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September 15, 2005

Yves Brun
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Dear Dr. Brun:

It is my great pleasure to write a letter of reference for Dr. Todd Lamitina. Todd's graduate work at Emory University focused on the genetic analysis of a difficult and important problem in germ cell developmental biology using the nematode *C. elegans* as a model system. During his graduate career at Emory, Todd developed a strong interest in physiology and physiology research. He took the Wood's Hole physiology course and did two rotations in epithelial ion channel laboratories where he worked on cytoskeletal regulation of ENaC and ENaC-CFTR interactions. Todd also worked in a renal physiology lab before beginning graduate school.

Todd visited my lab in January 2002 to discuss the possibility of a postdoctoral fellowship. His interest in physiology research and broad experience with *C. elegans* served as the foundation for his postdoctoral research training goals. Todd was intently focused on combining forward and reverse genetic analyses in *C. elegans* with state-of-the-art physiological tools to address important physiological problems. His long-term research interests are to define physiological processes in an integrated systems level fashion from single genes up to the whole animal.

My lab began working on *C. elegans* in November 1998. The decision to move into *C. elegans* was borne out of frustration over the lack of progress in understanding the molecular physiology of volume-sensitive anion channels that we have worked on extensively. Our success with *C. elegans* has been outstanding and all of the research projects and funding in my lab are currently focused on this organism. Todd's visit to the lab coincided with a recent decision I had made to begin exploiting *C. elegans* to identify the genes and genetic pathways responsible for cellular osmoregulation. This is an area we have had longstanding interest in and is also an important area of physiology research in organisms ranging from bacteria to plants and mammals. Detailed molecular understanding of how cells cope with osmotic stress has significant implications for understanding the broad problem of cellular stress biology as well as physiological and pathophysiological processes associated with cellular stress such as ageing.

Todd's progress since joining my lab in July 2002 has been spectacular. He quickly characterized the physiological response of *C. elegans* to osmotic stress providing an essential foundation for genetic and molecular studies. Specifically, Todd demonstrated that *C. elegans* survives and adapts to extreme hypertonicity by accumulating the organic osmolyte glycerol. He also demonstrated that glycerol accumulation is mediated by increased synthesis and that hypertonic stress induces a 20-fold increase in mRNA levels of the glycerol 3-phosphate dehydrogenase encoding gene *gpd-1*. This adaptation mechanism is analogous to accumulation of the organic osmolyte sorbitol in kidney cells. A paper describing these studies was published last year in the *American Journal of Physiology*.

Todd went on to create transgenic worm strains expressing a *gpd-1* GFP reporter. Under control conditions, GFP fluorescence is undetectable. Exposure of worms to hypertonic stress results in rapid and dramatic induction of GFP fluorescence in the intestine and hypodermis, which is part of the animal's excretory system or 'kidney'. I cannot overstate the significance of this observation. Using this 'ON/OFF' GFP reporter, it is now possible to carryout for the first time forward and reverse genetic screens to identify gene networks that allow an animal cell to sense osmotic stress and transduce that stress through specific signaling pathways into a regulatory response (i.e., increased *gpd-1* transcription and organic osmolyte accumulation).

Using the GFP reporter and RNAi feeding, Todd screened ~16,000 genes in less than four months. This screen identified 111 genes that when knocked down induce inappropriate transcription of *gpd-1* under non-stress conditions. Remarkably, 46 of these genes are involved in 'protein homeostasis'. Specifically, they function to maintain normal levels of properly folded and functioning proteins in the cell. This finding is particularly relevant because hypertonic stress causes protein denaturation and damage. Organic osmolytes help denatured proteins refold and they protect proteins from denaturing stressors.

Interestingly, Todd found that 31 of the genes he had identified also function to regulate age-dependent polyglutamine protein aggregation in *C. elegans*. Of these 31 genes, 22 function in protein homeostasis. Thus, knocking down protein homeostasis genes induces premature protein aggregation and triggers increased transcription of *gpd-1* in the absence of hypertonic stress. These findings suggest the novel and important hypothesis that genes involved in organic osmolyte accumulation are activated by protein damage through as yet unknown mechanisms. If this hypothesis withstands experimental testing, it will represent a paradigm shift in the field. Todd is currently writing this work up for publication.

Todd's RNAi screen also implicated *osm* genes as being important regulators of *gpd-1* expression. *osm* genes were previously identified as genes that regulate the ability of worms to sense and move away from a strongly hypertonic solution. Animals with mutant *osm* genes show defective osmosensing. Todd has shown that RNAi or loss-of-function mutations in three *osm* genes constitutively activate *gpd-1* expression and induce striking elevation of whole animal glycerol levels. Todd is currently cloning and characterizing these genes and will write this work up for publication shortly. His findings suggest an interesting and novel relationship between adaptations in osmosensory neuron physiology and whole animal osmotic balance.

