GENOMIC BIOLOGY AND PHYSIOLOGY OF BASAL METAZOANS & DEUTEROSTOMES

(Marine Genomics & Introduction of Novel Models for Experimental Biology)

Friday Harbor Laboratories Research Apprenticeship
Spring Quarter: March 29 - June 4, 2010 (10 weeks)

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CONCEPT:

Advances of modern genomic sciences and technologies are just beginning to be introduced into traditional zoological and comparative disciplines including marine biology. Moreover, there is an unfortunate divide between a few so-called "genomic" model organisms and a diversity of available species with unique developmental, functional characteristics but species lacking genomic information. This gap is even more dramatic when an experimental biologist starts to address complex evolutionary questions or mechanisms of adaptations in various ecosystems including marine habitants. It would be a long-desired goal of a biologist, to both use a wide diversity of species for physiological test and integrate these experimental tests (or evolutionary hypotheses) with sufficient genomic information for any given species (or even multiple of species) within months or even weeks.

Thus, we propose to develop an apprenticeship that will address these emerging challenges in genomic biology and marine genomics in particular. We would like to teach students how to integrate genomics and physiology; how to use non-traditional experimental preparations from less explored invertebrate groups and make them as powerful models with extensive genomic information. In fact, during the apprenticeship, we will make crucial experiments and introduce novel experimental models for genomic biology such as **basal metazoans** (Ctenophores, possible selected Cnidarians) and **basal deuterostomes** (such as Hemichordates, Echinoderms and Tunicates). Conceptually, we will integrate genomic and functional analysis for representatives of these two key lineages known to be crucial in our understanding of the origin and evolution animal organization; and specifically evolution of signaling pathways.

We will take advantage of the next generation sequencing technologies to obtain ~ 70-90% of transcripts expressed in specific tissues (including nervous and hormonal tissues) – this part of the work will be done before the start of the apprenticeship (the work is in progress now). For many basal prebilaterian animals (5 species of ctenophores and sponges) we already have most of required genomic information (+ The Moroz lab has started sequencing of the entire ctenophore genome – see below). As for hemichordates, *Saccoglossus kowalevskii* has been sequenced, and is closely enough related to the local hemichordate in Padilla Bay, *Saccoglossus bromophenolosus,* that RNa probes will cross-react. For *Glossobalanus berkeleyi*, we will obtain initial transcriptome information later this year (using our existing funds) as well as trying to raise funds for this initiative through NSF and NIH.

With all this molecular information in hands, we will design the apprenticeship in a very straightforward way. We will characterize the expression of genes crucial for overall body patterning, organization of nervous, muscular, hormonal and immune systems using *in situ* hybridization. We estimate that with these resources and our protocols it is possible to perform expression profiling of up to 20-30 genes in both ctenophores and basal deuterostomes (a least 2-3 species).

In parallel, we can perform functional/pharmacological/behavioral tests focusing on key signal molecules and possible neurotransmitters. Mapping nervous systems or components of immune and developmental signaling systems would be also straightforward and we will combine it with functional tests.

As a result, experimental analysis of a set of homologous genes and signal molecules within two lineages in parallel (e.g. ctenophores/cnidarians vs hemichordates) will give us interesting biological and evolutionary insights about major transitions in animals.

In summary, the ultimate research goal of this initiative is to enable students to identify and characterize evolutionarily conserved set(s) of genes by direct genome-wide comparisons across phyla. Second, students will learn of how to identity and functionally explore novel signal molecules; test their unique expressing in specific cells (including neurons or stem cells). We will be able to determine novel homologous cell lineages leading to formation of complex phenotypes and organ systems. The students will be exposed to a variety of organisms, with particular focus on groups that occupy salient points in animal evolution. The latter will include: basal animals such as sponges, placozoans, ctenophores, cnidarians, and basal deuterostomes, such as echinoderms and selected lophotrochozoans. The program will explore examples of modular organization of nervous and other signaling systems across phyla. We will also discuss hypotheses, identify trends and possible selective factors leading to independent origins of motor, sensory components as well as the central nervous systems during evolution in several animal lineages including ctenophores, cnidarians, molluscs and chordates.

In conclusion, we propose to take advantages of marine resources at the Friday Harbor laboratories during the spring 2010 with the objective to introduce participants to both larval and adult organization of basal animal lineages. Moreover we will encourage individual projects of students that address more functionally oriented questions and help place the question of a specific of cell signaling pathways in an appropriate ecological context of the life history transitions and its evolutionary implications. Finally, we are confident that some of organisms used in this apprenticeship will be further promoted and developed as novel model systems in neuroscience and genomic sciences.

