September 8, 2005

Dr. Yves Brun
Systems Biology Faculty Search
Department of Biology
Indiana University
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Dear Faculty Search Committee members,

Enclosed you will find my application materials for an assistant professor position at the Department of Biology, Indiana University. I am currently a Howard Hughes Medical Institute associate in Joanne Chory's lab at the Salk Institute for Biological Studies.

My current research focuses on understanding the biochemical and genetic mechanisms of the brassinosteroid (BR) perception and signal transduction in plant growth and development. In the long term, I plan to investigate the signaling networks of BRs and other signals through cell surface receptors by using approaches from various disciplines including genetics, biochemistry, and molecular and cellular biology, genomics, and proteomics.

Prior to joining the Chory lab, I received intensive training for a PhD degree in plant biochemistry and genetics in Dr. Brian Larkins' lab at the Department of Plant Science, the University of Arizona, and gained strong experience on amino acid metabolism, and enzyme purification and characterization. I believe that my training in these diverse fields enables me to approach fundamental biological questions from a somewhat different perspective.

I am ready to start a career as an independent researcher and educator. I have extensive experience in supervising undergraduate and graduate students, and I believe that my supervising and overall communication abilities have also been greatly enhanced through both my interactions with and my observations of my former and current research advisors, Dr. Brian Larkins and Dr. Joanne Chory.

I have enclosed a copy of my CV and research and teaching interests. Letters of recommendation are available from Drs. Joanne Chory, Tony Hunter, Joe Ecker, and Brian Larkins upon request. If you need my additional application documents or have any questions, please do not hesitate to contact me. I am looking forward to hearing from you.

Sincerely,

Xuelu Wang, Ph.D.
Plant Molecular & Cellular Biology Laboratory
The Salk Institute for Biological Studies

# RESEARCH INTERESTS AND FUTURE PLANS

As sessile organisms, plants response to numerous external and internal signals, and alter their development to adapt to prevailing conditions appropriately. Brassinosteroids (BRs) are a class of steroid hormones uniquely in plant kingdom and control a remarkable array of critical physiological processes, which include the stem elongation, seed size, fertility, flowering time, and resistance to biotic and abiotic stresses. BRs are perceived by BRI1, a cell-surface leucine-rich-repeat receptor-like kinase (LRR-RLK). Activation of BRI1 by BR binding initiates a signaling cascade, which ultimately regulates the expression of hundreds of genes. However, many fundamental mechanistic questions remain unclear in the BR perception and signaling pathway. Currently, I have been particularly interested in answering two questions: (1) what is the biochemical and molecular basis of BRI1 activation? (2) what are the signaling components between BRI1 and its downstream nuclear components? My short-term research focuses are to continue the projects initiated in the current lab to understand the underlying molecular and structural mechanisms of BRI1 activation and the BR signal transduction. My long-term goal is to understand how diverse signals, specifically abiotic stresses interact with BRs to control plant growth and development.

## Previous Accomplishments and Current Research

### I. The activation mechanism of BRI1

I have established a convenient ligand-receptor-bioassay system to determine the activity and physiological effects of BRI1 in the reference plant, *Arabidopsis*. Biochemical analysis of mutated BRI1 kinase *in vitro* and *in vivo*, and phenotypic analysis of transgenic *Arabidopsis* in BR-deficient and BR-perception mutant backgrounds with BRI1 variants revealed that phosphorylation of several residues in the C-terminal domain plays an essential role in activating BRI1. Furthermore, several lines of biochemical and genetic evidence supported that a preformed homo-oligomer is likely involved in the activation of BRI1, and BAK1 is not required for BL-binding to BRI1. These discoveries have led to a fascinating model to illustrate the molecular and biochemical mechanism of BRI1 activation, which should be broadly applicable for analysis of the hundreds of receptor-like kinases in plants (*Wang et al. 2005, Dev. Cell*).

