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9/28/2005

Dear Prof. Brun,

Attached please find my application material for the position in your department.
This includes:

- My CV and list of publications.
- A summary of research accomplishments and future plans.
- A statement of teaching interests.
- A list of referees whom I have asked to send letters of recommendations.
- A copy of my latest paper.

Please let me know if there is any additional information I can supply you with.

Sincerely yours,

Ido Golding

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RESEARCH INTERESTS AND FUTURE PLANS / Ido Golding

QUANTITATIVE STUDIES OF GENE REGULATION: SINGLE MOLECULE – SINGLE CELL – WHOLE POPULATIONS

The classic subject of genetic expression and regulation has seen a recent influx of new insights. This has been largely thanks to the application of quantitative approaches in both experiments and theory. This "quantitative revolution" encompasses a hierarchy of levels: From the single molecule (for example, using optical tweezers to study the interactions of biomolecules *in vitro* [1]), through the single cell (studying cell-to-cell variability in gene expression using fluorescence microscopy [2]), up to the level of whole populations (where DNA microarrays are used to quantify genome-wide expression patterns [3]).

After being trained as a condensed matter physicist, I have spent the last 4 years learning the experimental arsenal of modern molecular biology. I have chosen to study the *E. coli* bacterium and its virus, the bacteriophage Lambda. These two organisms serve as basic paradigms for gene regulation [4, 5]. I have developed and applied new quantitative tools for probing genetic interactions within these systems, ranging from individual molecules to the population as a whole. In all of the following, quantitative experimental work is accompanied by mathematical modeling, with a constant feedback between the two endeavors:

(1) Together with Prof. Edward Cox, I have developed a method for tagging individual RNA molecules in live bacteria [6]. The technique enables us - for the first time - to quantify the levels of messenger RNA (mRNA) and its encoded protein in individual cells, and to follow the life history of individual transcripts in the cell: The timing of message appearance, chain elongation during transcription, Brownian motion while tethered to the DNA template, motion of the released message, and RNA partitioning at cell division. Since these processes have not been directly observed in living cells before, many aspects of RNA dynamics are unknown, and our system offers a first glimpse into them. Some open questions that I hope to tackle include:

- What are the characteristics of transcription kinetics? Our results indicate that (counter to the text-book picture) transcripts are not made one at a time, in a simple Poisson process, but rather with a more complex temporal pattern, in a series of "bursting" events in which a finite number of mRNAs is made within a short period of time. Understanding mRNA kinetics can offer new insights into the microscopic workings of the transcription machinery in the cell, and help bridge the gap between the properties of individual RNA polymerase enzymes (as observed *in vitro* [7]) and population-level gene expression kinetics.
- Where in the cell does transcription occur? Does it happen all over the bacterial cytoplasm? Perhaps localized to a particular region (as was suggested for *Bacillus* [8])? One possible means of studying this question is to use Transposon insertions [9] to place the coding regions for the RNA targets at various chromosomal loci, and correlate these locations with the physical position in the cell where transcription is observed.

- After completion of transcription, what are the characteristics of mRNA motion in the cytoplasm? What can we learn from that motion about the physical nature of the cytoplasm? Our initial results suggest that mRNA motion is sub-diffusive, implying the presence of some quenched heterogeneity in the cytoplasm, with which the moving molecules interact. A possible source of that heterogeneity could be the cytoskeletal structures in the cell [10]. Alternatively, macromolecular crowding in the cytoplasm might lead to the anomalous motion [11]. In order to determine the relevant factors, cytoplasmic mRNA motion can be tracked under different growth conditions, in the presence of antibiotics, and in different mutant strains (e.g. deficient in cytoskeletal proteins).
- How is transcription coupled to other cellular processes, such as mRNA translation into proteins, DNA replication, and cell division? Our system enables us to pursue these relations, by correlating – in individual cells – mRNA levels with other relevant cellular indicators: the amount of protein encoded by the tagged mRNA; chromosome position and copy number (using the *in-vivo* DNA tagging technique developed by Straight and co-workers [12, 13]); cell size; etc.

The potential for future work is almost endless. The system we built is very modular, in that the genetic constructs can be modified to study gene expression from a bacteriophage, or from any gene in the bacterial chromosome. Of special interest would be genes whose transcripts (and therefore protein) exist in a low copy number in the cell. Such is the situation for many proteins that regulate gene expression [14]. An open question of much current interest is how cells maintain the "robustness" of their genetic circuits in the face of considerable stochastic fluctuations in the levels of these low-copy regulatory proteins [15].

