November 29, 2005

Yves Brun, Ph.D.
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 am submitting my application materials for the tenure-track faculty position in the Department of Biology and the Biocomplexity Institute at Indiana University. My studies directed towards elucidating regulatory mechanisms of chlamydial development appear to compliment departmental strengths. As such, I look forward to discussing my potential future in this department.

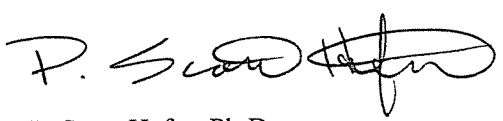
I have recently started my third year of postdoctoral training at the University of California, Berkeley. My research is focused on the elucidating global regulatory mechanisms involved in developmental conversion of the medically important human pathogen, *Chlamydia*. These studies have been performed under the sponsorship of Dr. Richard Stephens, Professor and Chair of the Division of Infectious Diseases, School of Public Health at UC-Berkeley.

To gain a better understanding of chlamydial developmental regulation and host-pathogen interactions, I have analyzed the function of an alternative sigma factor and cis-acting regulatory elements of the type III secretion system. Together these studies have provided important new insight into our overall understanding of the processes controlling formation of infectious *Chlamydia* and identification of potential virulence factors. Future studies are designed to build upon these findings and apply similar approaches to identify and characterize other developmental regulatory mechanisms.

Throughout my professional and educational experience, I have immersed myself in various aspects of cellular microbiology, virology and immunology. My prior research training and teaching experiences have provided critical growth as an interdisciplinary scientist with a focus on microbial pathogenesis. I anticipate that these will continue to prove invaluable both in the laboratory and classroom.

I am looking forward to the challenges and rewards associated with teaching and mentoring students. My experience has reinforced my desire to maintain an academic course of professional development. I believe that my excitement for science and its process will be transferred to the students that I have the privilege to serve in the future.

I have enclosed my curriculum vitae, statement of research plans, teaching interests and a list of professional references from which letters have been requested. It may be useful to note that I am also applying for the tenure-track position in the Department of Biology and Interdisciplinary Human Program. Thank you for considering my application and I look forward to your response.



P. Scott Hefty, Ph.D.
141 Earl Warren Hall
Berkeley, CA 94720
510-643-7651
pshefty@berkeley.edu

STATEMENT OF RESEARCH INTERESTS

Developmental regulation is a fundamental biologic process that has captivated my scientific curiosity. During my graduate studies, I was fascinated by the transformation *Borrelia* exhibited as it alters surface molecules according to the diverse environments of its life-cycle. *Chlamydia* is equally intriguing to me as it converts between two distinct morphologic forms, sequestered within a unique inclusion vacuole. Throughout, the same question has energized my investigations, "How are these virulence processes regulated?"

CHLAMYDIAE AND DEVELOPMENTAL BIOLOGY

Chlamydiae are successful bacterial pathogens that have an immense impact on public health. *Chlamydia trachomatis* is the most common sexually transmitted bacterial infection with an estimated 90 million new cases annually. In developing countries, *C. trachomatis* is the leading cause of preventable blindness. Additionally, growing evidence supports *C. pneumoniae* as a contributing factor of atherosclerosis and coronary disease, the second leading cause of human mortality. Despite its medical significance, many fundamental questions of chlamydial biology have yet to be elucidated, especially those related to developmental regulation and pathogenesis.

Chlamydiae are obligate intracellular bacteria that are perpetuated by a defining biphasic developmental cycle that is inexorably linked to pathogenesis. Intercellular transmission of *Chlamydia* occurs as a metabolically inert elementary body (EB) that, subsequent to gaining entry to the host cell, converts into a metabolically active, yet non-infectious, reticulate body (RB). Following numerous rounds of replication, reciprocal conversion occurs and the developmental cycle is repeated as EBs are released into the extracellular milieu. An important deviation from this cycle, especially clinically, is establishment of a persistent infection where RB to EB conversion is arrested. Relatively little information exists regarding specific extrinsic or intrinsic factors that regulate the developmental cycle and persistence. Moreover, the detailed events that occur during conversion processes are equally unknown.

