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Yves Brun
Systems Biology/Microbiology Faculty Search
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October 19, 2005

Dear Professor Brun,

Please find enclosed my application for the assistant level tenure-track position in the Department of Biology and the Biocomplexity Institute. My long-term research interest is to study small RNA-mediated posttranscriptional regulation of gene expression using the plant model system *Arabidopsis thaliana.* My specific areas of interest include microRNA transcript regulation, biogenesis, and target recognition, and the roles of small RNAs during plant development, viral pathogenicity and viral recovery.

Reference letters will arrive in the next few days from my postdoctoral advisor, David Bartel (Whitehead Institute, MIT), my doctoral advisor, Vicki Vance (University of South Carolina), a postdoctoral collaborator, Bonnie Bartel (Rice University) and a doctoral collaborator, Lewis Bowman (University of South Carolina).

Thank you for your consideration.

Sincerely,

Allison Mallory

Allison C. Mallory

## Statement of Research Interests

The long-term goal of research in my laboratory will be to advance our understanding of small RNA-directed posttranscriptional gene regulation using *Arabidopsis thaliana* as a plant model system. My specific areas of interest include microRNA transcript regulation, biogenesis and target recognition, and the roles of small RNAs during plant development, viral pathogenicity and recovery. This research will be of broad and significant interest to the RNA silencing and plant development communities and has the potential to generate tools that will have wide applications in agriculture, where viral disease leads to significant production and economic losses.

## Background and Significance

The discovery of non-coding small endogenous RNA in both plants and animals has revealed a fundamental process of endogenous gene regulation that is, at least in part, evolutionarily conserved across species. Among the first non-coding small RNAs to be discovered were the small temporal RNA (stRNA) *lin-4* and *let-7,* which were identified from a screen of *Caenorhabditis elegans* mutants exhibiting timing defects during larval development. Since this time, several labs have successfully cloned numerous 21-24-nt RNAs from worm, fly, mouse, human and plants. These small RNAs, including the original *lin-4* and *let-7* RNAs, were collectively named microRNA (miRNA). Thus far, miRNAs have been implicated in numerous processes including germline maintenance, stem cell differentiation, embryogenesis, organogenesis, programmed cell death and immune defense, and defects in the microRNA pathway have been linked to human diseases such as cancer and DiGeorge syndrome.

Mature ~21nt miRNAs are processed from imperfectly paired stem-loop precursor RNA transcripts by members of the RNAse3 family of proteins. Active miRNAs are known primarily to attenuate translation of mRNAs in animals and direct mRNA cleavage in plants. In plants, computational approaches have predicted miRNA targeted messages based on complementarity between the target mRNAs and the mature miRNAs. A

substantial fraction of these targets are transcription factors with putative or known functions in development, indicating that plant miRNAs have important regulatory roles during plant development and suggesting that many plant miRNAs function during cellular differentiation to clear regulatory transcripts from daughter cell lineages.

That plant miRNAs impart cleavage on their target mRNAs is reminiscent of the mechanism of RNA silencing, also known as RNA interference (RNAi) in animals. RNA silencing is a sequence-specific posttranscriptional regulatory mechanism that protects eukaryotic genomes against the invasion of foreign and endogenous nucleic acids, such as viruses, transgenes and transposons. The sequence-specificity of RNA silencing is guided by another class of non-coding small RNAs, the 21-24nt short-interfering RNAs (siRNAs), which are distinguished from miRNAs by their distinct biogenesis pathway. Like miRNAs, siRNAs guide the cleavage of complementary RNAs by incorporating into an RNA-induced silencing protein complex (RISC) that, in plants, includes the RNA slicing protein ARGONAUTE1 (Figure 1).