### II. Filling in the gaps in the brassinosteroid signaling pathway

Although several major components in the BR signaling pathway have recently been identified, the links between the BR receptor BRI1 and nuclear components remain a big mystery. I have identified several BRI1-interacting proteins using yeast two-hybrid screens. One of them, called BKI1 (BRI1 Kinase Inhibitor 1), has been characterized at the genetic, biochemical, and molecular level. BKI1 is a novel protein localized to the plasma membrane (PM) and cytoplasm. Its over-expression leads to a dwarf phenotype and significantly inhibits BR signaling. Remarkably, the PM-localization of BKI1-YFP is abolished by the BL treatment in the wildtype but not in a *bri1* null background. Phosphorylation assay by BRI1 kinase indicated that BKI1 is a good substrate of BRI1, and apparently their interaction resulted in an inhibition of BRI1 activity. These findings strongly suggest that BKI1 is a negative regulator specific for BRI1 and provides a novel level of regulation on this receptor kinase, and this work will be submitted for publication very soon (*Wang et al., in preparation*).

## Future Research Plans

### I. The structural and functional study of LRR-RLKs

**A.** *Structural and functional studies of the kinase domains of BRI1 and its homologs.* Our studies on the molecular mechanisms of BRI1 activation have put me in a unique position

to fully understand this process by solving its 3-D structure. In collaboration with Professor Joe Noel at the Salk Institute, I have been expressing and purifying several sets of recombinant proteins, and try to crystallize some of them and solve their 3-D structures. A structural modeling of the BRI1 kinase domain suggested that the C-terminus forms a α-helix and may fold back to contact with the active site to inhibit the kinase activity. The structural study proposed here will provide direct evidence of how the autoinhibitory conformation of BRI1 is maintained by this domain and by other domains, and how phosphorylated residues regulate the conformational change of BRI1 kinase.

**B.** *Identification of novel alleles of BRI1.* Besides the kinase and island domains, many other regions of BRI1 are also critical for BRI1 function. Our previous investigation indicates that the extracellular and transmembrane domains are possibly involved in the dimerization of BRI1 (*Wang et al., 2005, Dev. Cell*). I plan to employ the *Arabidopsis* Tilling project (*Till et al., 2003*) to rapidly identify additional *bri1* alleles in these unexplored regions, and this approach also has the virtue to identify both loss-of-function and gain-of-function alleles. Mutations showing altered BR-related phenotypes will be further characterized to understand their structural basis. For instance, the mutated proteins will be functionally analyzed *in vitro* and in transgenic *Arabidopsis* with our established ligand-receptor-biossay system.

**C.** *Understanding the desensitization mechanism of BRI1.* Phosphorylation is a major mechanism to reversibly control the function of proteins in signal tranduction. We have demonstrated that the ligand-induced BRI1 phosphorylation plays a key role in BRI1 activation. Therefore, I hypothesize that dephosphorylation of BRI1 is likely required for its inactivation or desensitization. In the *Arabidopsis* genome, there are around 100 predicted protein phosphatases, and most of them are Ser/Thr phosphatases. It is possible that some of these play a role in the desensitization of BRI1. I plan to test their interaction with both wild type and kinase-inactive BRI1 kinase domain in yeast using the Univector Plasmid-fusion System (UPS) (*Liu et al., 1998, Current Biology*), which allows a systematical and automated way (28 of them have pUni clones available from the *Arabidopsis* Biological Resource Center (ABRC)). For those protein phosphatases giving positive results, their roles in the BRI1 signaling will be further investigated *in vitro* and in planta.

## II. The regulatory mechanism of BKI1's role in BR signaling and in plant development

Another major focus of my future plans is to unveil the underlying biochemical, molecular, and cellular mechanisms of BKI1 in plant development and in the BR signaling. Our studies have suggested that BKI1 is an inhibitor of the receptor kinase BRI1. Several critical questions related to BKI1's function remain to be answered: what developmental cues or proteins participate in its regulation? Whether BKI1 is involved in the regulation of other signaling cascades? What's the structural basis for the inhibition of BRI1 by BKI1? In addition, BKI1 provides a great entry point to identify novel components in the BR signaling pathway.