RESEARCH GOAL AND SIGNIFICANCE

Defining regulatory components of chlamydial development will provide critical insight to basic biology and mechanisms of pathogenesis. Our global transcriptional analysis of the developmental cycle revealed that virtually all genes differentially expressed are up-regulated during the developmental stage correlated with RB to EB conversion. This highlights the governing role of transcriptional regulation during this essential developmental stage. EBs exhibit extensive infectious capabilities as they bind, facilitate entry and establish a unique non-lysosomal vacuole requisite for chlamydial growth. Furthermore, induction of persistent infections results in RB to EB conversion arrest. Therefore, **My primary research goal is to characterize the regulatory mechanisms of gene expression during chlamydial RB to EB conversion.**

Understanding control mechanisms employed during this developmental stage has four levels of significance. First, delineating the genetic regulatory mechanisms will generate critical information regarding molecular mechanisms of persistence or other relevant forms that promote pathogenicity. Second, revealing the regulatory components that control EB generation will provide specific targets for chemotherapeutic agents. Third, due to the current absence of a genetic exchange system for *Chlamydia*, defining trans-acting factors and cognate cis- acting elements will allow for the accurate application of heterologous systems that permit manipulation (e.g. *E. coli*). Fourth, chlamydiae are phylogenetically deeply rooted, diverging an estimated one billion years ago. Indicating that what is learned about chlamydial regulatory mechanisms may be widely retained in organisms that diverged after *Chlamydia* and may represent important basic biologic processes.

Ultimately, I envision determining how each trans-acting factor and cis-acting element contributes to RB to EB conversion independently and how they function in concert. However, it is imperative to first identify these components. Therefore, my immediate research goal incorporates two approaches; 1) elucidation of the regulatory components for the virulence determinant type III secretion system and 2) identification of alternative sigma factor gene targets and cis-acting elements for genes up-regulated during RB to EB conversion.

CHLAMYDIAL TYPE III SECRETION SYSTEM

Type III secretion systems (T3SS) are widely utilized by Gram-negative bacteria to inject virulence proteins into eukaryotic host cells. Chlamydiae encode a highly conserved T3SS that is apparently vital to development. The majority of chlamydial T3SS encoding genes are up-regulated during the RB to EB conversion process. Analogous to virtually all similarly expressed genes, little is known regarding chlamydial T3SS regulatory mechanisms. T3SS are typically regulated by a specific transcription factor that controls expression of core secretion machinery as well as the virulence proteins delivered to the host cell, termed effectors. Identifying this transcription factor and defining cognate DNA recognition sequences will reveal genes encoding candidate effector proteins. Furthermore, due to the relative paucity of regulatory components encoded by chlamydiae, the T3SS regulatory mechanism may be shared with other RB to EB conversion-associated genes.

My post-doctoral research was focused on defining the chlamydial T3SS operon structure. In all bacteria that harbor a T3SS, encoding genes are located in a single locus that also contains specific transcriptional regulatory factor(s) and occasionally effector proteins. Chlamydial T3SS gene organization is unique in that its 'locus' is fragmented and scattered throughout the genome. Many T3SS homologs are also separated by genes that encode proteins with unknown function. Genes contained within a transcriptional unit (operon) predominantly encode proteins involved in the same cellular function. My studies revealed that many genes encoding proteins with unknown function are transcriptionally-linked to T3SS homologs providing candidate T3SS regulatory and effector proteins.

Defining the T3SS operon structure also allowed for identification of promoter and additional cis-acting regulatory elements. Transcriptional start sites were determined for each operon revealing a classical housekeeping promoter. Functional studies employing heterologous systems (*E. coli*) demonstrate that T3SS promoters can be recognized by the primary sigma factor ($E\sigma^{70}$). Importantly, these data support that a repressor or activator regulates expression of the T3SS. Current studies are focused on identifying conserved T3SS cis-acting elements that is recognized by this regulatory factor.

