Eric J. Stewart, Ph.D.
Génétique Moléculaire Evolutive et Médicale
INSERM U571
Faculté de Médecine Necker-Enfants Malades
Université René Descartes-Paris V
156, rue de Vaugirard
75015 Paris, FRANCE
stewart@necker.fr

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

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Dear Dr. Brun and the members of the Systems Biology/Microbiology Faculty Search;

I am writing to apply for a faculty position in Microbiology and Systems Biology, in the Department of Biology and the Biocomplexity Institute. I am currently doing research as a Young Investigator at the University of Paris V, René Descartes. Although I am currently working in France, I am a US citizen and I plan to return to the US to take a permanent faculty position.

I have a B.S. in Microbiology from Penn State, and I received my Ph.D. in Microbiology and Molecular Genetics from Harvard University. I did my PhD research under the supervision of Jon Beckwith, at the department of Microbiology and Molecular Genetics. My thesis work is further described in my attached Research Goals.

I am currently working in the laboratory of Prof. Miroslav Radman with François Taddei in the Faculty of Medicine at Necker Children's Hospital, University of Paris. My research interests are in understanding how single cells grow, reproduce, and die. To this end, I have been working on a project studying the growth and death of individual cells throughout their life cycles. This project combines the newly available techniques of automated time-lapse microscopy with traditional bacterial genetics and molecular biology to study growing cells on an individual basis. Our knowledge of the dynamics of growth, division, and death for such model organisms as *E. coli* and *B. subtilis* comes almost completely from the study of populations, which can only reveal the average phenotype of all of the cells. Consequently, the variability between cells, the effects of phenotypic and epigenetic inheritance, and the patterns of cell death (including cellular aging) are all relatively unknown and answering these questions forms the basis of my work, including identifying the molecular mechanisms behind these processes. This project is fully described in my attached Research Goals. I plan to continue and expand this project once I leave the lab. I have the full endorsement of Prof. Radman to continue this project, and further, we intend to continue collaboration in this area to progress as rapidly as possible.

In addition to my research, I have had extensive experience in teaching as well. During my graduate career, I have had the opportunity to assist in the teaching of courses in microbial pathogenesis and general genetics to graduate students as well as concepts and laboratory practices in clinical microbiology to medical students. In addition, I have mentored rotating graduate students, helping them with their research and advising them in their course selection, as well as tutored students at a summer school on mathematics and applications in biology and medicine. More information is available in my attached Teaching Interests.

The questions that I am addressing and the techniques I am using have the potential to provide a significant complement to the research already present in the Department of Microbiology. In particular, there is a good potential for productive synergy between my research and that of Drs. Brun, Fuqua, and Kearns, just to name a few. Furthermore, I have found that once phenotypes are studied on the level of the individual, there is an abundance of unexplained and unappreciated variation. Indeed, a group of genetically identical single-celled organisms, all natural clones of a single progenitor, growing within a constant environment, will display emergent complex behaviors, and will give rise to individuals with widely varying phenotypes. It is the study of this complexity that makes me an excellent fit as a microbiologist of the Biocomplexity Institute, in addition to my interests in scientific interactions at all levels, from teaching to collaborations.

The following individuals will be sending letters of recommendation:

Jonathan Beckwith
Dept. of Microbiology and Molecular Genetics
Harvard Medical School
200 Longwood Ave.
Boston, MA 02115
Phone: (617) 432 1920
Email: jon_beckwith@hms.harvard.edu

Miroslav Radman
INSERM U571
Faculté de Médecine Necker - Enfants Malades
156, rue de Vaugirard
75015 Paris, FRANCE
Phone: 33 1 40 61 53 20
Email: radman@necker.fr

Richard D'ari
Institut Jacques Monod
2, place Jussieu
75251 Paris cedex 05, FRANCE
Phone: 33 1 44 27 69 43
Email: dari@ijm.jussieu.fr

Ivan Matic
INSERM U571
Faculté de Médecine Necker - Enfants Malades
156, rue de Vaugirard
75015 Paris, FRANCE
Phone: 33 1 40 61 53 21
Email: matic@necker.fr

Sincerely,

Eric J. Stewart

Eric J. Stewart, Ph.D.

Research Goals and Accomplishments

Project title: "Variation and epigenetic heredity in the growth of single cells"

My fundamental research interest is in how single cells grow, reproduce and die. Specifically, I am investigating the potential role of variability and polymorphism in the fitness of a population of clonal individuals. The evolutionary strategy that an organism takes toward reproduction (its life history) reflects the effects of the environment under which it evolved. Phenotypic variability in the absence of genetic variability may provide an organism with short-term adaptability in changing surroundings, while under relatively constant conditions, such polymorphism should decrease fitness. Therefore, it is possible that organisms will have been selected for genetic determinants that govern such plasticity. Studying the role of this variability and its source has been difficult in the case of single cells (whether bacteria or mammalian cells in culture) as most experiments require large numbers of cells, and consequently reveal the population average, rather than the variance around the average. In order to address the potential role of variability, it is necessary to first characterize it by examining single cells.

