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Dr. Yves Brun
Systems Biology/Microbiology Faculty Search
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Bloomington, IN 47405-7005

October 26, 2005

Dear Faculty Search Committee:

I am responding to your advertisement for a tenure-track Assistant Professor position in The Department of Biology and Biocomplexity Institute recently posted in *Science*.

As a postdoctoral fellow in the laboratory of Dr. William C. Smith at UCSB, I have been studying the developmental biology of ascidians and chordate evolution. My graduate and postdoctoral experiences have prepared me to conduct independent and original research as a principle investigator. During the past few years, two main focuses of my research have been: 1) to develop ascidians as a genetic model system to study chordate development, and 2) to study notochord morphogenesis through genetic, genomic, cell biology, modern imaging, and comparative embryology approaches. In my future academic career, I will continue and expand my work on elucidating the molecular details of notochord morphogenesis through molecular biology, genetics, and system biology approaches. I will also search for the differences at molecular and genomic levels that may account for the diverse notochord morphology and morphogenetic processes within tunicates. In addition, I will attempt to understand the adaptive value of different morphology and developmental pathways, and study how evolution shapes development. And finally, I will investigate the changes in developmental pathway that account for the emergence of notochord in early chordate history. I am confident that my research will provide some general insights in the mechanisms and processes that have been responsible for generating the astonishing diversity of animal and plant forms.

I believe that my research on ascidian developmental biology and chordate evolution can complement your institution's existing strengths in biological science. With my interests in teaching comparative embryology, developmental biology, and evolutionary biology, I would make a strong contribution to your educational program.

Enclosed with this letter are my curriculum vitae with a list of references, statements of my research and teaching interests, and representative publications. I would like to sincerely thank the search committee for considering my application. I look forward to hearing from you.

Sincerely,

Di Jiang, Ph D

RESEARCH INTERESTS

DI JIANG

INTRODUCTION

I am interested in how differentiated cells are organized into complex tissues and organs, and how such developmental events have evolved. I have chosen the ascidian notochord to study these developmental and evolutionary processes for two reasons. First, ascidians are the most primitive living organisms in phylum Chordata. They share the defining features of the phylum: a notochord, a dorsal nerve cord, pharyngeal gill slits, and an endostyle. Unlike their vertebrate counterparts which have gone through whole genome duplications, ascidians maintain a compact genome, which is likely close to the genome of ancestral chordates. Because of these features, ascidians provide a very useful system to investigate chordate bodyplan and genome evolution. Second, in contrast to the vertebrate notochord, which consists of thousands of still dividing cells whose positions and movements during development are difficult to trace, the ascidian notochord has only 40 cells, which are post-mitotic at the beginning of morphogenesis. During a process called convergence/extension (C/E), a four by ten sheet of differentiated notochord cells are organized into a single column of 40-stacked cells. Following C/E, notochord development continues as individual cells elongate and vacuolize along the anterior/posterior (A/P) axis. Finally, these vacuoles fuse to form a tube that runs the length of the larval tail. The simplistic nature of ascidian notochord development makes it an elegant system to study morphogenesis.

I have isolated a short tail mutant (*aimless,* or *aim*) from natural population that carries a mutation in ascidian homolog of drosophila planar cell polarity (PCP) gene *prickle*, and have shown that *prickle* is essential for C/E movement. I then discovered that individual notochord cells have, surprisingly, an A/P polarity that is evidenced by the anterior localization of prickle and strabismus (also in the PCP pathway) proteins and posterior position of the nucleus (see figure 1). With the use of the *aim* mutant, I was able to demonstrate a requirement of *prickle* for the A/P polarity of notochord cells. My findings reveal that the PCP pathway not only operates along the medial/lateral (M/L) axis during C/E, but also regulates notochord morphogenesis along the A/P axis at the elongation phase. The simple morphogenesis of the ascidian notochord, combined with molecular biology, genomic research, and modern imaging techniques, have allowed us to advance our understanding of the role of the PCP pathway in chordate development to a degree that has not been achieved using vertebrate model organisms.

