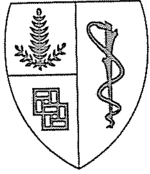


STANFORD UNIVERSITY SCHOOL OF MEDICINE

CENTER FOR CLINICAL SCIENCES RESEARCH
269 CAMPUS DRIVE • STANFORD, CA 94305-5174
(650) 723-6834 • FAX: (650) 723-2253

Department of Molecular Pharmacology

September 29, 2005

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

Dear Dr. Brun,

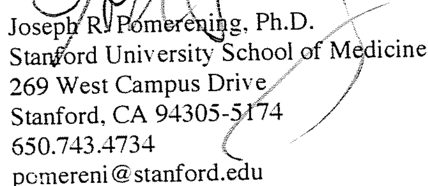
It is with tremendous interest that I am responding to an advertisement in a recent *Science* issue regarding the position of *Assistant Professor* in the Department of Biology and Biocomplexity Institute. I am completing my postdoctoral research in the laboratory of Dr. James E. Ferrell in the Department of Molecular Pharmacology at Stanford University, and I am looking forward to initiating an extensive academic research program, commencing summer 2006. The opening in your department calls for candidates "…enhancing our current strengths in biomolecular networks, including…signaling networks," and I find this to be an unequivocal match to my research interests and future scientific goals.

At the outset of my postdoctoral research, I was especially interested in studying cell signaling mechanisms and their regulation, but chose to approach it in a fundamentally different way: first, to focus on the behaviors that emerge from the wiring of a handful of enzymes, and then, to understand how the emergent properties of these circuits give rise to complex biological phenomena on the systems level. Using *Xenopus laevis* cell-free extracts, I discovered that the activation of the cyclin-dependent kinase, CDK1, is bistable – or has different on- and off-thresholds – in response to cyclin stimulus. This was a fundamental biological problem, and illustrated how a digital biochemical response can be derived from a graded stimulus. Building on these findings, I began to study the role of the positive-feedback loop that exists between CDK1 and its regulatory enzymes within the M-phase oscillator system. I discovered that the positive feedback involved in CDK1 activation is necessary for proper stimulation of the negative-feedback loop that directs CDK1 inactivation, and is therefore essential to provide sustained oscillations of CDK1 activity in the embryonic context. This work exemplifies that the systems-level behaviors that emerge from a subset of enzymes are as critical as the individual components of a biochemical circuit. My current work has expanded these studies into the context of the somatic cell cycle, and through the application of fluorescent biosensors, inducible expression systems, RNAi, and chemical biological and proteomic approaches, my lab will be poised to perturb, assess, and quantitate the input/output relationships of signaling pathways and interpret how they are wired to govern cell division and growth.

As I expand upon these ideas and findings to begin my own independent research program, I find a place in the Department of Biology and Biocomplexity Institute to be ideal. With a rich presence of molecular and cell biological expertise, I foresee endless possibilities for delving deeper into the signaling mechanisms and higher-order behaviors that arise within a multitude of biochemical and regulatory systems. What I find particularly exciting is the wide array of biological interests, including cell cycle regulation, development, and the signaling mechanisms that modulate these processes in numerous model systems, and I see it as a spectacular place to not only build upon that diversity, but also to contribute some molecular and systems-level insights into how signal transduction pathways are wired to establish functional biochemical systems within cells. I am eager to contribute to the research and teaching missions of the department and Institute, and aim to encapsulate and integrate methodologies and findings past and present within my research laboratory and classroom.

Enclosed with this letter are my CV, statements of past, present, and planned research, my teaching philosophy and ideas, representative publications, and a list of my referees. I would like to sincerely thank you and the faculty search committee for considering my application. I look forward to hearing from you.

Very truly yours,



Joseph R. Pomerening, Ph.D.
Stanford University School of Medicine
269 West Campus Drive
Stanford, CA 94305-5174
650.743.4734
pomereni@stanford.edu