(2) We have designed and generated a series of bacteriophage Lambda mutants expressing modified Repressor proteins[1] – each differing from the "wild type" in a known biophysical way, such as DNA binding affinity, folding properties, etc. In collaboration with other researchers, we are conducting *in vitro* single molecule studies to try and resolve key issues regarding protein/DNA interaction:

- How does the protein find its DNA target? In particular, one dimensional diffusion of the protein along the DNA has been claimed to play a major role in this target finding [16]. Yet, it has never been directly observed. One of our aims is to observe and quantify that motion.
- What are the microscopic kinetics of binding and dissociation? What is the role played by higher order processes (e.g. cooperativity, protein multimerization, long range DNA looping [17]) in the protein/DNA interaction?

The techniques we use include:

- Fluorescent Correlation Spectroscopy (FCS) in a "Zero mode optical waveguide" [18]. This work is led by H. Craighead, Cornell University.
- Imaging of GFP-labeled protein binding to DNA stretched in a nano-channel [19]. This Work is led by R. Austin, Princeton.
- Studies by Atomic Force Microscopy [20]. Led by P. Ong, Princeton.

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[1] Lambda Repressor is a DNA-binding protein, encoded by bacteriophage Lambda, that controls expression of a few phage genes, and determines whether the phage goes into a "lysogenic" (dormant, non violent) state in the host cell.

- Imaging of surface-bound GFP-labeled protein binding to DNA, by total internal reflection microscopy, with P. Ong, Princeton.

To complete the "multi-level" study, we would like to relate the molecular characteristics of each mutated Repressor protein with the phenotype of the bacteriophage and its host, and the selective fitness bestowed upon the organism within the population. At the single cell level, I intend to study the kinetics of viral infection and its two possible outcomes: the lytic (violent) and the lysogenic (dormant) pathways. For that purpose, I will use an *in-vivo* tagging of the lambda genome, as well as tagging of repressor mRNA and other transcripts of interest (using the technique discussed above). The construction of the phage DNA and RNA tagging systems is already in progress.

Finally, at the whole population level, we use classical microbiology techniques, as well as the more quantitative tools of DNA microarrays. We have constructed an array containing all *E. coli* and Lambda genes. Examining gene expression during lysogeny and induction (transition into "lytic", i.e. violent phase) of the wild type bacteriophage has already yielded surprising new results. For example, we have learned of previously unknown interactions between the lambda Repressor and metabolic functions in the *E. coli* host [21]. The next step is to use this tool to examine the phage mutants, and the way their different "molecular phenotypes" are manifested on the whole genome level.

(3) A subject which I have concentrated on during my Ph.D. studies (under Prof. Eshel Ben-Jacob), and which I would now like to re-examine with my newly acquired tools, is pattern formation by strains of *Bacillus* and *Paenibacillus*. Under adverse growth conditions – hard agar plates, low nutrient levels, the presence of antibiotics – colonies of these strains form elaborate macroscopic patterns, in a number of distinct morphological phenotypes ("morphotypes"): branching patterns, chiral patterns, and vortex-like patterns. These patterns reveal the different ways in which the colonies cope with the environmental stress [22]. Each morphotype is typical of specific growth conditions, and under appropriate conditions, a morphological transition can be observed, analogous to a physical phase transition. My earlier studies have concentrated on the physical mechanisms leading to the formation of the patterns, by building a theoretical model that reproduces the observed experimental results [23]. Now that I am familiar with the tools of microbiology, molecular biology and single cell microscopy, I would like to use this system as a possible model for bacterial cooperative handling of adversity. In particular, I would like to understand the genetic and physiological basis for the different morphotypes and the transitions between them, and the possible role of inter-cellular communication and chemotaxis in colonial stress response. I would also like to construct appropriate markers that will enable us to follow spatio-temporal gene expression during the lifetime of a colony.

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TEACHING INTERESTS / Ido Golding:

In general, I find great joy in teaching, and believe I can do so quite successfully. During my years of PhD studies, I tutored the course "physics for students of life sciences" for 8 consecutive semesters. As can be judged from the annual student polls, the students enjoyed my teaching and felt that they gained from it. Were I to get a position, I would be happy to teach this type of course or the "complementary" one – "biology for physicists". Another possibility is for me to give an introductory course about "mathematical ideas in biology". I'm also open to any other suggestions.