Based upon these findings, **future studies are designed to identify T3SS trans-acting regulatory factors.** Chlamydiae encode many homologous transcription factors that regulate T3SS gene expression in other organisms. These include two signal transduction systems and a transcriptional activator. Identifying the T3SS trans-acting factors will incorporate direct analysis of these components as well as DNA affinity and expression library methods to identify potentially novel transcription factors. In collaboration with Dr. Chester Price (University of California-Davis), the interaction of the terminal signal transduction component system (RsbW) with an alternative sigma factor (σ^{28}) was analyzed. It has recently been reported that the Rsb partner switching signal transduction system in *Bordetella* regulates the type III secretion system. This collaboration will continue in my new environment, as future studies will be directed towards determining targets of this signaling pathway and its potential role in T3SS regulation.

Identifying virulence factors are essential in elucidating mechanisms of pathogenicity. Without known exception, T3SS effector proteins have been defined as virulence determinants. **Future studies are focused on analyzing candidate effector proteins encoded within the T3SS operons.** In the absence of a system for genetic exchange, combinational approaches must be implemented to define chlamydial effector proteins. This includes demonstrating host cell cytosolic localization and type III dependent secretion within a heterologous system. The candidate T3SS effector proteins will be analyzed using these approaches. Functional studies would be initiated on proteins that met these criteria.

ALTERNATIVE SIGMA FACTORS

Alternative σ factors are widely utilized by bacteria to coordinately express entire gene subsets involved in diverse functions. All sequenced chlamydial genomes encode two alternative σ factors; σ^{28} and σ^{54}. The role of these σ factors is unknown, however data suggest that both are key in developmental regulation of RB to EB conversion. **My future specific research aims include identification of alternative σ factor regulons.** Identifying the gene targets for each alternative σ factors will result in four significant findings; 1) provide clarifying support for the role of these σ factors in RB to EB

conversion process, 2) elucidate what processes each regulates during this process, 3) identify potential virulence factors and 4) allow for charactization of each σ factor cognate cis-acting regulatory elements.

My post-doctoral studies have focused on elucidating the σ^{28} regulon. In the absence of a genetic exchange system, I enlisted the combination of *in vitro* transcription assays using genomic DNA and microarray analysis to identify σ^{28} gene targets. This is a powerful approach that circumvents potentially confounding pleiotropic effects associated with *in vivo* gene disruption or addition. While these studies are still ongoing, thus far these data support σ^{28} association with conversion processes and moreover, revealed new potential virulence factors. Through analyzing the transcriptional profiles generated from various structural genomic states (digested, relaxed, or supercoiled), these data reinforce that DNA topology may affect promoter activity *in vivo*. Importantly, we have also successfully applied a similar approach to identify gene targets of a chlamydial transcriptional regulator (ChxR). As a result, the binding sites of this trans-acting regulatory factor have been determined. These prior studies support the future utility of this approach to identify global gene targets of trans-acting factors.

While σ^{54} is typically associated with regulation of genes involved with nitrogen assimilation, it also is utilized for those determining many other unrelated metabolic functions. Defining the σ^{54} gene profile will establish a putative role in developmental regulation and reveal its association with metabolism. Unlike the σ^{70} family to which σ^{28} belongs, σ^{54} family members require an activator to initiate transcription. Therefore, two approached will be implemented to identify the σ^{54} regulon. First, σ^{54} capability to bind securely to its cognate DNA will be utilized to isolate specific DNA fragments. Second, the required σ^{54} activator will be modified using numerous methods previously demonstrated to generate transcriptionally competent σ^{54}. Once active, an approach similar to identifying σ^{28} regulon can be implemented. Moreover, generating transcriptionally active σ^{54} will allow for future studies to characterize the chlamydial σ^{54} cis-acting elements.

CIS-ACTING REGULATORY ELEMENTS

Cis-acting elements are essential molecular devices involved in transcriptional regulation of dynamic networks of gene expression. Promoter elements modulate transcriptional initiation while enhancers and operators increase or prevent transcription, respectively. Global transcriptional analysis of the developmental cycle has provided a precise list of genes up-regulated during the RB to EB conversion process. Identification and characterization of regulatory cis-acting elements for each of these genes will provide critical information regarding mechanisms of chlamydial developmental gene expression.