DOCTORAL RESEARCH

My research in the laboratory of Thomas Jacobs at the University of Illinois was initiated by identifying proteins that interact with cyclin-dependent kinase (CDK), and we discovered that the subunit eEF1Bβ of the eukaryotic translational elongation factor eEF1 is a physical and catalytic target of CDK. This study uncovered some interesting aspects of the functional organization of eEF1 in eukaryotes. While plants and animals possess homologues of eEF1Bα, akin to their evolutionary predecessor *Saccharomyces cerevisiae*, they also contain an additional subunit called eEF1Bβ for which no function had been elucidated. Bioinformatic analysis of these sequences revealed a distinguishing characteristic of eEF1Bβ: the presence of a conserved CDK site. I found that CDK could phosphorylate recombinant eEF1Bβ protein, but not eEF1Bα, or eEF1Bβ that was null for its CDK site. A functional study revealed that wild-type eEF1Bβ could not replace eEF1Bα in yeast and inhibited proliferation, whereas a version missing the conserved CDK site restored growth in eEF1Bα-deficient yeast. This study confirmed that eEF1Bβ evolutionarily succeeds eEF1Bα, and suggests that the CDK site in the former serves a regulatory role in plants and animals. Phosphorylation of eEF1Bβ by CDK may play an inhibitory role in metazoans, possibly to halt translation during mitosis. This work was presented at several national meetings and published in *Molecular Genetics and Genomics*.

POSTDOCTORAL RESEARCH

My research in the laboratory of Dr. James E. Ferrell, Jr. at Stanford University first focused on understanding how signaling molecules are integrated into regulatory circuits to elicit some of the complex biochemical behaviors that ultimately lead to cellular fates. Using the Xenopus egg extract system, I performed a quantitative study of the stimulus-response relationship between CDK1 and its regulatory subunit, cyclin. This work confirmed that the activation of CDK1 is switch-like in response to cyclin stimulus, and that it activates abruptly during a time-course after the addition of a supra-threshold concentration of cyclin. The major discovery of this work was that the threshold for inactivating CDK1 was found to be lower than the threshold required to activate CDK1; hence, there is hysteresis in the response of CDK1 to cyclin, and since CDK1 either remains active or is inactivated, its response is bistable. Computational modeling of this system revealed that the positive-feedback loops between CDK1 and Wee1 (its inactivating kinase) and Cdc25 (its activating phosphatase) are required to elicit this bistable response, and would sustain oscillations in a two-component model oscillator that could not oscillate in a system lacking this feedback. I presented this research as a speaker at the 2002 Cold Spring Harbor-*The Cell Cycle* meeting, and also at an invited seminar at the Mathematical Biosciences Institute of Ohio State University in 2003. This work was published in *Nature Cell Biology*, and both previewed in an article entitled, "Hysteresis meets the cell cycle" (*PNAS* (2003) 100: 771-772), and featured as an "exemplary" contribution of systems biology in an editorial entitled, "Scaling cell biology: all systems go!" (*Nature Cell Biol.* (2004) 6: 79).

My next major project progressed from considerations of the "steady-state" responses of this regulatory subcircuit to studying how positive feedback is important to the dynamical behavior of this oscillator. These experimental studies revealed that the positive-feedback loops embedded within the M-phase circuitry are essential to yield sustained oscillations of CDK1 activity. This feedback was determined to be essential to properly transit cycling egg extracts from interphase to M phase, since DNA replication was perturbed when M-phase control was placed under the control of a positive-feedback-insensitive CDK1. The transition from M phase to interphase also requires this positive feedback, since the negative-feedback loop that is responsible for cyclin degradation was discovered to not properly activate or inactivate without it. The finding that the positive feedback involved in the activation of CDK1 is required to properly establish both mitotic entrance and exit was unprecedented, and the use of cycling egg extracts in these experiments permitted the necessary observation of multiple cell cycles. These findings designated some new and unrecognized systems-level functions to this M-phase-specific feedback loop, and provided some unique insight into how more complex behaviors like oscillations can emerge from highly regulated subcircuits of enzymes that are not oscillatory. This research was presented as the second talk at the 2004 Cold Spring Harbor-*The Cell Cycle* meeting, and invited seminars at the World Conference of Nonlinear Analysts 2004 in Orlando, FL. It was published in *Cell* in August 2005, and featured in a preview entitled, "Shake it, don't break it: positive feedback and the evolution of oscillator design." (*Dev. Cell* (2005) 9: 309-310).

