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Yves Brun
Systems Biology/Microbiology Faculty Search
Department of Biology, Indiana University
Jordan Hall 142
1001 E 3rd Street
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Dear Dr. Brun,
In response to your announcement posted September 1, 2005, I enclose my CV, a statement of research interests, and a statement on teaching. On page 2 of this letter, I list colleagues willing to provide letters of reference.

My research interests consist of **developmental genetics** (using gonadogenesis in *C. elegans* as a model system) and **systems biology** (quantitative approaches, computer modeling and simulation of *C. elegans* development). I believe that these interests could thrive in the context of the Department of Biology and the Biocomplexity Institute.

I bring to this work 12 years of experience in *C. elegans* developmental genetics. My laboratory's work on mutants with proximal germline tumors has established two distinct roles for the gonadal sheath in promoting germline proliferation. We are pursuing the molecular basis for these interactions.

I also bring to this work four years of computer-based collaborations: (1) a sterility "phenome" database project (in collaboration with Kris Gunsalus and Fabio Piano), (2) a quantitative project addressing germ cell proliferation in *C. elegans* (in collaboration with Bud Mishra and members of group), and (3) a project on formal modeling and simulation of the egg-laying system in *C. elegans* using tools developed for system design of reactive complex systems (in collaboration with M. Stern at Yale and A. Pnueli and D. Harel of the Weizmann Institute and their groups).

Thank you very much in advance for your consideration of my application.

E. Jane Albert Hubbard, Ph.D.

RESEARCH INTERESTS

My research interests are in developmental genetics and systems biology.

I. Developmental/molecular genetics of germline proliferation in *C. elegans* as a model system to understand the interface between the control of cell proliferation and development. The animal germ line gives rise to eggs and sperm and, as such, forms the physical link between generations. During organogenesis, in addition proliferation/differentiation decisions, cells must decide when, where and how fast to proliferate. Improper control of cell proliferation can lead to developmental defects and cancer. We are focusing on the relatively unexplored area of the control of pattern and extent of germline proliferation as a model for this process, especially as controlled by signaling between the soma and the germ line during their co-development. Our entry into this problem is through mutants that display abnormal germline over-proliferation (germline tumors) and/or under-proliferation.

Recent research:

A germline tumor characterizes "proximal proliferation" (Pro) mutants: an ectopic mass of proliferating germ cells is found proximal to gametes. We have characterized Pro mutants in which the tumor derives from undifferentiated germ cells. Three of our Pro mutations encode novel gain-of-function amino acid substitutions in the GLP-1 receptor, a well-characterized receptor of the Notch family that promotes the mitotic fate in the *C. elegans* germ line (Austin and Kimble, 1987). **Pepper et al., 2003(a)** describes the genetic behavior of this new class of *glp-1* mutants, and **Pepper et al., 2003(b)** details the *glp-1(Pro)* phenotype, its implications for control of meiotic entry, and our subsequent cell ablation analysis of initial meiotic onset in the wild type. The *glp-1(Pro)* alleles were concurrently used in collaboration to investigate genetic interactions between *glp-1* and other genes in the context of continuous meiotic entry **(Hansen et al., 2004)**.

pro-1, another gene identified by a mutant that displays a germline tumor, acts in the somatic gonad (specifically the sheath lineage), not in the germ line (**Killian and Hubbard, 2004**). PRO-1 activity in the sheath lineage prompted further studies on sheath-germline interactions. We established (1) an early role of the distal pair of somatic gonad sheath cells in promoting pre-meiotic germline proliferation that is essential for fertility, (2) the connection between pre-meiotic proliferation and the timing of meiotic onset and (3) an activity that promotes tumor formation when the proximal sheath is inappropriately juxtaposed to pre-meiotic germ cells. **Killian and Hubbard (2005)** reports these results.

PRO-1 is a member of a highly conserved subclass of WD-repeat containing proteins. Related proteins are essential in yeast, and orthologs exist in plants, *Drosophila*, mice and humans – but no previous analysis has been carried out in a multicelluar organism. Recent work suggests a role for the yeast ortholog of PRO-1 in rRNA processing. Our genetic analysis and on-going molecular analyses support a similar role for PRO-1 in *C. elegans* as well as genetic interaction with the *C. elegans* retinoblastoma (Rb) ortholog. We recently cloned two additional genes based on their Pro mutant phenotypes: *pro-2* and *pro-3*. These genes encode orthologs of two additional highly conserved proteins involved in ribosome biogenesis. Based on these results, we tested other putative ribosome biogenesis factors by RNAi and found that they, too, can cause a Pro germline tumor phenotype. Thus many *pro* genes encode "general" factors yet have specific developmental defects. Analysis of these genes offers insight into the intersection between the control of ribosome biogenesis and development, and suggests that translation

must be optimized for proper somatic gonad development. It is unclear whether translation of many proteins or a small number of critical proteins is more critical (**Voutev et al., in prep.**).

