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Yves Brun
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Dear Professor Brun,

I am writing in response to your advertisement in the September 2 issue of *Science* for a tenure-track assistant professor position in Microbiology. Both my PhD (Microbiology, Cornell University, 2002) and post-doctoral research (Cornell University College of Veterinary Medicine) have centered on the molecular genetics of Gram positive bacteria.

I believe that my graduate training followed by four years of postdoctoral research have prepared me to be an independent investigator in the field of Microbiology. My graduate work with Dr. John Helmann focused on the roles of extracytoplasmic function (ECF) sigma factors in *Bacillus subtilis*. I approached the project from different angles: by investigation of the phenotypes of mutant strains lacking one or more ECF σ factor(s), by identification of target genes for each σ factor, and by characterization of signals that can induce expression of each σ regulon. This work has led to 7 first author publications and clearly defined the overlapping roles of three of the seven ECF σ factors. Of particular note, I developed ROMA (a technique that combines *in vitro* transcription and macroarray analysis) to survey the genome for promoters active *in vitro*. My paper on this work was highlighted twice in the journal of Current Opinion in Microbiology: as a paper of "Special interest" in Microbiology Paper Alert and as a paper of "Outstanding interest" in a review article.

For my post-doctoral studies I was able to apply my expertise in bacterial genetics and molecular biology to work with the food-borne pathogen *Listeria monocytogenes* with Dr. Helene Marquis. I have developed a new transposition system with higher efficiency and randomness to replace the *Tn917* system which has been used for the past 15 years. My current work has focused on characterizing bacterial factors involved in *L. monocytogenes* resistance to oxidative and nitrosative stress by transposon mutagenesis. This work will be the pedestal for my future research work in the relationship of oxidation, nitrosylation and *L. monocytogenes* pathogenesis.

During my graduate training, I had the opportunity to serve as a teaching assistant for fundamental biology lecture and laboratory courses and I supervised several rotation students. These experiences introduced me to the pleasure of passing knowledge to other people. I am confident that I can be a good educator as well as a good scientist.

I would enjoy discussing this position with you in the weeks to come. In the meantime, I am enclosing my Curriculum Vitae, statement of research and teaching interests, reprints of publications, and names of four references who will be sending letters. Thank you very much for your consideration.

Sincerely,

Min Cao

Research Interests: Regulation of *Listeria monocytogenes* pathogenesis by oxidation and nitrosylation

Background and Significance

L. monocytogenes is a ubiquitous gram-positive facultative intracellular pathogen that can cause serious food-borne infections (listeriosis) in immunocompromised individuals, neonates, and elderly individuals. The infection develops into a bacteremia, meningoencephalitis and possibly septicemia, whereas in pregnant women it causes spontaneous abortion(5). The mortality rate from listeriosis is about 20%, the highest of all food-borne illnesses reported. The pathogenesis of *L. monocytogenes* centers on its ability to survive and multiply in phagocytic host cells. The primary virulence factors responsible for the intracellular growth of *L. monocytogenes* are clustered in the PrfA regulon. PrfA is a positive transcriptional regulator and is essential for expression of genes within the PrfA regulon (8, 12).

Two very effective antimicrobial systems of phagocytic cells are the NADPH phagocyte oxidase and iNOS, which are responsible for generating reactive oxygen species (ROS) and reactive nitrogen species (RNS)(4, 10). The importance of nitric oxide and the oxidative burst in controlling infection by *L. monocytogenes* is well established. Mice deficient in either NADPH phagocyte oxidase (gp91-/-) or iNOS (nos2-/-) have enhanced susceptibility to *L. monocytogenes* infection(3, 7). One of the virulence traits of *L. monocytogenes* is its ability and efficacy at lysing the phagocytic vacuole. It was shown that in activated mouse bone marrow-derived macrophages, the production of ROS and RNS directly affects the bacteria's ability to escape from vacuoles. Although these activated macrophages inhibited bacterial escape from vacuoles, they were not listericidal indicating that *L. monocytogenes* is relatively resistant to oxidative and nitrosative stress(9).

I am interested in studying the role of oxidation and nitrosylation in the pathogenesis of *L. monocytogenes*. The specific hypotheses behind the proposed research are that (1) *L. monocytogenes* possess mechanisms of resistance to oxidative and nitrosative stress, and that (2) Oxidation and nitrosylation regulate the functions of proteins responsible for bacterial escape from vacuoles. My long-term goal is to elucidate the mechanisms by which *L. monocytogenes* survives oxidative and nitrosative stress, and those by which ROS, NO, and RNS prevent *L. monocytogenes* from lysing the vacuolar membrane.

Present Work

I have focused on the mechanisms involved in resistance of *L. monocytogenes* to oxidative and nitrosative stress by screening for sensitive mutants generated by transposon mutagenesis. However the transposons that are actually available for use with *L. monocytogenes* are far from optimal for performing genome scale transposon mutagenesis. Thus my postdoctoral work started with developing a good transposition system.