Transcriptional initiation is the primary step of gene expression and is regulated by specific protein-DNA interactions. The compilation and analysis of hundreds of experimentally-identified transcriptional start sites and upstream regions from both *E. coli* and *B. subtilis* was instrumental in the definition of canonical promoter elements. The paucity of experimentally-defined transcription start sites is the critical deficiency in elucidating chlamydial cis-acting regulatory elements. Importantly, of the described promoters, an extensive amount of divergence is exhibited compared to consensus promoters, highlighting the necessity to acquire more sequences. **Therefore, my future specific research aim is identification of transcriptional start site(s) for each gene upregulated during the RB to EB conversion process.** During my current studies, I have already mapped the 5' ends of over 20 genes. Surprisingly, virtually all are preceded by a σ^{70}-like promoter elements. This research is critical in defining sequences and motifs recognized by trans-acting factors that regulate this important developmental stage.

CONVERGING NETWORKS

In vivo, developmental regulation occurs through complex and interactive gene networks. By identifying gene targets for various trans-acting factors and defining cis-acting elements for complete gene sets, it is anticipated that much of this information will converge, expanding our comprehension of how these networks are regulated. By eventually expanding our analysis of regulatory mechanisms, including components responsible for sensing environmental changes and communicating this signal to affect gene expression, our understanding of chlamydial developmental regulation and concomitantly, host-pathogen

interactions, will be significantly advanced. Additionally, characterization of regulatory sensors should provide insight into the unique environment of the chlamydial inclusion and cues that trigger differentiation.

TEACHING INTERESTS

Academic scientists have a powerful influence on students. As such, it is important that they appreciate their role in teaching and mentoring. It was an instructor's enthusiasm for biology that initially inspired my interest in science. My utmost goal as an academic scientist is to provide a positive and enriching learning environment. I expect that my excitement for science and the scientific process will be conveyed to students that I have the privilege to serve.

Integration of various scientific disciplines is advantageous for progressive instructors. While my career has focused on Microbiology, numerous related disciplines have been emphasized including Molecular Biology, Microbial Physiology, Genetics, Pathogenesis, Virology and Immunology. Additionally, exposure to clinical diagnostic development and implementation has tethered my academic experiences to public health concerns. These have contributed to my development as an interdisciplinary scientist and will enhance my teaching capability.

Effectively communicating fundamental information is critical to the instructional process. This is usually achieved through fluency and preference for a given subject matter. As such, I would be effective as an instructor in the IU Microbiology 'Core' and Molecular Genetics courses. Many topics covered within these courses would be reinforced and placed in the framework of microbial pathogenesis through a graduate level Host-Pathogen Interactions course that I would also be interested in teaching.

The development of critical thinking and presentation skills is essential to scientific training. These skills can be fostered within a discussion course that dissects the scientific method in the context of current research publications. I would welcome the prospect of directing or participating in a course that includes group speaking and critical manuscript evaluation. The Critical Analysis of Scientific Literature - 'Paper Bashing course' appears to provide an appropriate discussion forum. Through these discussions, it is anticipated that students, especially graduate level, will become more effective in experimental design and manuscript preparation.

Science relies on the integrity and ethical practice of those who participate in its process. From data presentation to grant reviews, numerous situations require difficult ethical decisions to be made during the scientific process. Student exposure to these examples early in his or her scientific career will facilitate analysis and help navigate through complicated issues. I have had the opportunity to guest lecture for the Ethics and Scientific Integrity course offered at UC-Berkeley and would be interested in designing and incorporating a similar course at Indiana University.

While teaching and mentoring can be a challenge, it can be very rewarding. Much of my professional enjoyment is provided through student interactions, both undergraduate and graduate. I have had the opportunity both within my professional and academic careers to train and mentor numerous individuals. Observing an individuals progression as they integrate theoretical and technical concepts is especially rewarding. Therefore, I look forward to the challenges and enjoyment associated with classroom instruction and mentorship.