Washington
WASHINGTON·UNIVERSITY·IN·ST·LOUIS
School of Medicine

Sujin Bao, Ph.D.
Department of Molecular Biology & Pharmacology
Washington University School of Medicine
Campus Box 8103, 660 South Euclid Ave.
St. Louis, MO 63110
Phone: (314) 747-3998
Fax: (314) 362-7058
E-mail: sbao@wustl.edu

Dr. Yves Brun
Systems Biology/Microbiology Faculty Search
Department of Biology
Indiana University, Jordan Hall 142
1001 E 3rd Street
Bloomington, IN 47405-7005

November 11, 2005

Dear Dr. Brun,

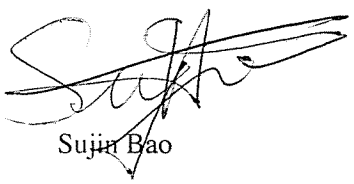
Please accept this letter and the enclosed documents as my application for the position of an assistant professor in the Department of Biology, as advertised at *Science*. Currently I am a post-doctoral fellow in the Department of Molecular Biology and Pharmacology at Washington University, working on cell adhesion and morphogenesis. Thus cell differentiation and developmental biology match my research interests.

The goal of my research is to understand how cell shapes and cell movements are regulated during development and how they contribute to organizing individual cells into the precise patterns required to build elaborate tissues and organs. I use the fruit fly *Drosophila* as a model organism. In the *Drosophila* eye, I found that heterophilic interactions between two cell adhesion molecules are essential for directing patterning of the retinal epithelium. Based on this work, I proposed a "Preferential Adhesion Model" as a general mechanism for patterning more sophisticated epithelia; this work is published in *Developmental Cell*, Vol. 6, 2005. In addition, I have several other interesting observations, and I am currently pursuing the mechanisms behind them. First, I have found that interactions between immunoglobulin superfamily members can promote junction formation, and I want to understand how. Second, cell-cell adhesion mediates cell competition and the levels of cell adhesion molecules can determine the survival/death fate decision. I want to address what the link is between cell adhesion and cell survival. Third, *Notch* signaling, better known for its regulation of cell fate, can also regulate cell adhesion during morphogenesis. Is this regulation direct or indirect? Can cell adhesion be translated into cell fate? My larger goal is to use these specific questions as stepping-stones to the larger issue of how cell adhesion and the cytoskeleton are coordinated to control morphogenesis. The details of my future research are in the attached Research Plan.

Your announcement points to a need for teaching. As described in the enclosed Statement of Teaching Interests, my teaching skills were first developed when I was a teaching assistant at Tsinghua University. These skills were fully applied when I was an associate professor in the Chinese Academy of Sciences and further developed at Washington University where I acted as a mentor for Ph.D. student rotation projects. My teaching philosophy emphasizes the mutual communication between the teacher and students. Based on students' feedback, my teaching was effective and enjoyable. And cell biology, developmental biology and biochemistry are my favorite teaching topics.

I am quite excited about the possibilities at Indiana University; its strong history of research would be an excellent match for my own goals. In return, I would bring the broad training to address a series of issues that are fundamental to both development and disease. I have enclosed my CV, Research Plan, Statement of Teaching Interests and one recent paper. The names of four references are listed in the next page. If you have any question concerning my application and my qualifications, please feel free to contact me. Thank you!

Sincerely yours,



Sujin Bao

Overview

I am interested in understanding how cell shapes and cell movements are regulated during development and how they contribute to organizing individual cells into elaborate biological structures. I use the fruit fly *Drosophila* as a model organism, and forward genetics combined with proteomics as my experimental approach to identify genes that are important for morphogenesis. In particular, I focus on *Drosophila* eye development.