Todd has a deep understanding of *C. elegans* biology and genetics and is an extraordinarily imaginative thinker. During lab meetings and informal conversations, he frequently suggests novel approaches for addressing physiological problems using *C. elegans*. For example, shortly after Todd arrived in the lab, he suggested that insulin-signaling mutants might show increased resistance to hypertonic stress. I thought the idea was farfetched, but agreed to let him test it. Todd's idea was correct. Mutations in the insulin receptor gene *daf-2* or genes in the downstream signaling pathway confer dramatically enhanced hypertonic stress resistance. These genes play a critical role in ageing and are highly conserved in all metazoan animals including humans. Using RNAi screening of 222 *daf-2* regulated genes, Todd identified 14 genes including heat shock protein encoding genes and genes encoding trehalose synthesis enzymes that confer hypertonic stress resistance in *daf-2* mutants. His work on *daf-2* was published this year in the *American Journal of Physiology*.

Todd is an excellent teacher and has worked closely with and trained several students. For example, Peter Agre recently sent one of his graduate students, George Huang, to the lab to characterize aquaporin physiology in *C. elegans*. I turned the day-to-day supervision of George over to Todd. George's progress has been outstanding and his thesis will be focused largely on *C. elegans* water

channels. He and Todd have already published an important methods paper and will publish one and possibly two major papers describing aquaporin physiology.

During the last nineteen years, I have trained over 55 students and postdoctoral fellows. I have been especially fortunate that the majority of these individuals were excellent to outstanding, and even brilliant investigators. However, only five of these individuals possessed the combination of intellect, imagination, technical skills, adaptability, and importantly, drive necessary to make a successful career in academic basic research. Todd is one of those five. He is brilliant, intellectually fearless and adaptable, and he is driven. He is an astute and mature observer of academic life and clearly understands what it takes to survive and be successful in this business.

Todd is an excellent colleague to everyone. I have little doubt that his understanding of and enthusiasm for *C. elegans* as a model system will be transferred into new projects in other labs with which he interacts in the future. Todd has an excellent sense of humor and knows how to roll with the trifling punches of academia. He is a superb teacher and readily shares his knowledge with those around him. His talks and lectures are always lucid and cogent. Having Todd in the lab has been a two-way street. I hope he learned as much from me as I learned from him and his research.

In summary, I can recommend Todd for a tenure track faculty appointment in your department without reservation. This is not a recommendation I make lightly. I strongly believe that most people in Ph.D. programs or postdoctoral positions are not well suited for life as a principal investigator. Todd is one of the rare ones who will make it. I have no doubt that he will become an internationally recognized leader in his field of research and that he will run a consistently productive and well-funded laboratory.

Sincerely yours,

Kevin Strange, Ph.D.
Professor of Anesthesiology, Molecular Physiology and Biophysics, and Pharmacology
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September 12, 2005

Dr. Yves Brun
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Dear Search Committee Members:

This letter concerns Dr. S. Todd Lamitina, who has applied for an assistant professor position in your department. Todd graduated in June 2002 from the Program in Biochemistry, Cell and Developmental Biology (BCDB) at Emory University and performed his thesis research in my laboratory. Consequently, I know Todd very well and feel that he is an outstanding young scientist that will be a successful Principal Investigator running his own research program. I will direct my comments below to Todd's time when he was a student in my lab and leave it to others to describe his successes as a postdoctoral fellow.

Todd was admitted to our Program during the Fall of 1997. During that first semester, Todd took my IBS 519 Foundations in Developmental Biology course. This is a combination lecture and student paper presentation course. Todd volunteered to be the first student to present, and his presentation was the best I had witnessed in the over 10 years I have been director of this course. He was confident, knowledgeable, witty and engaging of the other students (who can be passive if not prodded). There was a lot of space between Todd's two presentations and my candidate for the second best presentation of the semester. All of what I just said was made more impressive when I learned that this was Todd's first "public" presentation. Todd's second presentation and participation in this course were equally outstanding and he earned an "A" in the course. Shortly after his first presentation, Todd approached me and asked if he could rotate in my laboratory and I immediately agreed. Todd was curious about learning transmission genetics and we set up a project where he was to map and characterize several new spermatogenesis defective mutants by light microscopy.

Todd participated in the Physiology Course at the Marine Biological Laboratory, Woods Hole during the summer of 1998. As a former student of this course (1980), I know he faced an incredibly intense summer of research and course work. From discussions I had with Dan Kiehart (one of Todd's course instructors, from Duke), I

know that Todd made a very positive impression on the faculty and other students in his MBL class that summer.

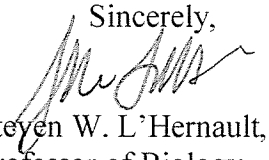
Todd's thesis research was to work on a series of dominant spermatogenesis defective (*spe*) mutants that are in my *C. elegans* strain collection. On the side, he also contributed to the positional cloning of a gene involved in fertilization (this was a hobby project; it has since been taken up by one of my postdocs and Todd will be an author on the paper that is presently being written). Dominant *spe* mutants present special difficulties and, during my first 10 years at Emory, I was not able to convince prior students to work on this project. They require a lot of attention, are easily lost and can have adverse effects on one's life style. However, dominant mutants allow genetic strategies for assembling morphogenetic pathways that are unavailable for recessive mutants. Todd realized the potential of these dominant *spe* mutants and was not deterred by the difficulties of working with these strains. His work on this project over the 5 year he was in my lab was impressive. He showed that six independent dominant *spe* mutants map to the same genetic interval and all have the same interesting light and electron microscopic phenotypes: spermatocytes do not undergo either cytokinesis or meiosis, yet they appear to "differentiate" in that certain spermatid-like nuclear and cytoplasmic maturation events occur. We tentatively named this gene *spe-37*. Our extensive characterization of recessive *spe* mutants has not identified any other mutants that show this collection of interesting defects. However, cell cycle mutants with analogous problems have been found to affect *Drosophila* spermatogenesis, suggesting that *spe-37* participated in a conserved process.

