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10/31/05

Yves Brun
Systems Biology/Microbiology Faculty Search
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Indiana University
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1001 E 3rd Street
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Dear Dr. Brun,

I am writing to apply for the faculty position in the Department of Biology. I believe that my work studying the molecular basis of axis specification and pattern formation during sea urchin development from the perspective of signaling and gene regulatory networks makes me a strong faculty candidate for your program. These processes are fundamental to all multicellular life, yet our understanding of them remains incomplete, especially in vertebrate embryos. My work has the potential to quickly unravel these processes at a molecular level, providing a detailed mechanistic model that will serve as a template for understanding more complex embryos and provide insights into the evolution of these processes.

I received my Ph.D. in Biochemistry and Biophysics from the University of North Carolina at Chapel Hill in 1998 in the laboratory of Dr. David Brenner, and after a short first postdoc, I joined Dr. David McClay's laboratory at Duke University. I have been independently funded throughout my graduate and most of my postdoctoral years, having obtained an NSF fellowship during graduate school and an NIH postdoctoral fellowship upon joining Dr. McClay's lab. I have also successfully competed for several short term awards, including a pilot feasability grant from CGIBD.

In Dr. Brenner's lab, I studied stress-associated signaling using in vivo models of liver disease. This work led to 4 first author papers and 16 middle authorships, as well as 5 review papers (one first author) and a first author book chaper. Although I developed a powerful arsenal of techniques during those years, I realized that clinical research did not fascinate me as much as developmental biology. For that reason, I made a major change in direction in 2000 when I joined the McClay lab. I continued to study stress-associated signaling in the context of sea urchin development. This work has led to one book chapter on gastrulation and two first author papers, the first of which shows that PI3K is required for larval skeletogenesis, while my second, major paper, which shows that the asymmetrical activation of p38 MAPK is the earliest known step in secondary ais specification and patterning in the sea urchin embryo.

I have developed a detailed network model of p38-dependent axis specification (Bradham and McClay, 2005 and unpublished results). p38 is also required for the induction of ectodermally-mediated mesenchymal patterning (Bradham and McClay, in preparation). This patterning process is dependent on filopodial-mediated cell-cell communication, and one of my future directions is to develop a complete molecular description of this communication event and the concomitant mesenchymal patterning process. Finally, my preliminary results suggest that

asymmetrically distributed mitochondria underly the symmetry breaking mechanism along this axis.

These studies will provide a novel depth and completeness to our understanding of these basic developmental and cell-cell communication events that will be relevant to the larger developmental community, and since many of the molecules and processes involved are evolutionarily conserved, my results will likely provide a template for similar events in more complex embryos.

Please find attached my curriculum vitae, my research and teaching statements, and selected reprints. Thank you for your consideration.

Sincerely,

Cynthia Bradham

Research Statement **Cynthia Bradham**

Summary

Axis specification and pattern formation are foundational aspects of embryonic development that are essential for all bilaterian life, but remain incompletely understood, particularly in vertebrate embryos. My work has produced a network model for secondary axis specification and pattern formation in sea urchin embryos. As a new faculty member, I will use this model as a framework for addressing fundamental questions about these early, critical processes, including (1) how are sub-populations of cells specified, in terms of signaling and gene regulatory networks? (2) what is the molecular basis for cell-cell communication and information exchange during pattern formation? and (3) how is axial symmetry broken? The sea urchin offers a simple model system that is particularly amenable to answering these questions. The results will provide fundamental insights relevant to the developmental and evolutionary communities at large, and which will have the potential to provide novel insights into disease processes in which cellular identity and behavior is perturbed.

Background

All embryos begin as a fertilized egg which ultimately becomes an organism with 3 body axes and an organized system of tissues. How this is accomplished remains a frontier in biology, and while significant advances have been made toward our understanding of the early embryonic specification and patterning events, a global picture has yet to emerge, particularly for vertebrate and mammalian embryos. Specification and patterning begin as molecular asymmetries, for which, in many cases, the nature and identity of the symmetry breaking mechanism remains unknown. As a consequence, distinct transcriptional regulatory networks are deployed, which separate cellular territories into distinct fates [1]. These territories subsequently interact via signaling relationships to induce subterritories with increasing specializations [1]. A complete understanding of specification and pattern formation will therefore require a global view of the transcriptional and signaling pathways which sculpt the organism, first crudely, into germ layers and body axes, and then with increasing refinement, to ultimately produce the complex patterns in an animal. For this reason, my work has focused on understanding these early processes by identifying the relationships between key signaling pathways and transcription factors.