In fact, the emerging fields of single cell genomics and microanalysis (areas of expertise of the Moroz lab), development and immunity (areas of expertise of the Swalla lab) as well as novel technologies for imaging of complex molecular machinery in living cells call for urgent changes in education with an integration of different disciplines under the unified training program. Thus, interdisciplinary and genome-wide approaches and technologies will be the leading philosophy of the course.

RESEARCH FOCUS:

The primary focus of the research program and individual projects will allow students to address a series of novel but critical questions: how to integrate genomic information about a given species to understand biology of an organism and how to design functional/physiological tests to understand gene functions in non-traditional experimental models. Proposed training and experimental projects will ultimately provide insight into how animals and signal systems have evolved. These major questions include:

i. How has independent evolution (different lineages) and the modular organization of signaling systems in animals lead to the formation of different types of hormonal, nervous systems and behaviors?

ii. How are multiple signaling pathways (and the activity of more than 20,000 genes) integrated into the activities of specific neurons, neural circuits and developmental programs?

iii. How have developmental mechanisms, signaling transduction pathways, nervous system and complex behaviors evolved?

iv. How have hormonal/secretory and other cell types been integrated into developmental and nervous system functions?

v. How have learning and memory mechanisms evolved? Do Ctenophores and Hemichordates learn and remember?

vi. How has the genome-scale organization of hormonal, developmental, neuronal systems and behavior participate in speciation and evolutionary events?

It is estimated that a diversity of types of signaling mechanisms in both basal deuterostomes and basal metazoans will form an ideal training and research foundation to address these questions both conceptually and experimentally.

Research long-tern objectives/lectures/discussion topics (examples):
- Organization and Evolution of Animal Genomes: insights from basal lineages
- Dynamic organization and operation of cellular genomes
- Comparative Genomics in Physiology and Development
- Novel genomic technologies for marine invertebrates and non-traditional experimental models
- Tools for marine genomics
- Cellular memory and its mechanisms
- Origin and Evolution of Animals
-Origin and evolution of developmental mechanisms
- Origin and parallel evolution of signaling, immune systems and organs
- Origin and evolution of signal molecules
- Origin of cell-cell communications;
- Origin and evolution of complex cell phenotypes
- Classification in Biology: from molecules to Organisms and Species
- Origin and Evolution of Nervous and Sensory Systems
- Comparative genomics, informatics and the development of new database management techniques
- Sensors and probes for monitoring gene expression and cellular biochemistry.

Practical training will include:
1. Introduction to the genomes, transcriptomes and epigenomes
2. Introduction to evolution of hormonal and nervous systems (dissection, microdissection and labeling of neuronal elements in representative of basal metazoans and deuterostomes)
2. Introduction to marine larval life histories
3. Induction and analysis of Metamorphosis and development
4. Confocal Microscopy and in situ hybridization
5. Practical approaches to study comparative signaling, repair and immune mechanisms
6. The methods and tool to reconstruct phylogenies; work with genomic databases to address questions about evolution of signal molecules and nervous systems
7. Identification of Signal Molecules, Neurotransmitters and hormones
8. Mass spectrometry and other quantitative methods
9. How to write a scientific paper and a report

It is expected that individual student projects will be organized in a way that each project will deal with one or two signaling pathways in representatives of at least two the key phyla (preferably Ctenophora and Hemichordata) with their comparative analysis. As a result, it will be possible to use the same technical expertise and compare organization of a giving signaling mechanism between two distinct animals. It will stimulate evolutionary thinking and, possible help to design an evolutionary hypothesis or a way to find out their evolutionary conserved functions, etc. Thus, different student projects will be mutually integrated. It will be a consensus across the group to combine data from different signal transduction pathways within one species as well as to compare different species for similar developmental/neuronal/immune markers. Training in making cell-/tissue specific genomic libraries will also be provided as a result we will extend the existing collection of cDNA libraries and screen them for the presence of neuron-specific markers. We also expect to test the possibility to culture cells from some groups for electrophysiological and imaging experiments.

Learning Objectives and Skills

At the end of this apprenticeship, apprentices will be able to have a thorough understanding of the organization of major organ systems in marine invertebrates (adult and larval). They will also have a solid foundation of mechanisms underlying cell signaling and development. Through the comparative nature of this apprenticeship they will learn about the evolution of animals and organ systems. Apprentices will learn basic immunocytochemistry, molecular techniques and genomic approaches. If time allows they will also be introduced to some functional knockout techniques such as RNAi and morpholinos. By reading primary literature and discussing their projects with the advisors, they will learn how to ask scientific questions and develop their own research project. Finally, they will learn how to present their data and write a scientific paper.