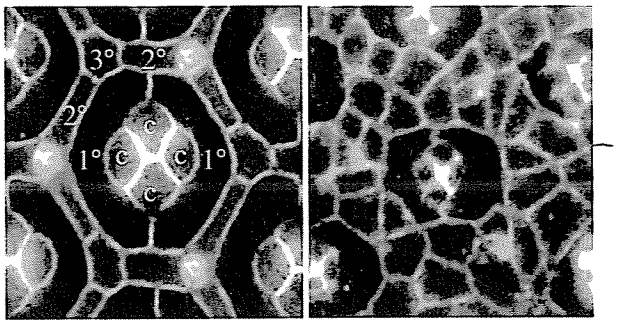


Figure 1. *Left*: The Drosophila pupal eye at 42 hrs. Interommatidial cells (shaded green) surround ommatidia (uncolored). c, cone cell; 1°/2°/3°, primary/secondary/tertiary pigment cell. *Right*: At 20 hrs, interommatidial precursor cells are unpatterned.

The simplicity of the developing *Drosophila* eye makes it an ideal model for studying morphogenesis. In the young pupal eye, the pool of interommatidial precursor cells (IPCs) rearrange into a remarkably precise hexagonal pattern that spans and organizes the retina (Figure 1). As a post-doctoral fellow, I have focused on understanding how a loose collection of cells is organized into interweaving precise hexagons in the pupal eye. A major finding of my work is that two heterophilic interacting cell adhesion molecules mediate formation of this precise hexagonal pattern. My work provides evidence that this process follows basic principles of physics.

Post-doctoral Research

My previous training in molecular biophysics has given me extensive experience in biochemistry, especially protein chemistry. As a post-doctoral fellow, I have combined this expertise with a strong grounding in genetics to explore morphogenesis and patterning from a unique perspective.

Cell adhesion and morphogenesis

Animals are composed of a remarkably broad array of different structures. As D'Arcy Thompson pointed out nearly a century ago, this richness likely arises from manipulation of a few basic patterning rules. We now understand this to be true, although how individual cells are organized into functional biological structures is still poorly understood. Cell signaling networks are known to regulate cell shapes and cell movements through cytoskeletal regulation. On the other hand, data from the dissociation and re-aggregation of embryonic cells suggests that cells can be organized by mechanisms that follow the principles of physics. For example, in the 1930s and 1940s, J. Holtfreter demonstrated that dissociated amphibian embryonic cells could re-aggregate and sort out from one another to reconstruct the tissues of origin, often arranged in their proper anatomical relationships, suggesting that different cell types possess differential affinity for each other. Similar re-aggregation studies led M. Steinberg to put forward the "differential adhesion hypothesis", which proposed that the sorting out of intermixed embryonic cells and envelopment of one embryonic tissue by another are driven by tissue interfacial free energies arising from differences in cell surface adhesive properties. However, there is very limited experimental data on the links between physical principles and patterning.

Hibris and Roughest are cell adhesion molecules in the immunoglobulin superfamily. In Ross Cagan's laboratory at Washington University, I found that Hibris and Roughest bind directly *in vivo*, acting as essential mediators of cell movements in the eye. Hibris is expressed in primary cells (1°s) and Roughest is partitioned to interommatidial precursor cells ('IPCs'); the result is that IPCs are 'pulled' into their proper niches. My work provided evidence that patterning is a natural outcome of minimization of free energy mediated by Hibris-Roughest affinity (Figure 2). Inspired by the pioneering ideas of M. Steinberg's "differential adhesion hypothesis", I proposed a "Preferential Adhesion Model" to better explain patterning of these cells (Bao and Cagan, 2005: *Dev. Cell* 8, 925-935). This model addresses how partitioning heterophilic adhesion molecules can serve as a powerful mechanism for driving fine patterning.

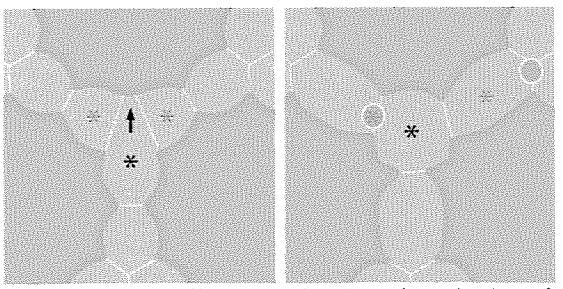


Figure 2. *Left*: Cells expressing Roughest (tan) push between neighbors to maximize contact with ommatidia expressing Hibris (green). *Right*: This competition promotes fine patterning.