This was initiated by my discovery that the asymmetrical activation of p38 MAPK (Fig. 1) is the earliest known step in secondary axis specification in the sea urchin [2]. p38 is required first for the specification of the oral (analagous to ventral) territory. Then, through its downstream targets, p38 induces the neurogenic subterritory via the expression of Chordin, as well as a distinct ectodermal territory that is important for pattern formation, the ventrolateral (VL) cluster site. This particular patterning process that is quite intriguing because it uses thin filopodia for the communication of information between two different cellular territories. We have demonstrated that a p38-dependent transcription factor, Otp, is essential for this patterning process, providing the first inroad toward a molecular description for this information conveyance. I have assembled my data into a signaling and gene regulatory network model (Fig. 2), which shows the specification of the oral territory, which is followed by subspecification of the neural and patterning regions, all of which occur downstream from the activation of p38. Interestingly, our data indicate that the asymmetric activity of p38 is induced by

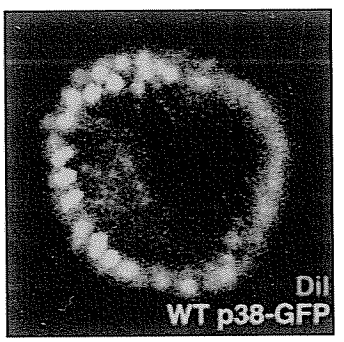

Fig. 1. p38 is asymmetrically activated in a transient manner. A single live embryo, with p38 activity shown by nuclear localization. The DiI lineage label was used to determine the oral fate of the activated region.

asymmetrically distributed mitochondria, which are a candidate for the symmetry-breaking mechanism along this axis.

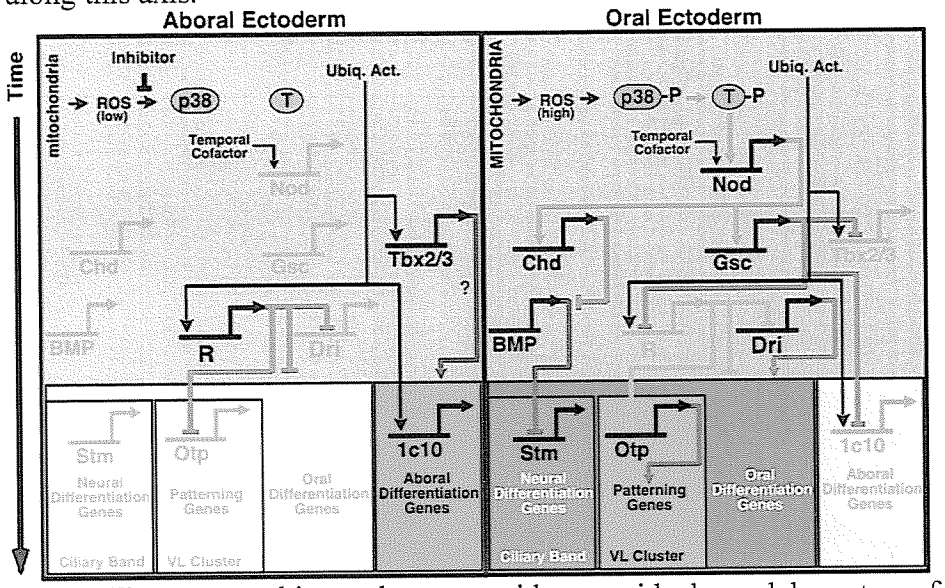

Fig. 2. Urchin Ectodermal Regulatory Network Model. In the default aboral compartment, a ubiquitous activator drives aboral genes and a repressor of oral genes (R). In the oral compartment, higher levels of reactive oxygen species (ROS) activate p38 MAPK, which induces Nodal through an intermediate transcription factor (T). Nodal drives Chd, required for neurogenesis and synapotagmin (Stm) expression, and the repressor Gsc, required for aboral suppression, oral differentiation, and the induction of patterning.