The embryonic oscillator project led to another major question: how different is logic between the somatic oscillator and the embryonic oscillator, particularly concerning the feedback that was found to be crucial in the context of the latter? Additional modeling that had been adapted from the *Cell* paper was used to probe whether or not a simple oscillator system that exhibits a period of nearly 24 h (in a somatic cell) versus 45 minutes (in an embryo) would necessitate positive feedback to sustain, albeit slower, oscillations. The model implied, barring many other considerations including subcellular localization, transport of proteins, and checkpoints (to name a few), that if positive feedback was essential, even partially short-circuiting the positive-feedback loops involved in CDK1 activation would have dire consequences on its somatic oscillator. Using numerous fluorescent biosensors, kinase assays, flow cytometry, and other molecular techniques, I performed quantitative experimental studies on the effect of a CDK1AF mutant on live HeLa cells, and the strong damping of oscillations that was predicted by our computational model was confirmed. This work is currently in preparation for submission to *Science*, and from this project extended another related computational manuscript that is currently in review by *Nature*. I presented the results of these studies at an invited seminar for the Division of Molecular Medicine at the Wadsworth Center of the New York State Department of Health in 2005, and will speak in the *Regulation of the Cell Cycle* session at the 45th Annual American Society of Cell Biology (ACSB) Conference in San Francisco, December 2005. This work also merited a travel fellowship for me to attend the 6th International Conference on Systems Biology (ICSB) at Harvard Medical School in October 2005.

INTRODUCTION

The biochemical circuitry of the cell cycle dictates periods of DNA replication and growth in cells, alternating with the dispersal of genetic and cellular material to their descendents and division. Cells initiate mitosis with cyclin B synthesis, the activating phosphatase Cdc25 reverses the Wee1-mediated inhibitory phosphorylation of cyclin-dependent kinase 1 (CDK1)/cyclin B complexes, and newly activated CDK1 promotes a positive-feedback loop by prompting additional Cdc25 activation. CDK1 then activates an anaphase-promoting complex (APC)-driven negative-feedback loop that targets cyclin B for proteolysis, which induces anaphase onset and mitotic exit. Feedback loops such as these are common in signal transduction pathways, but only recently is their functional prominence being studied in terms of the emergent properties that can arise from within the logic of a molecular system. My goal is to gain a quantitative understanding of how modules of enzymes are constructed to yield systems that can filter, switch, and oscillate, focusing particularly on M-phase regulation in embryonic and somatic cells, but also in scenarios where these networks have grown corrupt and can no longer properly regulate proliferation. This future work will be accomplished through quantitative cellular, molecular, and biochemical approaches, but also be underpinned by mathematical approaches in my own laboratory as well as through collaboration with other computational biologists. With this fusion of computational and experimental methods, I will be able to effectively assign and refine biochemical parameters, as well as design experimental rationale.

AIMS

At the outset of my research plan, I will begin addressing the following aims:

- Dissect the mechanisms that govern CDK1 activation and discern how the involved signaling players also interface with its APC-driven negative-feedback loop, determine if the M-phase phosphorylation of CDK1 targets is intrinsically linked to its kinetics, and learn the importance of feedback in sculpting the physiological output of a cell from its biochemical inputs during M-phase initiation, progression, and exit;
- Delineate the properties of a somatic cell that adapts its oscillatory behavior from that of the early embryo, determine what similarities and differences exist in the logic of these oscillators, and identify the roles that positive feedback plays in coupling progression of the cell cycle to CDK1 oscillations in the embryo and adult;
- Utilize developed as well as new molecular tools including fluorescent biosensors, inducible expression systems, RNAi, chemical biological, and proteomic approaches to identify kinase targets, study signaling kinetics, and quantitate the effect of signaling pathway perturbations within live cells and cell extracts.

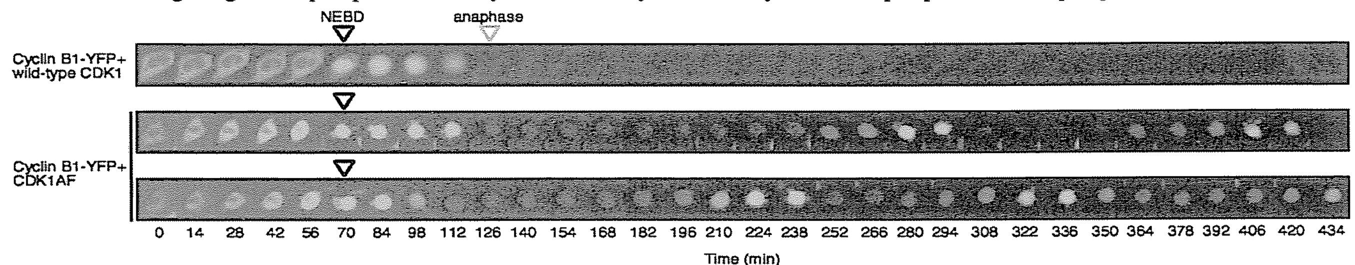
APPROACHES, METHODS, AND MODEL SYSTEMS

I want to gain a quantitative, systems-level view into how merely the synthesis of cyclin triggers the complex molecular behaviors that are involved in M-phase signaling and other associated pathways, and will study how feedback loops are integrated to regulate the mitotic oscillator system, whether in the rapidly proliferating cells of the zygote or inside the more deliberate divisions of somatic cells.