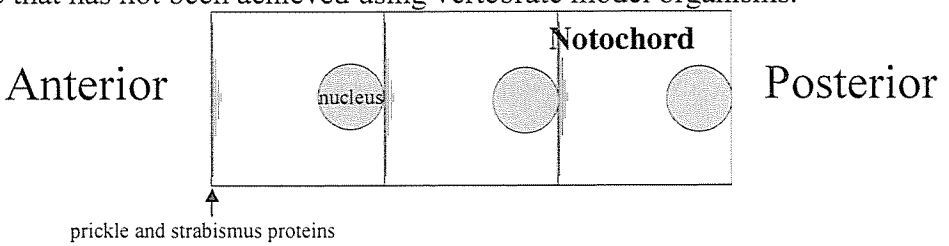


Figure 1 Anterior/posterior polarity in ascidian notochord cells

A survey of 12 species of ascidians and one species of larvacean (sister group of ascidians, also in Urochordate Subphylum) reveals that, A/P polarity is present in the majority of species, but is by no mean a universal characteristic of urochordates (figure 2). When this result is plotted against the urochordate phylogeny based on molecular and morphological data, the presence of A/P polarity seems to be an ancestral character, and it may have been lost secondarily in some species. This observation on one hand suggests an importance for A/P polarity, but on the other hand, presents some interesting questions such as why it is lost in certain species; how the genetic network regulating notochord morphology diverged to give rise to different morphology; what the physiological differences are as the result, and what ecological factors have been driving the evolution of this divergence.

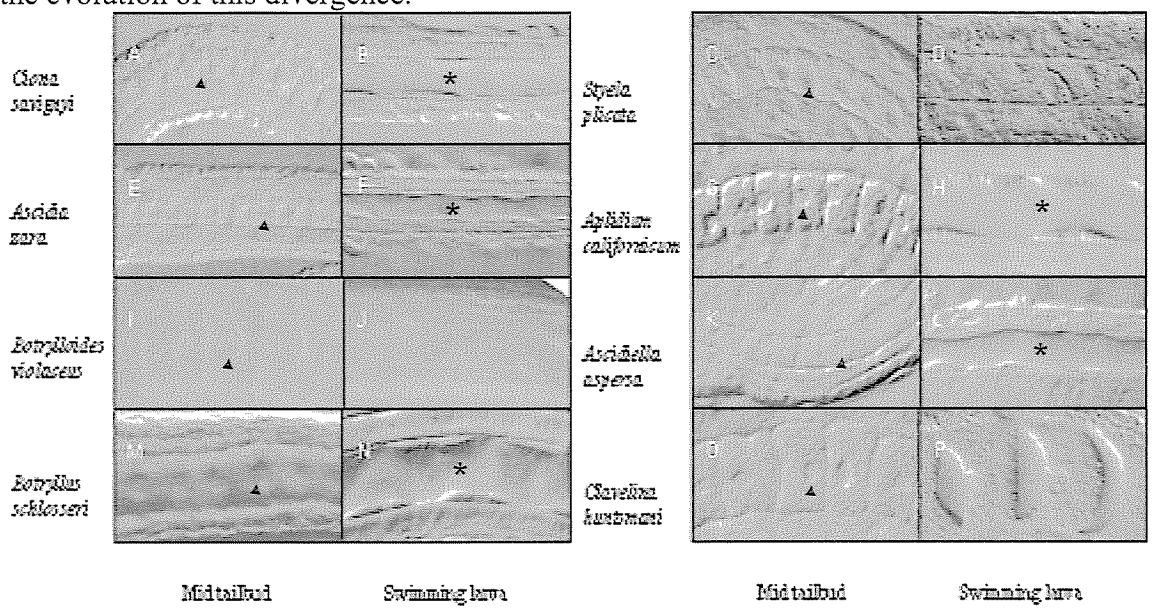


Figure 2 Morphology of notochord in a diverse group of ascidians
(Arrowheads indicate nuclei; stars indicate notochord tube)

PROPOSED RESEARCH

My future research will include three logically connected areas outlined below:

First, I will try to understand the molecular details of notochord morphogenesis in model organisms *Ciona savignyi* and *intestinalis*.