The sea urchin embryo provides an ideal model system for addressing these important developmental events. Urchin larvae are composed of only 11 major cell types ordered in a simple pattern, making this system quite amenable to molecular dissection and perturbation. The urchin offers a unique set of assets, as a fast-developing, transparent embryo that is robust, inexpensive, and available in large quantities year round. The embryos are easily injected at 2, 4, and 8 cell stages, as well as being surgically manipulable, allowing for the creation of many different chimeric embryos. Importantly, because the urchin is a deuterostome displaying regulative development, it has two key features that make this simple embryo most similar to more complex vertebrate embryos. The sea urchin genome has been sequenced and is now being annotated, and a gene regulatory network model has been established for the maternal specification of the urchin endomesoderm, providing the molecular framework underlying primary axis and concomitant germ layer specification [1]. This simple model system has great potential to quickly reveal the mechanistic underpinnings of basic developmental processes that are much less tractable in more complex models.

Past accomplishments

Graduate work. My graduate studies in the Brenner lab focused on biochemical aspects of signal transduction. I studied the response of the mammalian liver to transplantation using in vivo model systems manipulated by gene therapy. My publications from this period address oxidative stress, proliferation, and apoptosis as regulated by TNFα signaling. I found that both JNK and NF-κB are activated following liver transplantation [3], and that NF-κB is protective, preventing apoptosis [4], while JNK is pro-proliferative, inducing a regenerative response [5]. In addition, I found that the mitochondrial release of cytochrome c is an essential component of apoptotic signaling, rather than a consequence of apoptosis [6]. Although not developmentally-oriented, these studies have provided me with a strong background for studying signal transduction pathways and their subsequent cell biological effects using in vivo models.

Post-doctoral work. In the McClay lab, I found that PI3K is required in the primary mesenchyme for larval skeletogenesis [7]. I also discovered that asymmetrical activatation of p38 MAPK is earliest known step in secondary axis specification, which is accomplished through the sequential activation of

Nodal and Goosecoid (Gsc) [2]. This finding was surprising, since axial specification in response to an asymmetrically nuclearized kinase is unprecedented. In addition, I have shown that several distinct pathways are induced by p38 to specify subterritories along the O/A axis, and to express the instructive signals that result in mesodermal patterning. These studies are outside the main focus of the McClay lab, and will thus form the basis of my research as a new faculty member.

Future Directions

1. Neural Specification. My results show that Chordin is expressed downstream from p38 and Nodal. Chordin is restricted to the oral territory, but is not required for oral specification; instead it is required for specification of neurons in the ajacent ciliary band territory (Bradham, et al., in preparation). This result is surprising because in vertebrates, Chordin is important both for axial and neural specification [8]. Chordin is a well-established inhibitor of BMP, which signals through SMAD proteins and is also expressed orally. To understand the signaling dynamics of the interaction between Chordin and BMP, both of which are secreted proteins, I will assess SMAD activity in live embryos using GFP-tagged SMAD proteins. These and additional results will extend my network model in addition to having implications for the evolutionary relationship between the specification of neurons and the secondary axis.

2. Cell-Cell Signaling and Patterning. The larval skeleton is secreted by the primary mesenchyme cells (PMCs), but the skeletal pattern is dictated by unknown cues in the ectoderm that are detected by thin filopodia extended from the PMCs [9, 10] (Fig. 3). Using skeletal pattern as a readout (Fig. 4), I devised an assay [1] which I used to show that patterning requires Otp, a transcription factor that is expressed downstream of p38 and Gsc in a highly restricted spatial domain in the ectoderm (Bradham and McClay, in preparation). The patterning genes are therefore likely to be driven by Otp, and so I will identify Otp targets using genomic and array-based approaches. Identification of ectodermal patterning proteins will then allow the identification of their mesenchymal partners. Discovery of the apparatus for the communication of patterning instructions from the ectoderm to the PMCs will provide a molecular model for information conveyance by filopodial contacts. Recent studies have identified a critical role for such cytoplasmic extensions in long range patterning and morphogenetic processes in systems as diverse as the Drosphila wing disc, epithelial sheet closure, and axonal pathfinding [11-13]. Thus, such findings would be of broad significance to the larger developmental community.

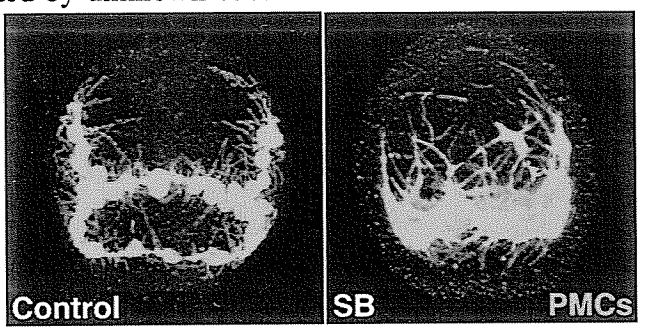

Fig. 3 Filopodia are extended from the PMCs in control late gastrula embryos, and are perturbed in p38-inhibited embryos (SB).