For studies of signaling within the context of embryonic stem cells, I will employ the early embryo system of the African clawed frog, *Xenopus laevis*. Xenopus egg cytosol can faithfully reproduce the biochemical context of the cell cycle. In the least, proteins can be depleted, RNA and protein can be added, and stable interphase and mitotic extracts can be produced, as well as cycling egg extracts that reiterate many of the biochemical events that would occur in a cleaving embryo. Multiple consecutive mitotic cycles can be achieved in this variety of extract and a bulk of material can be prepared for application in both traditional and more contemporary biochemical methods, and is thus extremely tractable for systems-level studies of the M-phase oscillator and other signaling pathways. Fertilized Xenopus embryos will be used to merge studies performed in extracts with the intact cellular scenario, and are also easily injected, pharmacologically treated, and otherwise perturbed and easily scored for defects in proliferation and development. Together, these traits make Xenopus especially powerful for my studies of the systems-level architecture and function of feedback loops and signaling in the embryonic context. One specific aim of mine is to quantitate the stimulus-response relationship between CDK1 and the anaphase-promoting complex (APC), and determine how cells direct M-phase exit with this negative-feedback loop with the same stimulus that initiates mitosis. These studies will be accomplished by measuring APC activity in egg extracts that are stimulated with cyclin B and/or modified with recombinant proteins, and the responses of particular APC

components can be tracked by their phospho-shifts and other readouts. Although many of these players have been identified, little is known about dynamical behaviors of APC activation and I will work to establish a systems-level link between CDK1 and APC in the M-phase oscillator circuit. A goal with some broader scope involves studying how feedback coordinates CDK1 activity towards its M-phase substrates, placing particular emphasis on how the kinetics of substrate recognition and phosphorylation by CDK1 might be defined by this feedback loop. This will be investigated by comparing the protein phosphorylation profiles that arise from egg extracts that contain wild-type CDK1, or the positive-feedback-insensitive mutant CDK1AF. A chemical biological approach implementing mutant versions of these kinases and a "bumped" ATP analog (as devised by Shokat; Bishop *et al.* (2000) Nature. Sept; 407:306-12) will allow an even clearer view of the targets of CDK that is driven with or without its stimulatory positive feedback, and may facilitate the classification of targets that necessitate this feedback loop. In summary, there exist numerous cell cycle-specific questions that can be investigated through experimental approaches that apply the Xenopus egg extract and embryo system, and this highly tractable system is unparalleled in terms of convenience and manipulatability.

My long-term research plans also involve quantitative studies of M-phase signaling in somatic cells. Several challenges have long existed for dissecting pathways in cells, including the need to chemically synchronize them to perform biochemical assays, the difficulty in observing and measuring their responses as a result of perturbation, and the inability to perform reverse genetic approaches. By applying live-cell microscopy, fluorescent biosensors, and RNAi, my laboratory will be empowered to perturb, assess, and effectively wire (or re-wire) the circuits that regulate proliferation and cell-cycle passage in somatic cells. I have successfully applied numerous cell cycle biosensors in my current studies, including fluorescent protein-tagged lamin A, cyclin B, gamma-tubulin, and an M-phase biosensor (Jones *et al.* (2004) Nat Biotechnol. Mar; 22(3); 306-12). An example of this was a quantitative study of oscillations in CDK1/APC activity in HeLa cells using cyclin B1-YFP as a sensor. CDK1AF was introduced to short-circuit the positive feedback involved in CDK1 activation and resulted in cells undergoing multiple periods of cyclin stability/instability without proper mitotic progression (see figure).