Todd used knockdown in *cis* to generate 10 ENU induced intragenic revertants of one dominant *spe-37* mutations. This generated recessive mutants that fall into three phenotypic categories. To make a long story short, Todd used a combination of positional cloning and a candidate gene approach to prove that *spe-37* encodes WEE-1.3, which is a negative regulatory kinase of Cdc2p. WEE-1.3 is a homolog of *S. pombe* Wee1p but, while Wee1p is a soluble protein found in the nucleus, WEE-1.3 is a one-pass integral membrane protein. WEE-1.3 has orthologs in all metazoa, but a genetic approach was not performed in any species prior to Todd's work. Todd sequenced all of the dominant mutants and the recessive suppressors. All six dominant mutations affect one of three residues in a region of four contiguous residues of SPE-37/WEE-1.3; in fact three mutations are identical, even though they are of unambiguous independent origin. The positions of the 10 suppressors are consistent with them being either reduction of function or null mutations. This analysis revealed that *wee-1.3* is an essential gene, apparently for most, if not all, mitotic and meiotic divisions in *C. elegans*. Apparently, the dominant *spe-37/wee-1.3* mutations permit its kinase activity to persist inappropriately during spermatogenesis, revealing that this universally used kinase is subject to tissue specific regulation. The first paper describing this work appeared in Development. Todd also characterized antibodies he raised to SPE-37/WEE-1.3. This paper has had some twists and turns, but is now being written and will be submitted very soon. I could go on, but you get the idea: Todd has generated a large body of high quality data on a very interesting biological problem.

Todd is incredibly motivated to do laboratory science and, in this category, is certainly within the top 5% of what I have seen among his peers in my 17 years at Emory. I would also place him in the top tier of graduate students I have known at Johns Hopkins (Carnegie Institution) and Yale, where I trained. He was an old-fashioned graduate student that was in it for the love of science, which seems to be an increasingly

rare quality these days. He worked long hours and, quite simply, did what it took to get a technique or procedure to work in his hands. He asked many questions in the lab and was always thinking about the experiments he was or would be doing. He aggressively sought out the help of others when necessary and quickly and thoroughly absorbed the information they provide. He had a positive attitude about science, did not complain, get discouraged or require a great deal of what could be generally referred to as "maintenance". I attend about 2-4 seminars (journal clubs, research progress talks, thesis committee presentations, etc) each week presented by graduate students, both in BCDB and the Genetics Program. Over the years, this has amounted to hundreds of such presentations. Todd easily grouped into the top 5% when compared to other students I have heard speak in these various forums. His presentations were clear, concise, inclusive and interesting. In short, Todd was and will continue to be productive and he will reflect well on our Program and Emory. It was a privilege to mentor Todd, and he will make an outstanding young investigator at the institution that is fortunate enough to attract him. He is smart, interactive, and incredibly enthusiastic about science; he will make a superb colleague. If there are any other issues you would like me to address, please do not hesitate to contact me at (404) 727-4204 or at bioslh@biology.emory.edu.

Sincerely,



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Nashville, September 12, 2005

To Whom It May Concern:

I am writing to you today in reference to Dr. Todd Lamitina's application for faculty position in your institution. I have known Todd since 2002 when he joined Dr Strange's laboratory for a post doctoral fellowship. I have personally interacted with Todd many times, both professionally and in private, and have greatly enjoyed working with him as a colleague and a friend.

Todd and I have collaborated on the identification of glycerol as one of the predominant osmolytes accumulated in *C. elegans*. During these studies I have come to know Todd as a highly energetic and curious researcher who does not shy away from tackling difficult problems. He is very persistent in the pursuit of his research goals and has no hesitation approaching new techniques to get to the right answer of his research questions. He is intellectually aggressive in a positive sense and has a great depth of knowledge, not only in molecular biology and genetics, but also in many other areas of physiology and biology. He has excellent capabilities to interpret his research results in regard to broader applications in human and animal studies and is highly motivated to expand and bridge his *C. elegans* studies to comparative studies in other species.

Todd has already shown a promising start in his academic publication record and will very likely be a prolific scientist in the years to come. In my opinion Todd is an outstanding individual and young scientist who has all the capabilities and training to be highly successful in an academic career. I recommend Dr. Lamitina without any reservation as a faculty member in your institution and support his application to the highest degree. If you would like to discuss his application further, please do not hesitate to call me at 615-322-1350.

Sincerely yours,

Gilbert W. Moeckel MD, PhD, FASN
Assistant Professor of Pathology & Medicine
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