Aside from exploring their ability to direct fine patterning, I also found that Hibris and Roughest promote junction formation and can regulate targeted programmed cell death (Bao and Cagan, 2005: *Dev. Cell* 8, 925-935). As discussed below in Future plans, I will continue to explore the mechanisms behind these observations in my own laboratory.

In addition, I have also investigated the role of programmed cell death in morphogenesis during eye development (Bao and Cagan, 2003: in *Essentials of apoptosis: A Guide for Basic and Clinical Research* pp145-161). In collaboration with Omar Jassim and Julia Cordero, we observed an early wave of cell death in the pupal eye that required Wnt/Wingless signaling (Cordero et al, 2004: *Mech Dev* 121, 1523-30).

Notch signaling and morphogenesis

Notch signaling is an evolutionarily conserved, intercellular signaling pathway essential for proper embryonic development. The Notch receptor is a single-pass transmembrane protein. Upon ligand binding, the intracellular domain of Notch is cleaved and translocated into the nucleus to activate *Notch* target genes. Due to a broad requirement for *Notch* signaling during development, it is not surprising that perturbations in the *Notch* signaling pathway lead to the pathogenesis of several human diseases such as leukemia and Alagille syndrome (reviewed in T. Gridley, 2003: *Hum. Mol. Gen.* 12, R9-13). However, we do not really understand how a single signaling pathway can have such profound effects on so many developmental processes. *Drosophila* has often been the model system of choice for studying *Notch* signaling during animal development. In the *Drosophila* eye, *Notch* is required for successive cell decisions (Cagan and Ready, 1989: *Gene Dev* 3, 1099-1112). For example, cell fate specification of the (glial-like) cone cells requires signaling inputs from *Notch* (Flores et al, 2000: *Cell* 103, 75-85); by contrast, *Notch* activity is also required for other cells ('IPCs'; see below) to physically move within the epithelial plane during the same developmental stage (Cagan and Ready, 1989: *Gene Dev* 3, 1099-1112). The mechanism underlying this latter process is unclear.

I am interested in understanding how Notch regulates morphogenesis during development. In the pupal eye, the ligand Delta (Dl) is expressed in photoreceptors and cone cells while the Notch receptor itself is expressed dynamically in all cell types (Kooh et al, 1993: *Development* 117, 493-507; Parks et al, 1995: *Mech Dev* 50, 201-216). *Notch* activity is essential for the morphogenetic process of interommatidial precursor cells (IPCs). Reducing *Notch* activity led to (i) loss of primaries and (ii) loss of Hibris expression; the result is a disrupted interommatidial pattern. Conversely, increasing levels of *Notch* activity in primaries yielded elevated levels of Hibris and ectopic activation of *Notch* induces ectopic Hibris expression in cells that do not normally express this protein. That is, *Notch* signaling is both sufficient and necessary for the expression of the cell adhesion molecule Hibris, which in turn is required for normal cell-cell adhesion and patterning. Interestingly, replacing *Notch* by Hibris is sufficient to direct cells into the primary fate, as assessed by morphology (Figure 3). I am currently examining primary-specific molecular markers to determine if adhesion can be translated into cell fate. Thus, my data suggest that *Notch* can regulate adhesion through its regulation of Hibris, an adhesion molecule that then directs patterning of the neighboring IPCs. My work links *Notch* activity to cell adhesion, a novel activity for *Notch*, and postulates a novel method for *Notch* to regulate cell fate through activating expression of the cell adhesion molecule.

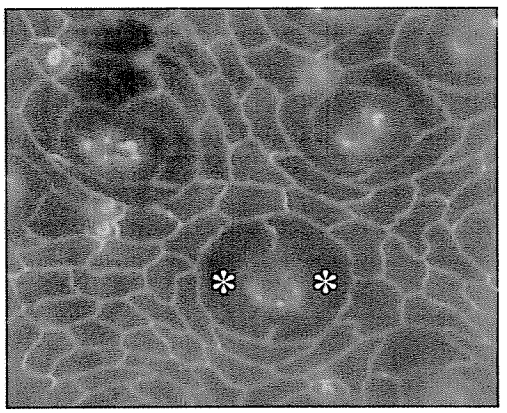


Figure 3. *Notch* regulates cell adhesion. In a *Notch* mutant eye, primary cells do not form properly. When Hibris is introduced into two cells (*), these cells now are able to enwrap cone cells as primary cells.