We are keen to identify the pathway(s) by which the sheath communicates the proper level of proliferation in the underlying germ line. Recent results suggest that the insulin pathway may be involved, and a non-biased genome-wide RNAi screen is underway (see below).

Current/Future goals of germline proliferation studies – (short term) sheath/germline interactions that affect germline proliferation and (long term) connections to aging and metabolism:

A **genome-scale screen using RNA-mediated interference (RNAi)** is underway in several different mutant backgrounds in parallel to identify and distinguish specific germline defects that are soma-autonomous and germline-autonomous. We are simultaneously screening for RNAi-induced enhancement and suppression of the Pro phenotype to identify genes responsible for the proliferation-enhancing functions of the distal and proximal sheath, respectively. In parallel, we are determining the *specific* gonad/germline defects in RNAi treatments known to confer sterility (Ste and Stp genes). This screen is aided by a web-based digital scoring system we developed with Kris Gunsalus and Fabio Piano and their groups. We document (by generating high-magnification Quicktime© movies/stacks of specimens in multiple Z-focal planes) qualitatively distinct "sterile" phenotypes induced by RNAi. In addition to facilitating our own projects, this "genome-wide" view of germline development will be available to the community on a searchable public web-based database (Gunsalus, et al., 2004). Digital signatures we generate will be used for Phenocluster and PhenoBlast analyses (Gunsalus et al., 2004; **Allan et al., in preparation)**. Importantly, the digital information can also be more readily processed for computer modeling projects (see below).

Several recent studies from other laboratories point to functional interactions between germline development, **aging and metabolism**. *C. elegans* offers a tractable system to probe these connections, and our work on germline proliferation is poised to interface with these aspects of animal life history.

II. Computer modeling and simulation of *C. elegans* development

Because, unlike humans, computers never forget, are not flummoxed by complexity, do not make (nor tolerate) logical errors, and can take the logical consequences of a given state or state change to the bitter end, tools that enable biologists to take advantage of computers will become essential for the future of biological research that is increasingly faced with unmanageable volumes of data.

A. Computer-assisted studies on C. elegans *germline proliferation*

We wish to understand how early germ cells begin and maintain the proper level of proliferation and how the proliferation of germ cell tumors compares to normal germline proliferation. Unlike the somatic lineages in *C. elegans*, divisions within the germ line do not occur in a reproducible pattern. Thus, the spatial and temporal dynamics of germ cell division within the mitotic zone are not well defined. No markers exist to label putative stem cells, the cell cycle in the adult mitotic zone is slow, and, with current technologies, it is not possible to follow divisions in live animals. Concurrent with our development of new experimental technologies to address some of these issues, we took a wet/dry quantitative approach to investigate the dynamics of cell division of the germline proliferation zone. A manuscript is submitted **(Maciejowski et al., submitted)**, and a paper that places this project in the

larger context of computational approaches to biological phenomena is published **(Mishra et al., 2003)**. Our studies validate a quantitative approach and led to the unexpected result that the frequency with which cell divisions are observed within the mitotic zone is not uniform with respect to distance from the ligand source – in fact, cell division frequency is lower in cells that directly contact the distal tip cell. These results are consistent with a hypothesis for a niche/stem cell/transit amplifying system similar to mammalian stem cell systems such as the gut crypts. This quantitative approach can be extended to additional mutants and life history stages.

B. The application of system design tools to modeling biology

Motivated by consideration of a complex biological problem – control of proliferation in an ever-changing developmental context where many independent but interacting cellular processes converge (e.g., cell cycle, cell-cell signaling, and anatomical changes), in a collaboration with a fellow *C. elegans* researcher at Yale University (Michael Stern) and a group of scientists from the Weizmann Institute of Science in Israel (David Harel, Amir Pnueli, Irun Cohen), we are developing and testing computer modeling tools and applying them to *C. elegans* development. In particular, our approach aims to incorporate traditional condition/result biological data and methods used in the design of complex reactive systems. The computer scientists involved in the collaboration are leaders in their fields. (Amir Pnueli received the 1996 Turing Award for his work in the introduction of temporal logic into computing and in program and systems verification.) Moreover, together with Irun Cohen, they pioneered the application of their methods to biology (e.g., Kam et al., 2001; Efroni et al., 2003, Efroni et al., 2005).