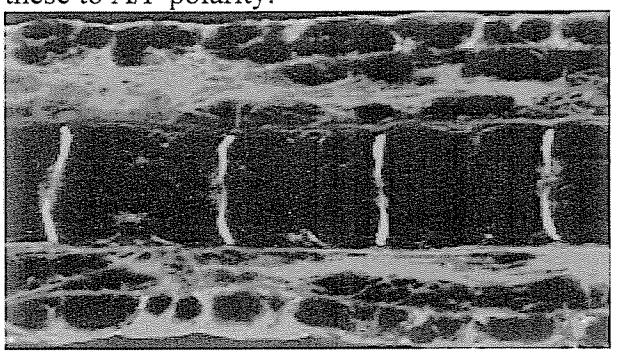
Second, I will search for the differences at molecular and genomic levels that may account for the diverse morphology and morphogenetic processes.

Third, I will attempt to understand the adaptation value of different morphology and developmental pathways, study how evolution shapes development, and investigate the changes in developmental pathway that account for the emergence of notochord.

1. Molecular details of notochord morphogenesis in model organisms *Ciona savignyi* and *intestinalis*

1.1 Investigation of notochord cellular architecture and morphogenesis in model ascidian *Ciona intestinalis* and *savignyi*

Elongation of the tail after notochord cell convergence is achieved by cell shape changes, cell rearrangement, and extracellular matrix formation, and tubulization. Several basic cell biology events are taking places: cell growth, possibly polarized secretion of matrix, cell neighbor exchanges. Bio-mechanic forces are working to reshape and move cells in an organized manner; cell-cell and cell-matrix adhesions have to be remodeled to accommodate these processes. Confocal imaging technique allows us to visualize these events at high resolution. I have begun to use this approach to examine the localization of regulatory proteins (including more PCP proteins), cytoskeletons, secretory pathways, and adhesion apparatus in notochord (figure 3), in an attempt to reveal the dynamic changes in notochord elongation. Once we have this information, we can begin to understand the molecular and bio-mechanic mechanisms underlying these processes, and relate these to A/P polarity.



Green: F-actin; red: α-tubulin

Figure 3 Cytoskeleton architecture of notochord cells

1.2 Function genomic approaches to study the genes essential for notochord morphogenesis

1.2.1 Identification of pk downstream genes through microarray analysis

Prickle has been traditionally thought as a cytoplasmic molecule that functions through protein-protein interaction in a signal transduction pathway. However, one recent report has demonstrated convincingly that prickle can act as a transcription factor. Nevertheless, its downstream targets are completely unknown. I have begun a microarray analysis to identify prickle targets by comparing wild type embryos and *aim* mutant embryos. Because the only phenotype in *aim* is in notochord morphogenesis, I believe this type of analysis can identify a cohort of genes that are specific for notochord and important in regulating morphogenesis.

1.2.2 Identification of pk interacting genes through genetic screen

The small genome of ascidian, and its simple yet chordate-type development, makes ascidian a very attractive system for genetics. My current research has demonstrated the feasibility of genetic approach in identifying genes and novel functions of genes in chordate development. I believe we can take ascidian genetics to a next level, by conducting a more focused screen aimed at developmental pathways one at a time. Very fortunately, homozygous *aim* mutant is completely viable in the laboratory. This will allow me to perform enhancer and suppressor screens to identify other genes that genetically interact with *aim/prickle*. I am particularly excited by suppressor screens (see Figure 4 for screen scheme) because I believe that PCP pathway is much more complex than currently thought, and we know little about the players that act antagonistically to *aim/prickle*. Because the *aim* phenotype is very obvious and fully penetrant, the screen for a reduction of this phenotype should be very easy to score, and therefore is suitable for an undergraduate project.