A. Construction of a *Himar1 mariner*-based transposition system for *L. monocytogenes*

In recent years, *Himar1 mariner* has been used as the transposon of choice to perform high throughput mutagenesis in many different bacterial species(1, 13). The *mariner* requires no factors for transposition other than its self-encoded transposases and its requirement for insertion is the dinucleotide TA(6, 11), which makes it perfect for transposition into low GC content organisms such as *L. monocytogenes* (39% GC). I have constructed two *mariner*-based transposon delivery vectors. Each vector contains: (1) the *Himar1 mariner* transposase gene (*tnp*) directed by a strong *Bacillus subtilis* promoter (P_{mrgA} or P_{katA}), (2) the Gram-positive erythromycin resistance (*ermC*) gene flanked by the 29-bp inverted repeat elements of the *Himar1 mariner*, (3) the kanamycin resistance (*kan*) gene

as a screening marker for loss of the plasmid, (4) the temperature-sensitive replication origin (ts *ori*) derived from pLTV3(2) and (5) the *E. coli* low-copy-number replication origin (p15A *ori*)and the Gram-negative chloramphenicol resistance (*cat*) gene. Both vectors have been tested in *L. monocytogenes* and worked equally well. This transposition system has high transposition efficiency and is highly random. I believe that this *mariner*-based transposition system will be a great tool not only for the project described herein, but also for the entire community of scientists working with *L. monocytogenes*.

B. Identification of *mariner* insertional mutants sensitive to oxidation and nitrosylation

I have started to search for factors that are crucial for defense against oxidative and nitrosative stresses by screening libraries generated by the *mariner*-based vectors. Twenty independent libraries (~ 15,000 erythromycin-resistance mutants) were generated in the *L. monocytogenes* wild-type strain 10403S. I have used acidified nitrite and sodium nitroprusside (SNP) to study the nitric oxide and nitrosative stress. Hydrogen peroxide and diamide (a thiol-specific oxidant) were used to study oxidative stress. I have screened 9,000 colonies with acidified nitrite and only got one sensitive mutant. 3,000 colonies were screened with SNP and 21 were sensitive mutants. 6 out of 6,000 mutants and 3 out of 3,000 mutants were found to be sensitive to hydrogen peroxide and diamide individually. Current and future work involved the characterization of the mutants, the genes so identified, and their roles and regulation. Note that this work is completely distinct from the long range plans of Dr. Marquis' lab, and this project is one that I am free to take with me to a new position.

Future Research Plans

1. Characterization of factors involved in *L. monocytogenes* resistance to oxidative and nitrosative stress.

The *L. monocytogenes* genome contains approximately 3,000 ORFs: my initial screening is obviously not saturated. I will screen for more sensitive mutants. Meanwhile, I will characterize the sensitive mutants (obtained from initial screening) by sequencing. Subsequently, non-polar mutations will be generated in identified genes for further studies in activated macrophages. I will measure survival rates of bacteria in activated bone marrow derived macrophages from wild-type mice, phox-/- mice (absence of NADPH phagocyte oxidase) and nos2-/- mice (absence of iNOS). Alternatively, macrophages from wild-type mice will be treated with inhibitors of oxidative and nitrosative response.

2. Determination whether *L. monocytogenes* factors involved in bacterial escape from primary vacuole are targets of oxidation and nitrosylation.

I will collaborate with Dr. Marquis to look at the bacterial factors that mediate membrane lysis of the primary vacuole. These factors are the pore-forming hemolysin listeriolysin O (LLO) and the phosphatidylinositol-specific phospholipase C (PI-PLC). I will also look at the positive transcriptional regulator (PrfA), which is essential for expression of these factors. I will check the transcriptional and translational levels of these genes, as well as the activity of the proteins.

3. Identification of *L. monocytogenes* factors that are susceptible to modification by nitrosylation.

I will use bacterial broth cultures to study protein modification by nitrosylation. NO donors will be added to the bacterial culture for a short period of time. Modified proteins will be identified using an established biochemical technique. Genes encoding these proteins will be mutated to study their biological functions and correlation with the pathogenesis.

