the usefulness of this mouse strain for conditional inactivation of genes.

This work was published in *Developmental Biology* in 2003 and garnered the cover. We are also in the process of finishing a paper describing *Hand2* expression in the heart (including the secondary heart field) and comparing that expression with *Hand2-Cre* expression. I expect that paper to be submitted within two months.

In the *Dlx5/6-Cre* project, Bruno showed that the *Dlx5/6* enhancer used to drive Cre was only expressed in a 24-hour window, though the daughter cells subsequently formed the entire mandible bone. He further showed that inactivation of a conditional *Ednra* allele using *Dlx5/6-Cre* mice does not affect mandible bone development, suggesting a function of *Ednra* before embryonic day (E) 10.5 in the mouse (*Genesis*, 2004).

In the second project, we set out to examine the cellular and genetic basis of the *Ednra* phenotype in cephalic NCCs. This project took almost two years of very careful analysis of both cellular and molecular changes in overall neural crest development, but yielded a very nice paper in *Development* (2004) that was one of several articles chosen for review in that issue. In his third project, Bruno examined both the timing and function of *Ednra* signaling through a conditional gene inactivation approach. His results demonstrate that *Ednra* function is required between E8.0 and E9.5. Further, based on analysis of craniofacial malformations present in the conditional knockouts, it appears that *Ednra* signaling was likely one of the key events in the evolution of the vertebrate hinged jaw. This work is currently submitted for publication.

More recently, Bruno has been involved with a detailed investigation into the timing of *Ednra* signaling in NCCs using a non-peptidic *Ednra* antagonist that is administered to pregnant wild type mice at different gestational ages. This study has been a tour-de-force due to the number of litters required for such a project. However, we are close to finishing and should have a paper submitted within 6 months.

Professionally, Bruno is a very motivated scientist who works quite hard at his research. He pursued several small grants last year, receiving a Post-doctoral award from the Heart and Stroke Foundation of Canada. Personally, Bruno is a very pleasant person to have in the lab. He gets along well with others (technicians, students and other post-docs) and is very interactive. At our departmental seminar series, he routinely asks questions to our invited speakers. While English is his second language, one would not know this from one-on-one interactions with him. In a group setting, such as a seminar, Bruno can rush to find words, ending up saying something that is incorrect. He is aware of this and continues to work on his presentations.

Bruno and I have talked extensively about his research plan. I think his proposal is the perfect fit between his interests in mechanisms of gene regulation and developmental biology. To date, no one knows how *Ednra* gene regulation is achieved. Identifying the elements involved would be a huge accomplishment and would lend itself to many projects. Further, it is not clear how differential regulation of NCCs occurs and the role that different G-proteins might have on this process. Elucidating the affects of these different proteins would be of interest to multiple scientific fields, especially if he is able to show developmental affects of transgene overexpression in different lineages of NCCs. I am interested in Bruno's continued development as a scientist and expect that we will continue to interact at a scientific level.

However, the projects he describes are his to take with him and develop. I do not have any plans to conduct similar projects in my lab.

In closing, I would strongly recommend Bruno for your faculty opening. I believe that his demeanor and expertise would contribute much to your Department. Please contact me if you have any questions.

Sincerely,

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BIRTH DEFECTS CENTER

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September 20, 2005

Yves Brun
Systems Biology/Microbiology Faculty Search
Indiana University, Dept of Biology
Jordan Hall 142
1001 E. Third Street
Bloomington, IN 47405-7005

Dear Dr. Brun:

It is a pleasure to write in recommendation of Dr. Louis-Bruno Ruest for a position in your department. I have known Dr. Ruest since 2002 when he joined the Department of Molecular, Cellular & Craniofacial Biology and University of Louisville Birth Defects Center as a postdoctoral fellow in the laboratory of David Clouthier. Inasmuch as our laboratory collaborates with that of Dr. Clouthier's, I've had the opportunity to interact closely with Bruno and observe his development into an able, creative scientist with a substantial amount of potential to become an outstanding research scientist and scholarly academician. His work currently is focused on craniofacial and cardiovascular development, particularly characterizing signaling pathways regulating neural crest cell patterning. Specifics and productivity of this research program are outlined in his *curriculum vitae*.

His recent work has been published in well-recognized peer-reviewed scientific journals, and is of great importance for the scientific community as it helps provide a better understanding of certain aspects of neural crest cell patterning. His work may eventually contribute to the development of diagnostic tools and the development of corrective treatment for children born with craniofacial or cardiovascular malformations.

Bruno has developed a wide repertoire of both molecular and biochemical experimental approaches. Moreover, he is a careful, conscientious and zealously honest investigator. Most importantly, rather than being technique-oriented, he demonstrates a problem-oriented approach to his research. Each postdoctoral fellow in our department is required to present an annual formal research seminar. In these forum's, I have found Bruno's scientific originality as well as his ability to organize and communicate scientific data to be superior. He has always been responsive to criticism in a constructive fashion, learns and develops new research techniques quickly, and has the discipline and perseverance to conduct and complete a research project in a scientifically orderly manner.

During the past several years I have recruited a large number of junior investigators to our department. Aside from the obvious things we all look for in a successful candidate, I've learned that you hire people, not research programs. People with pride in themselves and a drive to

succeed that will not allow them to fail. I believe that Bruno is that kind of person! Based on his demonstrated abilities, and his desire to excel as a scientist, I have full confidence that Bruno will continue to be an innovative and productive member of the scientific community. I would like very much for Bruno to continue as a member of our departmental research milieu. However, I also understand his desire to seek a more secure position than the soft-moneyed position he now holds here. I offer my most enthusiastic endorsement of his candidacy. Should you wish to contact me regarding Bruno, please feel free to do so at any time.

Sincerely,

A handwritten signature in black ink, appearing to read "R. M. Greene". The signature is fluid and cursive, with the first letters of the first and last names being capitalized and prominent.

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