I began this project with a series of specific questions: How much variability is there between clonal cells growing under identical conditions, and what is the source of this variability? Is this variability heritable in a phenotypic or epigenetic manner; for example, if a mother cell is growing slowly, will one or both of its daughters grow slower (or faster) than the other cells? How are cell components, other than the genetic material, segregated in dividing cells? In apparently symmetric divisions, are some components unequally divided, leading to differences in cell potential? Could such inequalities manifest as aging in symmetrically dividing organisms, contrary to theory?

Aging in symmetrically dividing organisms

In order to answer these questions, it is necessary to follow single cells and their offspring as they grow and divide over time. I have developed techniques to accomplish this using time-lapse fluorescence microscopy to follow cells as they grow from one to up to 1000 cells in a microcolony. To begin with, I chose to use the model system of *Escherichia coli* for the genetic tools available, its speed of growth, and specific growth properties that it possesses. These cells grow in the cavity of a microscope slide on a nutrient medium-agarose surface at a controlled temperature. To identify molecular mechanisms responsible for these processes, I have developed microscopy-based screens that can identify mutants with increased variability in phenotypes such as cell death frequency. In addition to studying wildtype cells under benign conditions, I am also following cells from stationary phase, after heat shock, and under other conditions, looking for effects on variability and its heredity.

The time-lapse microscopy generates a series of images that can then be analyzed by a computer to identify and track the lineage of all the cells produced from a single starting cell to the 300 to 1000 cell stage. While the cells divide into two apparently equal offspring, thus making the assignment of "parent" and "offspring" difficult, I have been able to track the orientation of the cells throughout their growth (*E. coli* grows as a rod), such that the old pole of each cell can be followed through each generation. The old pole provides a very useful macro-cellular marker that allows the specific ends of any given cell to be followed throughout a lineage.

This effort led to the surprising discovery of aging in *E. coli*, an organism that reproduces by symmetric division, with no juvenile phase. This work was published in *PLoS Biology*, and has been written up as both an Editor's Choice and News of the Week in *Science*, and appeared in the

online news section of *Nature*, among other journals. Beyond the results already published, basic elements of cell growth are also becoming apparent; we have determined that individual cells grow exponentially with their length, even during cell division, contrary to some models. The fact that two individual cells appear to have the same growth potential as one cell twice as long indicates that the decision of when to divide must be determined by other life history factors, such as the geographic spread of individuals, the survival of stresses and pathogens, and the like.

Testing the cell damage theory of aging

One potential hypothesis to explain aging in single cells states that the reduced life potential in an old cell is caused by a process of segregating damaged cell components preferentially to the old pole. With Ariel Lindner, a postdoc in the lab, we have tested this hypothesis by generating strains with inclusion bodies consisting of aggregated, misfolded proteins. These aggregates are used to represent one type of damaged cell component; their advantage is that inclusion bodies can be followed in phase contrast microscopy through their highly refractive nature, as well as in fluorescence microscopy, when labeled with a fluorescent protein. A strain expressing such a marker has been analyzed by time-lapse microscopy, during which the inclusion bodies were tracked from cell to cell by an automated system during the growth of the microcolony. We have found that such aggregates are indeed located in the old pole, and through statistical analysis, we have determined that a cell that inherits such an aggregate will grow slower than expected otherwise. This growth defect is apparently responsible for approximately 40% of the aging phenotype associated with old poles, and represents the first example of asymmetric inheritance of protein damage leading to aging. A manuscript describing these results has already been submitted, and should be published soon.

Identifying other molecular mechanisms of aging

In order to identify further mechanisms involved in the aging process, I have developed screening procedures that can identify mutants in a library that produce either greater or lesser numbers of dead cells when grown on solid media, allowing those mutants to be isolated and characterized. With Fanette Fontaine, a student in the lab, we have used these systems to identify and characterize a number of mutants of each class. The mutants that do not produce as many dead cells during the course of growth are of particular interest, as they are likely to lead us to the systems normally responsible for cell death, and possibly aging. Aside from identifying the genes affected in these mutants, they have been characterized in several ways. First, time-lapse films of these mutants have been generated that can be used to compare them to wildtype cells for the phenotypes of cell death, aging and cell-to-cell variance. Second, they have been analyzed in growth competitions with wildtype cells, to determine if there is a cost associated with the change in the number of dead cells generated. For example, life-history evolution theory postulates that if a strain produces fewer dead cells (possibly through increased maintenance), there should be a trade off in other aspects of its physiology. Our competitions have revealed such a trade off, which may indicate why such mutants are not already more prevalent. So far, we have identified mutants that appear to affect the global balance of gene expression, shifting cellular resources between the competing regulons of maintenance, growth, and expansion. This manuscript is currently in preparation, and will be submitted shortly.