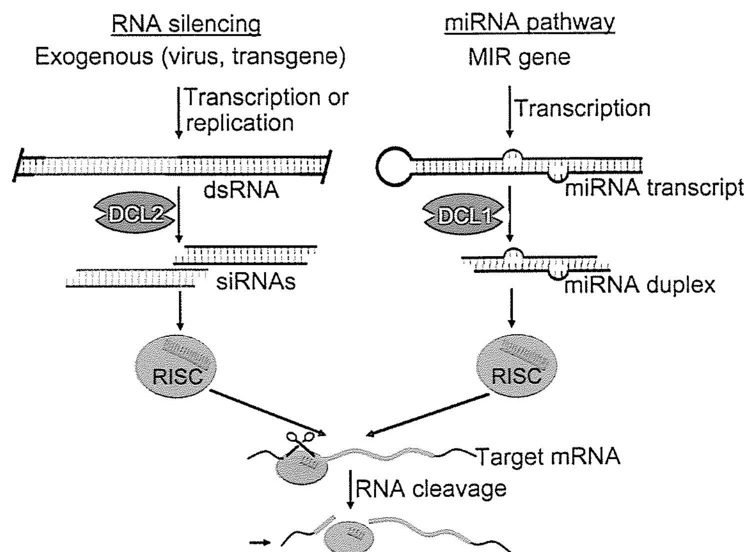


**Figure 1.** A simplified schematic of the miRNA and RNA silencing pathways in plants emphasizing the role of small RNAs and the steps in common to both pathways. During RNA silencing, long double-stranded RNAs resulting from viral replication or transgene transcription are diced to siRNA populations by DCL2 or an unknown DCL, respectively. Imperfectly paired miRNA transcripts produced from MIR gene transcription are diced to a single miRNA:miRNA* duplex by DCL1. Both siRNAs and miRNAs serve as guides in a RISC complex, directing cleavage of complementary RNAs. Not depicted are the roles of genetically or biochemically defined proteins such as RNA-dependent RNA polymerase 6 (RDR6), the double-stranded RNA binding protein HYL1 and the RNA methyltransferase HEN1.

Compatible interactions between plants and viruses either lead to successful infection or resistance. In both cases, local immune responses activated in the plant can include the production of cis-acting siRNAs originating from and targeting viral RNAs, a RNA silencing process referred to as virus-induced gene silencing. The local response can be accompanied by the production of a mobile signal that originates at the site of viral infection and propagates through the plant to elicit systemic resistance. This phenomenon is referred to as recovery. One way viruses can establish a successful infection is by encoding viral suppressor proteins that counteract RNA silencing and, in some cases, impact plant development. The recovery mechanism is poorly understood, but it likely involves components of the RNA silencing machinery. The mechanisms by which viral suppressors counteract the immune response are also poorly understood, but it is presumed to involve disabling the RNA silencing machinery.

## Research Plan

My long term goal is to advance our understanding of small RNA-directed gene regulation during development and viral defense using *Arabidopsis* as a model system. My previous experiences studying plant-virus interactions, small RNA-mediated regulation, and the role of miRNAs in plant development have given me a strong scientific foundation to lead a research group that will utilize genetic, molecular, biochemical and bioinformatic approaches to gain insight into small RNA-directed regulatory pathways.

### miRNA biogenesis and action

My laboratory will design genetic screens to identify novel components of the miRNA pathway involved in the regulation of MIR genes, the biogenesis of miRNAs and the action of miRNAs. Several mutants among the millions that exist in *Arabidopsis* have phenotypes similar to those of known miRNA pathway mutants. One example is *serrate*, a mutant that exhibits a phenotype similar to plants with mutations in HYL1, a double-stranded RNA binding protein implicated in miRNA biogenesis, or HEN1, a protein that methylates mature miRNAs and siRNAs *in vivo.* The axial patterning defects of *serrate* mutants are reminiscent of those of plants expressing miR165/166-resistant HD-ZIP genes. Indeed, mutations in SERRATE negatively affect miR165/166 transcript processing, resulting in misregulation of miR165/166 HD-ZIP target genes *PHABULOSA* and *PHAVOLUTA*. I will screen existing libraries for mutants that have developmental defects overlapping with that of known miRNA mutants, attempting to link novel genes to the miRNA pathway. I also will mutagenize known miRNA mutants and screen for enhancing or suppressing mutations.

### Small RNA-directed regulation

Identifying the gene repertoire that is influenced by small RNA-directed regulation is essential for our understanding of plant development and defense. Computational miRNA target predictions have relied on a high degree of complementarity between plant miRNAs and their mRNA targets, assuming that mRNA cleavage is the major mode of miRNA-directed regulation in plants, unlike in animals where miRNAs primarily direct

translation attenuation and exhibit less complementarity to their targets. However, one example of miRNA-mediated translational control has been suggested in plants, and. discrepancies between mRNA and protein levels have been noted during RNA silencing, suggesting the contribution of translational control. However, it is unknown if translational control acts synergistically with mRNA cleavage during endogenous small RNA-directed regulation or if it controls the expression of a unique gene set that remains to be identified. To address whether small RNA-directed translational control in plants occurs by a mechanism analogous to that in animals, I will introduce an animal-based miRNA:target mRNA system in plants and test the efficacy of this system in mutant backgrounds known to affect miRNA-directed mRNA cleavage or plants expressing components of animal RISC. To address the contribution of small RNA-mediated translational control in plants, I will investigate the basis of small RNA:target mRNA interactions to define parameters that will advance the identification of novel endogenous targets.