**A.** *Suppressor and enhancer screening of BKI1 over-expression lines.* Over-expression of BKI1-YFP led to a semi-dwarf phenotype. Screening mutants to suppress or enhance its dwarfism might lead to the identification of loci regulating the activity of BKI1 and/or playing roles in the BR signaling. To obtain both loss-of-function and gain-of-function mutations, I am utilizing two ways to induce mutations: one is the EMS-treatment; the other is the activation tagging. The isolated putative suppressors and enhancers could be classified into different categories based on the subcellular localization of BKI1-YFP and the BES1 phosphorylation status, which act as powerful cellular imaging and biochemical markers of BR signaling, respectively. This also helps me to determine whether the mutated genes are involved in the early or late events of the BR signaling cascades, or in other signaling pathways.

**B.** *Identification and characterization of novel BKI1 interacting proteins.* Given that a small domain of BKI1 is necessary and sufficient for BRI1 interaction, the other domains of

BKI1 may have specific unknown functions by interacting with other proteins. I have been performing yeast two-hybrid screens using BKI1 as a bait and cDNA libraries from different sources to rapidly identify its interacting candidates. In addition, the established transgenic lines with BKI1-FLAG and BKI1-YFP have given me the opportunity to explore whether BKI1 complex is present, and if it is, whether it is regulated by BL. Then a proteomic approach will be employed to identify the components in the complex by SDS-PAGE and mass spectrometry.

**C.** *Structural and functional study of BKI1.* BKI1 can be phosphorylated by BRI1 kinase. I will identify these phosphorylation sites and mutate these sites to understand how their phosphorylation regulates BKI1's subcellular localization and function. Together with the structural study of BRI1 kinase domain, the recombinant proteins of the full-length and the BRI1-interacting domain of BKI1 will be expressed and purified in a large scale, and could be crystallized in the absence or presence of the BRI1 kinase domain. Then the corresponding 3-D structure could be solved. This study will provide the structural evidence of how BKI1 contacts with BRI1 and inhibits its activity.

**III. The signaling networks between BRs and other signals**

In the long term, I am interested in elucidating the genetic, biochemical, molecular, and cellular mechanisms of the interaction between BRs and various signals, particularly abiotic stresses. It is known that the application of BRs increases the resistance of plants to many abiotic stresses, and the stimulatory effects of BRs on plant growth are more obvious under stressful conditions than under optimal growing conditions (*Krishna, 2003*). However, we know little how plants coordinate growth-stimulatory signals, such as BRs, with growth-inhibitory signals, such as abiotic stresses, to precisely control their growth and development. It is logical to expect that these signals may undergo interactions at different levels. First, they may interact through the early signaling events proximal to the PM. For instance, the PM-associated components are essential for the perception and initiation of BR signals, and a recent study reported that a cell surface LRR-RLK, RPK1, is involved in the early events of ABA signaling (*Osakabe et al., 2005*). It is also possible that downstream components of BR signaling and abiotic stress pathways might be involved in cross-talk. For instance, BR-treatment enhances the translation of heat-shock proteins (HSPs) and increases the resistance to heat stress by limiting the loss of essential components of the translation machinery (*Dhaubhadel et al., 2002*). In addition, a *BIN2* homolog, *AtGSK1* plays a role in the NaCl stress signaling pathway (*Piao et al., 1999*). Finally, one signaling pathway may affect the other secondarily by regulating the expression of genes for the biosynthesis of signals and signaling components. I have initiated a set of experiments to determine at which levels the cross-communication between BR and stress signaling networks may occur, and to develop strategies for genetic screens.