Current projects and the future

These techniques, facilitated by improvements in fluorescence microscopy and automated computer technology, will enable me to continue to address difficult and novel questions in microbiology. I am currently engaged in a collaboration with an optical physicist, where we are utilizing a microscopic laser trap that will allow us to follow a single cell as it grows and divides, through many more than the ten divisions that are currently possible.

The formation of a microcolony has been likened to development, and while *E. coli* may have no apparent developmental plan, there are other cells and even other bacteria that do. My first investigation into these processes is through a collaboration with a research group that studies *Bacillus subtilis*. Together, we are investigating the systems that determine the developmental fate of individual cells in this bacterium. As *B. subtilis* grows, the cells differentiate from each other by entering the pathways of sporulation and competence. The timelapse system described above is allowing us to detect these cells as they enter these pathways, and identify their place in the microcolony lineage. From this, we expect to gain insight into the evolutionary pressures on the life history traits that govern this phenotypic variability in a clonal organism.

I am also working with several students in the lab on a project that is exploiting the polarity found in the intracellular pathogen *Shigella flexneri* in order to harvest aged bacterial cells. The protein responsible for polymerizing actin (IcsA) and propelling the pathogen within a host cell is found predominantly at the old pole; if it were present at both poles, the cell would not undergo net movement. By adhering cells to a column through IcsA binding and subsequently eluting them, populations enriched in older cells can be obtained. These populations can then be used to determine the kinetics of very old cells, and additional mechanisms of cellular aging.

Further improvements in microscopy will also hopefully lead to the ability to follow microbial growth in three dimensions, and open up the real-time study of biofilms and other structured microbial environments and behaviors. In addition, there are bacteria and other microorganisms that form specialized structures such as fruiting bodies, and these are ideal candidates for such a developmental 3D analysis.

Finally, these techniques may also allow us to probe the symmetries and variance in the growth characteristics of other types of cells. For example, it should be possible to follow the growth of cells such as transformed and untransformed human cells. Untransformed cells experience senescence, limiting their growth. Transformed cells manage to pass through this phase, and divide apparently without restriction. We may be able to identify both the lineage pattern of the senescence as well as which cells in a lineage may eventually escape it and become transformed. The goal of these investigations is to use the information gathered to determine the role of variability in these organisms in the context of evolution and life history.

Prior work

During my graduate career in the laboratory of Jon Beckwith at Harvard Medical School, I studied the model organism *E. coli*. To complete my thesis, "The role of two thiol-disulfide redox pathways in *Esherichia coli*", I used bacterial genetics, molecular biology and biochemistry to complete two projects involving the oxidative stress systems in this organism. In the course of these projects, I identified, cloned, knocked out, and determined the role of a previously unidentified gene, and extensively modified another through directed mutations and gene fusion techniques to determine its function and structure, using biochemical methods. Each of these projects resulted in publications in the *EMBO Journal*. In addition, I created software to analyze whole-genome sequences according to an algorithm (designed in the lab) that identifies the sub-cellular location of proteins.

Teaching interests

I entered Penn State University as a biology major, and like many undergraduates, I wasn't sure how I could translate my general interest in the subject into an interesting and fulfilling career. In my second year, however, I encountered my first microbiology lectures and labs, and was instantly smitten. I was fascinated by this mostly unseen world, the organisms that inhabited it, and the clever ways that had been developed to study it. I immediately changed my major to microbiology, and I have enthusiastically followed that path ever since.

Perhaps not surprisingly, my greatest interest in teaching is passing on the discovery of microbiology to new students, and sharing the excitement of microbial research with advanced students. In particular, I find microbial physiology, life cycle, and life history studies to be rewarding and topical subject areas to share with students. Beyond this, I feel that there is a strong need for teaching the techniques and systems of data analysis to intermediate and advanced students. This is due to the rapid advances in experimental techniques such as high-content microscopy; gene, protein, and other arrays; and automated systems in general, which now are capable of generating overwhelming amounts of data very quickly. As new researchers, today's students are going to increasingly use these types of experiments, and being able to effectively analyze their results will be a great advantage.

I am also interested in teaching genetics at all levels; and to advanced students, critical reading and analysis of research papers. In a similar vein, I found practical courses in proposal writing very useful as a graduate student, and I feel that these could be extended to writing research papers as well. These are obviously fundamental skills for researchers, and students would benefit from learning about them.

I have experience teaching from graduate school and my time as a postdoc. At Harvard, I helped teach the class "Principles of Genetics" to younger graduate students, and I taught laboratory courses in "Immunology, Microbiology and Infectious Disease", and "Microbial Pathogenesis" to medical students. As a postdoc, I have been an instructor at the "Summer Mathematical Research Center on Scientific Computing and Its Applications: Mathematics and Applications in Biology and Medicine."

Given my interests and experience in teaching, I feel that I would make a very productive contribution to student education, and I look forward to the possibility of having the opportunity to do so.