Future Plans

The epithelium comprises a single layer of epithelial cells. In order to function as a tissue, epithelial cells must have the right shape and structure to pack together with neighbors. In fact, epithelial cells in multiple-cell organisms recognize their neighbors, adhere to them and form intercellular junctions. Cell junctions play an essential role in various cellular processes including mediating cell shape change, cell movement, migration, proliferation and cell survival. Among junctional complexes, adherens junctions play a key role in maintaining epithelial integrity. Complete disassembly of adherens junctions result in a loss of the polarized, poorly motile epithelial characteristics and acquisition of a migratory phenotype as seen in metastasis in cancer. However, very little is known about how adherens junctions are regulated.

My previous work indicates that binding between cell adhesion molecules Hibris and Roughest promotes formation of cell junctions (Bao and Cagan, 2005: *Dev. Cell* 8, 925-935). In my own laboratory, the first question I will try to address is how cell-cell adhesion modulates cell junctions. I will also address two related questions: how are cell adhesion molecules transcriptionally regulated, and how does cell-cell adhesion affect cell survival?

1. Cell-cell adhesion and the regulation of dynamic cell junctions

To explore the mechanistic basis through which Hibris and Roughest stabilize junctions, I will identify proteins that physically associate with Hibris/Roughest. My goal is to understand the molecular basis through which cell-cell adhesion is linked to cell junctions and provide insights into how this process regulates cell shapes and cell movements.

i) Proteomics: To identify members of the Hibris/Roughest complex, Hibris/Roughest proteins will be subjected to immunoprecipitation, initially from the embryo and the pupal eye. Samples will be subjected to proteomics analysis. An important advantage of this approach is the identification of interacting proteins precipitated directly from the tissues in which they are active. As a backup, I will consider two approaches: 1) yeast two-hybrid screening and 2) the split luciferase assay (Luker et al, 2004: *Proc Natl Acad Sci USA* 101, 12288-93).

ii) Genetic modifier screen: Genetic modifier screens are powerful tools for identifying functional interactions. Hibris is normally not expressed in interommatidial precursor cells (IPCs). When hibris is mis-expressed in IPCs, ectopic junctions form between IPCs and the hexagonal pattern of the eye is disrupted, leading to a "rough eye" phenotype (Bao and Cagan, 2005: *Dev. Cell* 8, 925-935). I will use this phenotype to screen for genetic modifiers that worsen (enhance) or ameliorate (suppress) this defect.

Candidate genes identified by using the above approaches will then be subjected to further phenotypic and genetic interaction analysis. While the details of analysis will depend on the specific loci identified, I have received extensive training in these techniques in the Cagan laboratory and will bring my analysis to single-cell resolution. This will include immunohistochemistry, live imaging, and standard biochemical analysis. I will also emphasize establishing a cell culture model to complement my *in vivo* work.

2. Signaling circuitry and the regulation of cell adhesion molecules

Fine regulation of cell adhesion molecules can play a central role in regulating changes in cell shape and cell movements that direct development; my work has emphasized the importance of regulating their expression dynamically. My preliminary data suggest at least two signaling pathways are directly involved in this process.

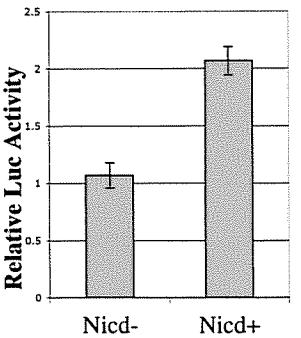


Figure 4. Activated Notch (Nicd) promotes expression of a *hibris-luc* reporter *in vitro*.

2a. Notch signaling pathway:

Notch signaling has been implicated in a broad array of developmental processes. During cell movements in the pupal eye, *Notch* is required for correct localization of Roughest protein. However, it remains unclear whether this is due to a role of *Notch* in regulating cell fate or a potentially new role for Notch: adhesion. My preliminary data indicates that *Notch* regulates adhesion by activating *hibris* expression. In addition, I have preliminary data suggesting that *Notch* signaling directly targets *hibris* transcription. To further address this issue, I will refine my work to isolate *hibris* regulatory

fragments and assess the ability of Notch/Su(H) to directly regulate these fragments *in vitro* and *in vivo*. An example of *Notch* activation of an upstream *hibris* fragment is shown in Figure 4.