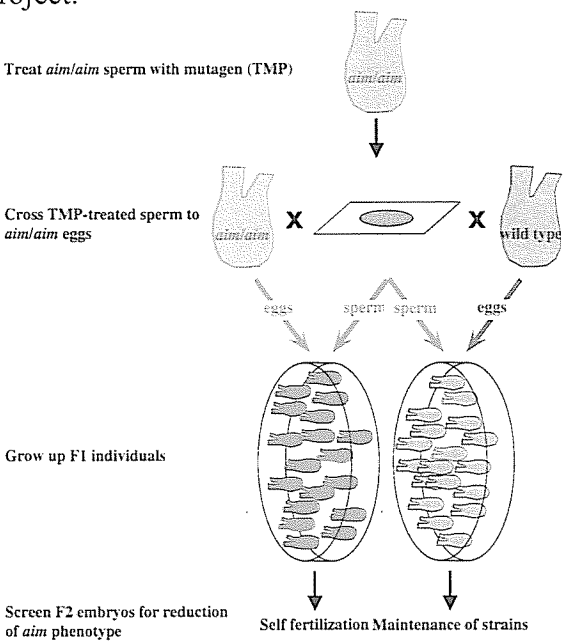


Figure 4 Genetic screens for modifiers of *prickle*

1.2.3 Identification of Prickle interacting partner through proteomic approaches

Both microarray analysis and genetic screen will yield a large amount of genes functionally involved notochord morphogenesis. Some of these genes should have homologues in other model systems, and their identity and functions have been studied. By studying the function of these homologues, especially their protein-protein interaction profiles through system biology approaches, I will build an interactome in which all protein-protein associations and interactions will be delineated. The result of this work will guide my future study on how complex molecular network operates in the establishment of notochord morphology and morphogenesis.

2. Comparative molecular embryology approaches to study the molecular differences underlying phenotypic diversity

Diversity in notochord morphology reflects different organism's adaptation to its unique environment. The genetic program underlying notochord morphogenesis must have been modified in evolution at several levels, including transcriptional and translational levels, but ultimately the genomes of different extant species must have been altered, from common ancestor, to bring about different morphology. Once we know the molecular details of one model system, in my case, *Ciona*, and its genomic structure of the genes involved in notochord development, I will investigate, both molecularly and functionally, the homologues in species that are morphologically different, using the approaches highlighted above.

3. Adaptation, developmental diversity, and evolution of notochord

3.1 Adaptation and developmental diversity of notochord

What is the adaptive value associated with different morphology? Why do some ascidians have an A/P polarity and some don't? Why do some ascidians develop tube-shaped notochord and some never in their larval stage? Related to this, and even more interesting questions are: why do chordate make a notochord and what does notochord offer to chordates and perhaps give them a chance in the struggle of life? And what genomic and morphological raw materials did ancestral chordates use to create a notochord and how did they do it? I believe one can look into tunicate tadpoles and their genes for answers. To begin to approach these questions, I will try to understand the life styles of diverse group of ascidians and correlate them with their morphology. I have already noticed that the absence of A/P polarity seems to be associated with tadpoles with large size; and the lack of tubulation may be more prevalent in colonial ascidians. It may be that different notochord morphology correlates with the need of dispersal involving swimming. Large tadpoles produced by colonial ascidians tend to swim very little and settle near the parents, and they more likely have a notochord without A/P polarity and tube formation. I will expand this comparative study to further test this conjecture.

The above observation underscores the importance of notochord in chordate swimming. However, we know every little about the bio-mechanics of ascidian notochord, the simplest notochord in living chordates. We know that tubulized notochord provides turgor to the tail, but we don't know what the material is in the tube that is created and surrounded by the notochord cells, and how it does it (figure 5). I have recently discovered from ascidian genomic database that notochord cells express a group of genes encoding proteins commonly found in extracellular matrix. This observation suggests that the space within the tube may have some orderly and mechanically meaningful structure related to the function of notochord. My future research will include studies on microscopic structure of extracellular materials in notochord tube; bio-mechanical properties of these materials; how they contribute to the turgidity; what diversity exists in ascidians that reflect different adaptive strategy to environment.