Prof. B. Swalla has done frequent graduate summer courses and two undergraduate apprenticeships at FHL, one in 2001 and one in 2005. Each of these research apprenticeships had seven students, and most of those students are now either in Professional or Graduate Schools. She has extensive experience in teaching molecular developmental and evolutionary biology in marine labs around the world (Italy, France, Australia, Japan). Working with emerging model systems and refining in situ hybridizations for different species are her forte.

It is increasingly important for students to be able to understand and tap into genomic resources that are being generated at unprecedented rates. All students in this apprenticeship will be trained in complex computer skills, regardless of the outcome of their research projects. A mix of field projects, laboratory experiments and database searching has proven to be a very successful formula to excite students about research. Students will be encouraged to think in an interdisciplinary way about the animals that they are using for their projects.

Prof. L. Moroz is one of the organizers and leading lecturers for the course "Comparative Neurobiology" for biomedical graduate and undergraduate course. He has 10 years of experience to teach of this course at the University of Florida. He also was the organizer of NIH neurogenomic workshops (2004-2006; two weeks; and NSF genomic workshop, 2009) for undergraduate and graduate courses and one of PIs for NSF funded REU summer program at the Whitney Laboratory for Marine Bioscience in 1999-2009.

In addition, he was one of the leading instructors of tropical and marine neurobiology summer course in Puerto Rico (2 months) in 1999, 2001, 2002, and the apprenticeship at FHL in 2008. In fact, a lot of molecular, genomics and comparative data were obtained during that apprenticeship. However, because of the seasonal nature of selected animals and their access at FHL only, we would like to coordinate our efforts to complete a number of projects initiated earlier at FHL.

All of these courses used a wide diversity of marine invertebrates from all major phyla. The concept of the courses was a multidisciplinary approach that integrates molecular, genomic, morphological, physiological and behavior approaches to reveal principles of neuronal organization in relatively simpler nervous systems of invertebrates.

"***Nothing in Biology Make Sense, Except in the Light of Evolution***" is the title of the most influential paper about biological education, written more than 30 years ago by Theodosius Dobzhansky. In 2005, evolution was identified as the breakthrough of the year by the AAAS journal Science. In 2009, we celebrate 200 years of Darwin's birthday and 150 years of his "Origin of Species". Today evolution is the foundation of all biology, so basic and all-pervasive that scientists usually take its importance for granted. Despite this, evolution is rarely integrated into undergraduate/graduate biomedical training programs, particularly those in the neurosciences and cell signaling. Genome-scale Cell Signaling and Nervous systems are some of the most complex machines in our universe. For example, the myriad of signaling mechanisms within nerve and immune cells, the billions of nerve cells and trillions of connection (most of them are unique) are truly wonders of enormous proportions. Yet extant nervous systems are the way they are because they have been molded by millions of years of evolution. Thus, excluding evolution from modern training programs in the neurosciences removes the opportunity to fully understand the modern manifestations of nervous systems by tracking the lineages and constraints that have molded them.

The absence of rigorous evolutionary and genomic approaches to understanding nervous and hormonal systems is particularly worrisome in light of much of society's acceptance, of "intelligent design" as the only way to explain the enormous complexity of life. Indeed, for many, the complexity of the human brain and phenomena such as "the soul" and "the mind" are, for many, the strongest arguments in favor of intelligent design. An understanding of how nervous systems have evolved to produce the complexity of the complex brain will go far toward countering this attitude.

Achieving this goal is not merely a matter of providing students with courses in evolutionary theory and an appreciation of animal lineages. It requires both genomics and a systems biology approach that will combine the efforts of biologists with mathematicians, computer scientists, chemists, physicists and engineers working on biological questions as part of multidisciplinary teams. To achieve this we propose to initiate an integrated training program that builds from evolutionary and genomic platforms. Such foundation could also be used as a basis both for teaching biological principles to non-biologists, and for ensuring that students of biology are fully aware of, and able to take advantage of the influences evolution has had on the processes they are studying. Here, we propose to develop a program specifically designed to address these issues within the context of genome biology, neurobiology and development of two key animal lineages representing major transitions in the animal organization. The same concept could, and should be used for other disciplines.

As a result, students need to be directly exposed and provide a practical training using organisms representing an enormous diversity of neuronal, hormonal and developmental designs. Ideally, these organisms should be also available for behavioral experiments and studies in natural habitants. Clearly, the Friday Harbor Laboratories provide a unique place for this type of teaching such an apprenticeship proposed due to the availability of a large diversity of organisms (especially planktonic ctenophores - they are inaccessible in most of the universities and research centers) and excellent research facilities (such as imaging, physiology and molecular biology). Both, Prof Leonid Moroz and Prof. Billie Swalla Heyland have visited collection sites and FHL many times before and are familiar with specific location where organisms can be found. Most importantly we already established molecular and genomic resource that will provide a solid foundation for the genomic approaches to study evolution of complex functions and development in major animal lineages.