2b. EGFR signaling pathway:

Similar to *Notch*, the *EGF receptor (EGFR)* signaling pathway is required reiteratively during *Drosophila* eye development. In the pupal eye, *EGFR* expression overlaps *roughest* expression. Recently, a *roughest* enhancer was isolated by Karl Fischbach's laboratory; interestingly, it contains a binding site for Pointed, the transcription activator for *EGFR* signaling. Thus, *roughest* may provide an interesting connection between *Notch* and *EGFR* signaling. I will assess the role of *EGFR* on *roughest* expression by the methods discussed above.

3. Cell-cell adhesion and cell survival

Programmed cell death is an important component of development. Misregulation of programmed cell death has serious consequences in animal development as well as in disease such as tumor growth. Although it has been proposed that cell signaling plays an important role in regulating cell death, it is not clear how some cells are removed while their neighbors are spared. One strong possibility is that competition between cells drives cell death. But what is the nature of this competition? My work suggests a model in which levels of Roughest in individual retinal cells determine their capacity to compete for survival (Bao and Cagan, 2005: *Dev. Cell* 8, 925-935). And the Drosophila eye provides a unique opportunity to explore it in detail: cells are removed to refine the hexagonal pattern (Figure 5).

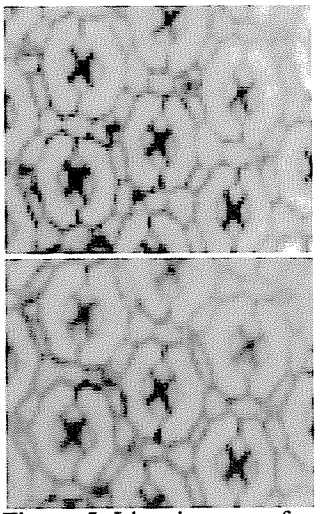


Figure 5. Live images of a developing eye, 90' apart. Cells in red are removed by death to refine the pattern.

One hypothesis is that differences in the levels of Roughest affect the ability of cells to establish junctions with neighboring ommatidial cells; this may in turn lead to removal of cells with lower levels of Roughest. I will test this hypothesis by manipulating the level of junctional proteins (e.g., E-cadherin) at single cells to assess whether this will affect the outcome of cell competition in comparison with the effects of manipulating growth regulators (e.g., *myc*). In the long run, I am also interested in examining whether regulation of cell survival/cell death by cell-cell adhesion is a broadly utilized mechanism underlying organ size control.

4. Broader impact of my work: the role of cell-cell adhesion in shaping the developing mouse kidney

Accumulating evidence indicates mechanisms for regulating cell shape and cell movements are largely conserved from fly to mammals. Interestingly, genes essential for patterning the Drosophila eye are also found to function in shaping the mouse kidney, e.g., *Nephrin, Nephl, Eya, WT1,* and *Pax2*. Nephrin and Nephl are mammalian homologs of Hibris and Roughest. Disruption of either Nephrin or Nephl results in failure of forming the filtration barrier in the kidney – the slit diaphragm, leading to leak of proteins into urine. However, the mechanisms by which Nephrin and Nephl contribute to the formation of the slit diaphragm and how these cell adhesion molecules are regulated during development are poorly understood. Recently, mammalian *Notch-2* has been implicated in the kidney development, raising the possibility that Nephrin and *Notch* signaling are functionally linked during kidney development. To test this hypothesis, in collaboration with Raphael Kopan, we are exploring the role of *Notch* signaling in kidney development using (i) DNA microarray to identify *Notch* target genes and (ii) immunohistochemistry together with *in situ* hybridization to examine Nephrin expression when *Notch* signaling is inhibited. While working on mouse kidney development is outside the initial focus of my laboratory, one long-term goal would be to collaborate with other laboratories to determine whether results from my laboratory's fly work can inform issues of mammalian kidney development.