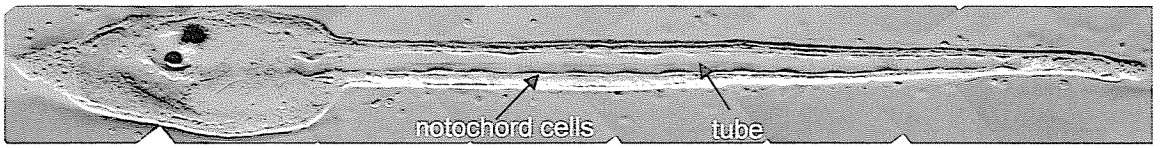


Figure 5 *Ciona savignyi* tadpole

3.2 Emergence of notochord as the result of changes in the regulatory regions of mesoderm genes

Based on available paleobiology, molecular phylogeny, and developmental biology evidence, I postulate that chordate ancestor was most likely a worm-like benthic organism that possesses a bipartite body which resembles the fossil vetulicolian deuterostomes discovered recently in ChengJiang Fauna in China. Its anterior half has a pharynx perforated laterally by gill slits and underlain ventrally by an endostyle, while its posterior half features muscles but lacks a notochord. The acquisition of notochord in chordate lineage conferred an advantage of swimming and allowed them to escape from benthic environment and occupy the pelagic realm which was becoming more inhabitable during Cambrian period. But how did new tissue such as notochord arise in evolution? One possibility is through a "trans-differentiation" process in which an old tissue type is transformed in evolution to a new tissue type. In the case of notochord, I argue that notochord was derived from muscle tissue during evolution for the following reasons. First, both notochord and muscle belong to the same mesodermal germ-layer. Second, ascidian notochord expresses several genes homologous to muscle-specific genes in vertebrates. Third, notochord in cephalochordates (another lineage of invertebrate chordates) has contractile property similar to that of muscle. Fourth, initial muscle development is independent of notochord in vertebrate embryos. And last, muscle has been a more primitive tissue present in all bilaterians, while notochord is a later-comer only in the chordate lineages.

What was the developmental switch that shifted the fate of a subset of muscle to notochord? Some observations on ascidian development provide hints. Ascidian *brachyury*, the "master-control" gene for notochord differentiation, has two sets of cis-regulatory elements: positive elements conferring the expression in all mesodermal tissues: muscle, mesenchyme, and notochord, and negative elements restricting the expression only in the notochord. I postulate that the acquisition of these negative elements may be accountable for the historical separation of cell fates and the emergence of the notochord. In non-chordate deuterostomes, *brachyury* transcriptional regulatory region should lack these negative elements and brachyury, as the result, is expressed in general mesoderm such as muscles and mesenchyme. In my future research I will compare the cis- regulatory region of *brachyury* gene in non-chordate (Xenoturbellum, Hemichordates, and Echinoderms) and chordate (Ascidians, Larvaceans, Cephalochordates, and Vertebrates) deuterostomes and identify traces of changes that may explain different expression patterns. Molecular phylogeny methods will be employed to find conserved and distinct elements. Expression analysis of different promoter elements will be carried out to test each element in development functionally.

TEACHING INTERESTS AND PHILOSOPHY

DI JIANG

My teaching interests are Comparative Embryology, Developmental Biology, and Evolutionary Biology.

My primary motive for teaching is to share the stories of nature and science with others. This goes hand in hand with one of my purposes for conducting scientific research: so that I have stories to tell.

I have found teaching to be both challenging and satisfying. As a teaching assistant for the undergraduate Immunology and Genetics courses in the George Washington University, I acquired skills in leading discussion, creating and evaluating exam and essay questions, and relating course materials to students' interests. An equally rewarding teaching experience has come from one-on-one mentoring in the fundamentals of experimental biology. As a senior graduate student at National Institutes of Health, I mentored two undergraduate summer apprentices in a wide variety of biochemical, immunological, and cell biological techniques.

My method for teaching will be to draw examples from different groups of organisms to provoke discussion centered on important and controversial biological and evolutionary theories/hypotheses. I will cover as many organisms as possible, representing all major and fundamentally distinct body plans, discussing their morphology, physiology, cell biology, development, and ecology, so that my students will have a broad appreciation of animal diversity and not just a few model systems in the laboratory. I also think that teaching the process of biological discovery is as important as providing students with information. To achieve this type of teaching, I will incorporate the historical contents of classic discoveries in the history of zoology, developmental biology, and evolution, and ask students to interpret the findings with and without their historical limitations. Scientific thoughts evolve often at a different pace from the fact collection. I feel that encouraging students to think about the process of scientific progress will arouse their interest in development and evolution.