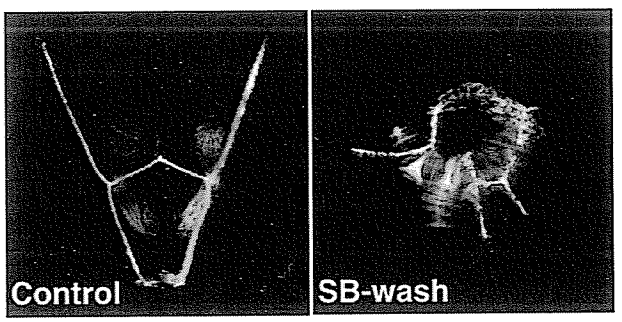

Fig. 4 Skeletal pattern, illuminated with plane-polarized light, is normal in control larvae, and perturbed when p38 activity is temporarily inhibited (SB-wash).

3. Symmetry breaking. Mitochondria are physically distributed in an asymmetrical manner prior to fertilization, and this asymmetry is maintained throughout cleavage in sea urchin embryo. I have found

that the asymmetric mitochondria correspond spatially with active p38 (Fig. 5) and indeed are required for the asymmetric activation of p38, which is known to respond to mitochondrially-derived reactive oxygen species [14, 15]. This is a critical specification event, responsible for driving all the downstream pathways discussed in herein (Fig. 2). My graduate work with mitochondria [6] will facilitate the further exploration of this process. For example, I will perform gain of function experiments by asymmetric delivery of reactive oxygen species to the mitochondrial-sparce side of the embryo. Completion of these studies will determine whether the asymmetrical distribution of organelles provides a novel mechanism for symmetry breaking during axis specification.

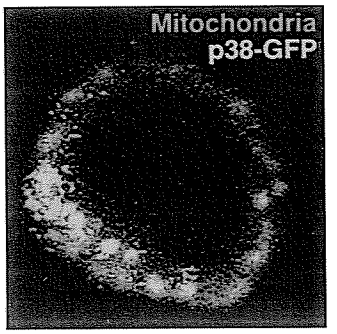

Fig. 5. Mitochondria correspond spatially with asymmetrically active p38. Here, the mitochondria are labeled with MitoFluor Red.

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Teaching Interests **Cynthia Bradham**

I enjoy teaching a great deal, and am looking forward to the opportunity to teach in a faculty position. I worked as a tutor during four semesters of my undergraduate years, teaching various chemistry courses (general, analytical, organic, biochem). I was employed by the Department of Learning Skills, which provided free tutors to students in need. In that capacity, I taught small groups that ranged from one on one, to a section of 27 students, and worked with more than 200 students overall. I won awards for best new tutor and outstanding tutorial service, based on student reviews. I think that successful teaching relies on two main aspects: the ability to communicate clearly, and enthusiasm for the topic. In my tutoring years, I worked frequently with students who were failing the courses, and the most important thing for turning the majority of them around was instilling some degree of enthusiasm for chemistry and science in general.

As a graduate student, I worked as a TA for several semesters, teaching biochemistry for nursing students, nutrition for dental hygenists, and finally cell biology. I enjoyed leading discussions and working with the students to clarify difficult points and generate study summaries to help with exam preparation.

I have also had a number of training and mentoring experiences in the lab, both as a graduate student, and as a postdoctoral fellow (7 undergraduates, of whom 4 decided to pursue graduate studies, in addition to a large number of M.D. fellows and technicians). I have thoroughly enjoyed introducing students to benchwork and a practical approach to science, and look forward to working with both undergraduate and graduate students in the lab.

In the summer of 2005, I taught a Cell and Developmental Biology, an advanced undergraduate course in the Biology Department at Duke. Again, I enjoyed the experience and have gained an appreciation for what is involved in teaching at the faculty level. I am excited by that prospect and look forward to creating challenging and interesting courses for undergraduate and graduate students. I would enjoy teaching an introductory biology, cell biology, or developmental biology course for undergraduates. I would also enjoy teaching a graduate course focusing on signal transduction during embryonic development. Eventually I would like to lead seminar courses in developmental topics.

As a graduate student, I was required to take 2 courses, one on grant writing, and the other on giving presentations. I have been grateful for that instruction ever since, and would be pleased to help with similar pre-exisiting courses or to create new ones.