Summary

The specific goal of these experimental programs is to understand how cell-cell adhesion modulates junctions and how it is developmentally regulated. My larger goal is to understand how cell adhesion and the cytoskeleton are coordinated to control morphogenesis and patterning during development and the impact of aberrations on diseases.

I enjoy teaching. I find that teaching can help maturate one's knowledge and thinking. And more importantly, my experience tells me that good ideas can emerge from direct interactions with students during teaching.

When I was an undergraduate student at Dalian University of Technology, I participated in a volunteer group to help freshmen study chemistry, physics and mathematics. Twice a week, I explained to them some theories taught in class, helped them solve some difficult problems and answered their questions. The positive feedback from students made me enjoy teaching and prompted me to seek more efficient ways to interact with students. Due to my interactions with other students and my performance in my own class, I won the First-Class Scholarship (1986, 1987 and 1988), the most prestigious honor for undergraduate students at Dalian University of Technology.

When I joined the Master's program at Tsinghua University, I was a teaching assistant for both a Thermodynamics class and a Principles of Heat and Mass Transfer class in the Department of Thermal Engineering. I took charge of monthly 2-class exercise sessions in which I explained to students the most common mistakes from their homework, which I had evaluated, and answered their questions. Due to my enthusiasm and efforts, together with my service in my own class, I won the Jiang NanXiang fellowship (1991), one of the most prestigious honors for graduate students at Tsinghua University. During this period of time, I began developing my own teaching skills: facing students, looking into their eyes, and keeping class interactive through stories and questions from time to time.

As an associate professor in the Chinese Academy of Sciences, I taught undergraduates eight-class lectures of Protein Crystallography in the Department of Biological Science and Biotechnology at Tsinghua University. My teaching skills developed earlier were fully practiced. Based on students' feedback, my teaching was effective and enjoyable.

At Washington University, I have been a mentor for Ph.D. student rotation projects. For Ph.D. students, I developed a different strategy. First, I trained students to be excellent technicians. For some special techniques used in *Drosophila*, e.g., eye dissection, students were asked to go through a certain amount of practice. They needed to show their good performance before moving to the next new technique. Second, I trained them to be good students. I encouraged them to use more than one approach to deal with each task, and I expected them to find the best approach after each experiment. And the best approaches were compiled, shared and updated frequently. Third, I trained them to be good thinkers. I encouraged them to read extensively and raise questions. I organized regular discussions. And particularly, I encouraged everyone to bring hypotheses. So far, two students I have mentored are very active in the laboratory and they work very efficiently.

My current and future research interests are directed to cell adhesion and morphogenesis. Thus, cell biology, developmental biology and biochemistry are my favorite teaching topics for undergraduates or graduates. I will use class as an opportunity for me to apply my teaching skills and interact with students. I also believe my own teaching skills will be further developed through the classes.

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Department of Molecular Biology & Pharmacology
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Campus Box 8103, 660 South Euclid Ave.
St. Louis, MO 63110
Phone: (314) 747-3998
Fax: (314) 362-7058
E-mail: sbao@wustl.edu

Enc.)

Four references

Reference 1:
Ross Cagan, Ph.D. and Professor
Department of Molecular Biology and Pharmacology
Washington University School of Medicine
Campus Box 8103, 660 South Euclid Ave
St. Louis, MO 63110, USA
Phone: (314) 362-7796
Email: cagan@wustl.edu

Reference 2:
David M Ornitz, Ph.D., Professor and head
Department of Molecular Biology and Pharmacology
Washington University School of Medicine
Campus Box 8103, 660 South Euclid Ave
St. Louis, MO 63110, USA
Phone: (314) 362-3908
email: DORNITZ@WUSTL.EDU

Reference 3:
Richard W. Carthew, Ph.D.
Owen L. Coon Professor of Molecular Biology
Department of Biochemistry, Molecular Biology and Cell Biology
Northwestern University
2205 Tech Drive
Evanston, IL 60208
(847) 467-4891
Email: r-carthew@northwestern.edu

Reference 4:
Karl-Friedrich Fischbach, Professor and Dr.
Institut für Biologie III
Schänzlestr.1
D-79104, Freiburg
Germany
Telephone: +49-761-203-2730
Fax: +49-761-203-2866
Email: kff@zum.de