My laboratory will develop additional sensors that facilitate the monitoring of cell cycle progression and cellular physiology. This will permit a unique *in vivo* view into the logic of the somatic oscillator, both in normal (within primary cells) and abnormal (within cancer cell lines) contexts. Combined with these biosensor studies, RNAi will be utilized to knockdown individual or complexes of proteins of the M-phase circuit, so that the *in vivo* oscillator can be re-engineered with modified versions, and their impact on M-phase entrance, progression, and exit will be determined. There are several known enzymatic regulators of mitosis (e.g. the Polo kinase) that are recognized to modulate the activities of multiple targets, but how they fit into the dynamical behaviors of the complete system – as outside regulatory factors, or as central and critical regulatory nodes – is not well understood. With a combination of live-cell, cytometric, and biochemical methodologies, we will be afforded the opportunity to assemble and refine logical maps of these complex systems, and further our knowledge into how nature has evolved its very complicated yet efficient signaling systems from a basic assortment of motifs and modules. Application of these methodologies will facilitate our inquiry into some fundamental cellular and molecular questions concerning the regulation of cell division and growth, but will also mediate the interface of these basic studies with analyses of the processes that lead to the abrogation of proper cell cycle controls.

During my years of teaching lectures, laboratories, and discussions, I was inspired to develop numerous theories and practices to maximize student learning and teacher effectiveness. I taught in several different classroom environments with varying student interests and abilities, and mentored new teaching assistants as well as several faculty on techniques to improve their communication and overall rapport with students. After being rewarded with consistently excellent reviews of my teaching from students and faculty, and receiving awards at the course, department, college, and campus-wide levels, I recognized that it would be important for me to delineate how the vocation of being a student and teacher of the sciences could be idealized. In order to summarize my teaching endeavors at the University of Illinois, I contributed some of my experiences and insights into a book chapter on the need for students to actively integrate and facilitate lecture, laboratory, and discussion periods; and likewise, that faculty must also promote to students the benefits of this hands-on time with professor and teaching assistant.

I am a strong advocate of active learning in the classroom, where students are richly engaged in the learning process. No lecture, large or small, is an effective teaching or learning tool if it remains passive. In the academic context, there is constant tension between content coverage and comprehension. I value the importance of both, but I am especially devoted to ensuring that my entire audience is provided with strong foundation for their cognitive development and retention of the material in question. One of the most important lessons I learned from my graduate education and my experience as an educator is that students accepted to institutions of higher learning are certainly bright, but their individual abilities and skills are displayed in broad spectrums of experience and learning styles. Too often, student performance in the traditional classroom seems to result in the ubiquitous bell curve. Is it how students learn, how we instructors teach, or a combination of both that contribute to the serious differences in student performance? I respond to this question with the determination to build upon the knowledge of every student, and I take an active approach to promote this development by directly engaging the differences of students in my classroom. Teaching with blends of visual and verbal, as well as inductive and deductive styles, not only connects the material to diverse learning groups, but also affords crucial repetition.

The biological sciences are studies of life. What better way to teach these courses, than having students look at themselves and all of the interactions and processes that occur around, between, and inside them? I enjoy teaching advanced topics on the undergraduate and graduate level because it permits me to hone my knowledge within and outside my research focus, but I especially welcome the challenge of teaching introductory classes. Courses I have taught covered a wide range of topics, ranging from the molecular to the organismic, and beyond. All biological foci can become quite complex, but I apply a bottom-up approach to pose the essence of these to my students; sometimes beginning simply as a series of yes-or-no questions, but always providing some fundamental rules about the process in question that they can follow and fall back on if their thought process demands it. Using this method, students are less intimidated to begin learning about an entire biological mechanism no matter how complex they initially perceived it to be, and as the details and complexities of the process increase, the continuum of principles and concepts from which they can build should remain evident.

Whether in the undergraduate or graduate classroom, the maintenance of student interest is also a crucial aspect to facilitating this continuum. Conceptualization, analogy, and real-life examples enrich the classroom experience and draw students closer to the true nature and eventual application of what they are learning. By using the power of information technology and multimedia I try to illuminate the expansive world of the biological sciences along with the traditional chalk and slate of the classroom. Biology itself has evolved to extend its reach to a global scale with the advent of high-throughput experimentation and systems-level approaches, and it begs that the methodologies and information gathered from this forefront is integrated with our classical lessons. Research that formerly took years is now accomplished in days, and a whole genome or proteome worth of data can be collected during one experiment. By bringing examples past and present right into the classroom, I will integrate lessons of conventional cellular and molecular biology with recent technological and scientific advances, and will work to keep my students attuned to what is happening at the forefront of biology. From the undergraduate learning fundamental biology, to the graduate student working to refine their studies and understanding of cellular signaling, I will maximize the levels of insight that students can attain by actively connecting them with the material, by building upon their existing knowledge base, and by providing them views of biology's horizon.