**A.** *Physiological and genetic studies on the BR-related mutants.* I have conducted experiments to assess the response of various BR-related mutants to diverse stress signals. My preliminary results indicates that lines overexpressing BRI1 or DWF4, a BR biosynthetic enzyme, are more resistant to ABA in seed germination and more resistant to NaCl, while mutants with reduced BR signaling are more sensitive to these stressful conditions. At the same time, the early marker BKI1-YFP and the downstream marker BES1 will be used to predict at which level the two signals possibly interact. Similarly, the ABA-related mutants will be characterized under different BR conditions.

**B.** *Genetic screens to identify components involved in the cross-talk between BR and ABA signaling networks.* It is known that the ABA-dependent pathway plays a major role in the signaling of most abiotic stresses and well documented that ABA and BRs have antagonistic effects on seed germination. Therefore, unraveling the mechanisms of their interaction is a reasonable entry point to investigate the cross-talk between BRs and abiotic stresses. I have been developing genetic strategies to identify loci, which may be involved in both BR and ABA signaling. The germination of *bri1-5* and BKI1-overexpressor is almost

completely inhibited by 1 μM of ABA, though most of wildtype seeds can germinate under the same condition. Thus, using the mutagenized seeds of *bri1-5* and BKI1-overexpressor, I plan to select mutants, which can normally germinate in 1 μM of ABA. Hopefully, I could eventually identify and clone genes involved in both ABA and BR signaling.

In summary, the mechanistic study on BRI1 and BKI1, and functional characterization of their interacting proteins will provide significant insights into the regulation and activation of plant receptor kinases and elucidating the BR signaling events proximal to the plasma membrane. A number of putative mutants have been isolated from the BKI1 suppressor screening and are being characterized and cloned. My research will have a great contribution to the BR biology and ultimately have a significant agricultural impact. In my long-term plan, I will investigate the molecular and biochemical mechanisms of signaling network between BRs and other signals in plant growth and development.

## References

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# Teaching Experience and Interests

*Xuelu Wang*

I have served as a principal research advisor for a group of undergraduate students throughout the duration of their thesis work in the springs of 1991-1995, when I was a research associate at the Chinese Academy of Agricultural Science. Upon moving to the United States to continue my graduate work at the University of Arizona, I was able to gain more experience as a teaching assistant for a general plant biology course, *Plant and Our World*. At the Salk Institute for Biological Studies, I have trained several students to gain basic knowledge and experimental skills in molecular biology, biochemistry, and genetics. The students include a high school student, two rotation graduate students, an undergraduate student, and a volunteer trainee. Besides personal experience, I believe that my teaching and overall communication abilities have been greatly enhanced through both my interactions with and my observations of my former and current research advisors, Brian Larkins and Joanne Chory, and through collaboration with other principle investigators, and colleges at the Salk Institute and other institutions.

I believe that instructor's enthusiasm is the most important component of effective teaching, and that it can be contagious. I take great joy in teaching, and I think that the best way to encourage students to learn about science in particular is to go beyond the textbooks and capture some of the excitement of the scientific discovery process to bring right into the classroom. I intend to do my best to install an appreciation for how the great discoveries really come about, to extol the virtues of perseverance, to share the electricity of success, and to emphasize patience with failure.

I believe that my training background and research experience in genetics, biochemistry, molecular biology, and plant physiology will enable me to contribute a more broad and deep perspective to teach most classes in the biological sciences ranging from general topics in these fields to more specific topics within the realm of plant biochemistry and signal transduction. Whatever the subject material, I plan to combine textbook materials and current primary literature, with most of the emphasis placed on the latter for the graduate courses.

As for students in my laboratory, I will train them to solve a given biological problem using many different experimental approaches and with own thoughts. I would hope to guide them to be generalists, to be able to design experiments with a panoramic view of the field, yet rooted in the fundamentals. To that end, I will encourage them to read extensively, to immerse themselves with all kinds of scientific information, including important papers that might ordinarily be considered outside the boundary of plant biology.