References:

1. **Bae, T., A. K. Banger, A. Wallace, E. M. Glass, F. Aslund, O. Schneewind, and D. M. Missiakas.** 2004. Staphylococcus aureus virulence genes identified by bursa aurealis mutagenesis and nematode killing. Proc Natl Acad Sci U S A **101:**12312-7.
2. **Camilli, A., A. Portnoy, and P. Youngman.** 1990. Insertional mutagenesis of Listeria monocytogenes with a novel Tn917 derivative that allows direct cloning of DNA flanking transposon insertions. J Bacteriol **172:**3738-44.
3. **Dinauer, M. C., M. B. Deck, and E. R. Unanue.** 1997. Mice lacking reduced nicotinamide adenine dinucleotide phosphate oxidase activity show increased susceptibility to early infection with Listeria monocytogenes. J Immunol **158:**5581-3.
4. **Fang, F. C.** 2004. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. Nat Rev Microbiol **2:**820-32.
5. **Farber, J. M., and P. I. Peterkin.** 1991. Listeria monocytogenes, a food-borne pathogen. Microbiol Rev **55:**476-511.
6. **Lampe, D. J., M. E. Churchill, and H. M. Robertson.** 1996. A purified mariner transposase is sufficient to mediate transposition in vitro. Embo J **15:**5470-9.
7. **MacMicking, J. D., C. Nathan, G. Hom, N. Chartrain, D. S. Fletcher, M. Trumbauer, K. Stevens, Q. W. Xie, K. Sokol, N. Hutchinson, and et al.** 1995. Altered responses to bacterial infection and endotoxic shock in mice lacking inducible nitric oxide synthase. Cell **81:**641-50.
8. **Mengaud, J., S. Dramsi, E. Gouin, J. A. Vazquez-Boland, G. Milon, and P. Cossart.** 1991. Pleiotropic control of Listeria monocytogenes virulence factors by a gene that is autoregulated. Mol Microbiol **5:**2273-83.
9. **Myers, J. T., A. W. Tsang, and J. A. Swanson.** 2003. Localized reactive oxygen and nitrogen intermediates inhibit escape of Listeria monocytogenes from vacuoles in activated macrophages. J Immunol **171:**5447-53.
10. **Nathan, C., and M. U. Shiloh.** 2000. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proc Natl Acad Sci U S A **97:**8841-8.
11. **Plasterk, R. H., Z. Izsvak, and Z. Ivics.** 1999. Resident aliens: the Tc1/mariner superfamily of transposable elements. Trends Genet **15:**326-32.
12. **Portnoy, D. A., T. Chakraborty, W. Goebel, and P. Cossart.** 1992. Molecular determinants of Listeria monocytogenes pathogenesis. Infect Immun **60:**1263-7.
13. **Rubin, E. J., B. J. Akerley, V. N. Novik, D. J. Lampe, R. N. Husson, and J. J. Mekalanos.** 1999. In vivo transposition of mariner-based elements in enteric bacteria and mycobacteria. Proc Natl Acad Sci U S A **96:**1645-50.

Min Cao

Statement of Teaching Philosophy and Teaching Interests

"*A teacher for a day is like a parent for a lifetime*". - Ancient Chinese proverb

I believe that good teachers play an indispensable role in the development of a young person's life. To me, being a teacher will be the best way to express my appreciation to all the teachers who offered me knowledge, support and encouragement.

Being a student for more than twenty years, I am acquainted with the feeling of anxiety when entering a new class: "What if I don't know anything?" "Will the teacher think that I am stupid?" "The teacher is so knowledgeable and inapproachable." Now, from a teacher's view, I will try to relieve those feelings by making myself available and approachable to students. I will let my students know that they can count on me for prompt, helpful, and reliable answers. Teaching is a process of encouraging students to make connections between their day-to-day experiences and abstract concepts. I believe that all students are capable. Each student may bring some new insights to a subject or raise questions that have not yet been considered.

Students taking basic Microbiology courses always come from a diversity of backgrounds, and some parts of Microbiology (like metabolism and microbial genetics) can be very intricate to them. The instructor must grasp the fundamentals of the discipline so that he/she can pass them to the students in a plain way. Besides, a good instructor should be aware of the up-to-date proceedings of the ongoing research in the field. He/she should be actively engaged in research and participating in classes, conferences, and workshops.

I believe that classes should be "learner-centered". I would encourage students to debate scientific issues. Small group discussion is a very useful format that offers each student a chance to express thoughts and to learn from their peers. I have noticed that students usually learn more and faster if the information comes from one of them. I think that a social and interactive learning environment is the most effective way to transfer knowledge.

I am interested in teaching Microbial Genetics at both undergraduate and graduate levels. Genetics is a complex field and can be very difficult to understand. But on the other hand, it is such an elegant art involving deliberate thinking and logical reasoning. I have learned from my own experience that there is no better way to understand genetics than by experiential and tactile learning in a lab setting, where students can learn basic principles and techniques best through practices. For graduate students, I will include comprehensive readings and discussions from recent research papers so that they can gain experience in critical analysis. By evaluation of others' work, one can learn to critique the strengths and weaknesses of one's own research.

"Open Doors, Open Hearts and Open Minds"- This is Cornell's statement on diversity and inclusiveness. I will also adopt it as my teaching philosophy. Being a Cornellian, I will pass this spirit to my colleagues and work hard to generate and maintain an open environment for my students.