We employed a methodology recently developed by the Weizmann group (the language of "live sequence charts" (LSCs) with the "play-in/play-out" process) to formalize and query genotype/phenotype and ablation/result datasets and the inferences derived from them. We started with previously published experiments on *C. elegans* vulval development as a test case. Our approach is described in **Kam et al., 2003(a,b).** Further results of this analysis are in preparation for publication **(Kam et al., in preparation)** and were presented in a plenary talk at the International *C. elegans* meeting in late June 2005. A post-doctoral researcher in the group used a different methodology, Statecharts, to model a subset of the vulval data **(Fisher et al., 2005)**. A student in the group has recently modeled the AC/VU decision using both LSCs and Statecharts, linking them with Interplay (Barak et al., 2004).

My immediate interest is to develop models for co-development of the somatic gonad and germ line. Towards these aims, a post-doc in my lab is developing a statechart model of the cell lineage of *C. elegans* **(Kugler et al., in preparation)** and is exploring methods for encoding genetic interactions. The post-doc is from the Weizmann computer science group and his work also includes further enhancement the Play Engine tool for challenges that lie ahead.

A longer-term goal of this project is to further develop and apply these and other related methods to the entire egg-laying system of *C. elegans*, for which an **NIH R24 GLUE grant** proposal was funded in 2003. We are optimistic that in the long run, the methods we are developing can be extended to additional types of data (e.g., microarray data), to the whole worm, and to systems beyond. Much in the way that model system genome sequencing led to the development of new computational tools, our modeling efforts can serve as a platform for the integration and testing of many modeling approaches.

Please see my laboratory's website "research interests" link for additional details on all projects: http://www.nyu.edu/classes/hubbard

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TEACHING INTERESTS

I am interested in teaching genetics, developmental biology, and/or selected topics in systems biology. I received New York University's Golden Dozen Award in 2003 for teaching and undergraduate mentorship, and obtained an NSF grant to equip a new undergraduate laboratory course in genetics (see below) that uses *C. elegans*. I taught all but 2-3 guest lectures (out of 28) in a new graduate course (see below) and all laboratory classes of my undergraduate course (with the assistance of a TA). In addition, I am involved in the Developmental Genetics program in collaboration with the NYU Medical Center and taught several lectures/labs with Ruth Lehmann and Scott Clark in that program. I also team-taught in the undergraduate Principles of Biology course, the Molecular and Cell Biology course and the graduate Molecular Control of Form and Function course.

Courses developed and implemented:

Graduate "Advanced Genetics" course took an historical approach to genetics. In contrast to a "facts" or "survey" approach, it emphasized the experimental methods by which genetic principles and concepts were developed and the elegant application of those principles to biological research. The course relied almost exclusively on the primary literature. The first part of the course included papers from the early literature (Mendel's pea paper, classic Drosophila, maize, and microbial studies), covering patterns of inheritance, the connection between chromosomes and traits, the recognition and use of mutants, the nature of mutations, and the genetic consequences of changes in chromosome structure and gene dosage. The second part of the course considered the genetic experiments in yeast, bacteria, and phage that were used to parse metabolic pathways, recognize DNA as the genetic material, support the "one gene, one enzyme" hypothesis, understand the nature of the genetic code and demonstrate the co-linearity of gene and protein structure. The last part of the course took on special topics in genetics (such as genetic screens, genetic epistasis, suppressors and enhancers, unlinked non-complementation, synthetic phenotypes, pleiotropy, genetic mosaics, and transposable elements) by dissecting relatively recent papers from the yeast, fly, and worm fields. Although most of the information content was not new to the students, this was a highly effective course. By using the primary literature, this course prepared students to read original papers critically and to evaluate and interpret genetic data. By studying genetic data from many different systems, the students learned to focus on the genetic methods and reasoning regardless of the system to which they are applied. By taking an historical approach, the students appreciated the thoughtful transitions from observation to hypothesis to more observation to synthesis and back again through the process - i.e., the very essence of experimental science.

Undergraduate "Laboratory in Genetics" course covered genetic principles by means of a project-based laboratory using *C. elegans*. Students identified and characterized new mutants, analyzing dominance, linkage, recombination, dosage effects and complementation. The second part of the course addressed genetic approaches made possible by the availability of complete genome sequences (genomics) using RNAi.

Students selected a gene of interest (e.g., a gene similar to one connected to human disease). Using gene databases available on the internet, they identify a closely related gene in the *C. elegans* genome and, using the RNAi technique, they remove the function of that particular gene of interest from the worm and note the consequences in several related mutant backgrounds.