27th October, 2005

Yves Brun
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 would like to apply for the position of Assistant Professor in the Department of Biology and the Biocomplexity Institute as advertised in the 2nd September issue of *Science*. Currently I am a Research Associate in the laboratory of Professor N. Patrick Higgins at the Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham.

My research is focused towards elucidating the molecular architecture of the bacterial chromosome and its consequence to cellular and metabolic processes. As a graduate student I established the importance of nucleoid organization as a regulator of gene expression in response to environmental stress. In the present laboratory I have determined the location and spread of bacterial chromosomal domains. These studies have elucidated the relationship among domains of DNA supercoil, transcription and transposition. I have also examined the regulation of transposon target selection with possible implications ranging from evolution of our genome to gene therapy.

I intend to establish an interdisciplinary, highly energetic, and extramurally funded research group that would pursue questions in chromosome biology and functional genomics. With a BS in Physics, MS in Biotechnology, doctoral schooling in classical genetics and a postdoctoral training in high-throughput technologies I am well qualified to take on problems that are at the forefront of biology today. Besides my experience in research, I have supervised graduate and summer students in Prof. Higgins lab and taught supplementary courses to graduate students at UAB, both of which I enjoy immensely. With my educational background and proven publication record, I strongly feel that I will be a suitable candidate for the advertised faculty position in your department.

Please find enclosed my curriculum vitae, copies of five selected publications, statement of future research interest and names and addresses of four referees. I would be happy to arrange letters of recommendation sent when needed. In the event of any other supporting material needed, please do not hesitate to contact me at the following address.

Thank you for your consideration,

Dipankar Manna
Department of Biochemistry and Molecular Genetics
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## Society Membership:

**Genetics Society of America**, Member since 2000
**American Society for Microbiology**, Member since 2000

## Research Expertise:

Experienced in classical bacterial genetic approaches including transduction, conjugation, transposon-mutagenesis and chromosome modification using bacteriophage lambda recombineering system. Well versed with phage genetics of bacteriophage λ, bacteriophage P1, bacteriophage P22 and bacteriophage Mu. Proficient in all routine protein biochemistry and molecular biology techniques. Skilled in DNA microarray fabrication, processing and data analysis.

## Current Research Interests:

My research is focused on understanding the folded structure of the bacterial chromosome. Towards this goal, I have developed a variety of approaches to study chromosome structure in vivo. Among these, bacteriophage Mu has served as the most important tool in probing chromosome structure and dynamics. While studying Mu transposition in *Escherichia coli*, I identified a set of transposition hot spots at the transcriptional control region of the *bgl* operon. An AT-rich DNA sequence was the sequence determinant in creating these hot spots but was itself a cold spot for transposition. Subsequently, comparison of in vitro and in vivo transposition pattern indicated that nucleoid organization affects Mu target selection. (**J. Bacteriol. 183**:3328-3335).

In a second project, I studied the mechanism by which Mu avoids certain targets. In a phenomenon known as transposition immunity, Mu rarely targets its own genome. This protection requires Mu DNA ends and MuB protein. Using a novel mobile target, I proved that transposition immunity is not limited to the phage DNA alone; it spreads in cis to the adjacent host DNA. Immunity decays as a function of distance from Mu ends and the decay function is indicative of a dynamic chromosome in which distant sites frequently interact with each other through a supercoil dependent serpentine motion of the intervening DNA. (**Mol. Microbiol.** 32: 595-606).

Recently, in collaboration with Nicholas Cozzarelli's laboratory at the University of California at Berkeley, I constructed a whole genome map of Mu transposition targets in *Escherichia coli*. This is the first whole genome target profile for any transposon. Target profile revealed over a 1000-fold bias in Mu transposition at the gene level. Interestingly, hot spot genes tended to be clustered and so were the cold spot genes. Analysis revealed that transcription negatively impacts transposition. In collaboration with Michael McClelland of Sidney Kimmel Cancer Center (San Diego, CA), I have now generated a similar target profile for Mu in *Salmonella typhimurium*. Target profile in *S. typhimurium* demonstrated the role of protein binding and spreading on DNA in regulating DNA accessibility and further enhanced our understanding of DNA organization in vivo. (**PNAS, USA. 101**:9780-9785, **J. Bacteriol. 187**:3586-3588 & Manuscript in preparation).

**Future Research Interests:**

Classically bacteria have proven to be an excellent model system to discover the fundamentals of biology. However, our knowledge of the organization of its chromosome is far behind our understanding of the ordered structure and the regulated condensation-decondensation of eukaryotic chromosomes. Over the last five years I have combined classical genetics and high throughput molecular biology and developed a new set of tools to study DNA organization and dynamics in living bacteria. With the help of these new set of tools, I intend to focus my research in three major areas:

**(1) In vivo chromosome organization and dynamics**. Work on Mu target preference indicated that Mu target preference is an effective tool in probing chromosome organization and dynamics. A whole genome transposition profile is the net result of local 'attractors' and 'deflectors' of transposition machinery. I plan to carry out detailed biochemical characterization of the local attractors such as the AT-rich sequence at the *bgl* promoter in *E. coli* and of deflectors such as the *parS* site in *S. typhimurium*. I am also interested in the dynamic nature of the nucleoid in vivo. In this context, I would study the effect of changing metabolism and stress on chromosome organization.

**(2) Mechanism and regulation of DNA transposition**. Mu transposition follows a distinct cut and paste pathway during lysogenization as opposed to the replicative transposition during lytic growth. The details of this cut and paste process and its regulation during lysogenization have not been studied. I would develop assays to study the intermediates in this process and determine the molecular mechanism of DNA integration during lysogenization. Results from these studies would unravel factors that make the switch between the two transposition pathways possible. These studies would also shed light on the interaction between phage and its host factors in regulating transposition.

**(3) Functional genomics**. Till now DNA microarray has been a popular platform mostly to assay gene expression. However, several studies have proved that it is also suited to ask other questions such as DNA methylation pattern of the whole genome. I am interested in using array platform to study the correlation between the organization of bacterial chromosome and its function. My own data suggested that transposition follows a periodic pattern. This is most likely related to the short and long range domain organization of the nucleoid. I plan to use a combination of DNA transposition, chromatin immunoprecipitation and high density tiled DNA array to study the fine structure of bacterial chromosome and its relation to the functional organization of the genome.

Together these studies will elucidate the structure of bacterial chromosome and the links between chromosome organization and the dynamic processes of transcription, replication and recombination including the spread of mobile genetic elements.

**Teaching Experience:**

I believe that a career in science necessarily includes the responsibilities of inculcating a scientific attitude among colleagues and preparing the next generation of scientists. I have always enjoyed teaching science and have loved scientific discussions. During my masters studies I was assigned to teach courses in biophysics and molecular biology to juniors. While pursuing my doctoral studies, I delivered many supplementary lectures on advanced topics in biophysics to bacterial recombination. During my postdoctoral training I have been taking an active part in advising undergraduate and graduate students, instructing them on basic and advanced techniques in molecular biology and genetics, and introducing them to practical and theoretical aspects of scientific methodology. I have also been an active and longest running member of a weekly science journal club at UAB, which imparts students with critical thinking and knowledge in all fields of modern biology.

I am fully competent to teach undergraduate and graduate students topics in bacterial genetics, phage genetics and molecular biology. I have lectured on the principle and use of DNA microarrays to undergraduate and graduate students in several departments in UAB. In my own field I plan to develop courses on prokaryotic genetics, DNA-recombination and functional genomics. Interaction with colleagues and students is important and I plan to pursue it in order to establish my own active research group.

**References:**

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