**Small RNA-directed plant defense**

Recovery from viral infection likely relies on components of endogenous RNA silencing pathways. Initially I will employ a candidate gene approach to identify components of the recovery pathway. I will test the expanding collection of mutants impaired in RNA silencing pathways for their ability to undergo recovery. In addition, I will develop genetic screens to identify new mutants with either an increased or decreased ability to mediate recovery. This screen will be based on the differential capacity of wild type and mutant plants to recover from viral infection.

A complementary approach to identify components of the recovery pathway will be to determine the cellular targets of viral suppressors. To this end, a genetic screen will be performed on plants that express viral suppressors to identify mutants with either increased or decreased suppression of the immune response. These experiments will provide insight into both the mechanism underlying the innate immune response to viral infection as well as the control of plant development by viral infection.

## Research Accomplishments

Viruses have evolved strategies to counteract plant defense mechanisms, such RNA silencing. As a PhD student, I contributed to the identifications of both the first viral suppressor of RNA silencing, Helper Component-Proteinase (HC-Pro), and the first endogenous plant suppressor of RNA silencing, rgs-CAM, a calmodulin-related protein that interacts with HC-Pro. To gain understanding of how HC-Pro suppressed silencing, I conducted a molecular analysis of transgenic *Nicotiana tabacum* plants expressing HC-Pro and revealed that HC-Pro inhibits siRNA accumulation associated with transgene-induced RNA silencing, but does not interfere with the systemic silencing signal or DNA methylation. Moreover, our studies revealed that the inhibitory effect of HC-Pro on small RNA accumulation could not be generalized to all types of silencing. Although HC-Pro suppresses virus-induced RNA silencing (VIGS), its expression leads to increased accumulation of siRNAs associated with VIGS, adding a layer of complexity to the relationship between plant defense and viruses. I also showed that HC-Pro affects the miRNA pathway, indicating that HC-Pro alters endogenous small RNA pathways as well. Because miRNAs have critical regulatory roles during plant development, this result suggested a molecular basis for the developmental abnormalities caused by virus infection.

My postdoctoral research has focused on two areas: 1) The contribution of miRNA-directed gene regulation during plant development, and 2) The mechanisms of small RNA biogenesis and target recognition. By exploring the regulatory roles of miRNA:target mRNA pairs in the model system *Arabidopsis*, my work has revealed developmental roles for two conserved miRNAs, miR164 and miR160, and has exposed a novel posttranscriptional regulatory mechanism for members of the two transcription factor families that these miRNAs regulate. miR164-directed regulation of the NAC-domain transcription factor family member CUP SHAPED COTYLEDON 1 (CUC1) is essential for proper embryonic, vegetative and floral development. miR160-directed regulation of the transcription factor family member AUXIN RESPONSE FACTOR 17 (ARF17) also plays critical roles during embryonic, root, vegetative, and floral development. Furthermore, impairment of miR160-directed regulation implicated ARF17

as a regulator of *GH3*-like early auxin response genes, which encode auxin-conjugating proteins. The observation that proper miRNA-directed regulation is essential for plant development adds a new layer of complexity to the gene regulatory mechanisms that control cell differentiation and organ development in plants and points to a mechanism that could be exploited for agricultural improvements.

To increase our understanding of the overall role of miRNAs during plant growth and development, it is necessary to develop more robust methods for miRNA target prediction. Understanding how a miRNA recognizes its target mRNA is essential to this process. To this end, I determined the nucleotide positions within miRNA complementary sites of targeted mRNAs that are most important for maintaining miRNA regulation. Using the experimental model miR166 and its target mRNA *PHABULOSA* (*PHB*), which encodes a member of the HD-ZIP domain transcription factor family that specifies leaf polarity, mismatch scanning revealed more tolerance for mismatches at the center and 3' end of the miRNA compared to mismatches at the miRNA 5' region. These nucleotide pairing requirements, previously only appreciated for animal miRNAs that direct translation attenuation, established a new parameter for small RNA target prediction in plants. My ongoing postdoctoral research incorporates these parameters to define new miRNA targets in plants.

I also have joined in a collaborative effort with the laboratory of Hervé Vaucheret at INRA to investigate the roles of the four DICER-LIKE (DCL) RNase3 proteins in *Arabidopsis*. We implicated DCL4 as the primary processor of *trans*-acting siRNAs (tasiRNAs), a novel class of small regulatory RNAs that, like miRNAs, direct the cleavage of mRNAs that have little overall resemblance to the RNAs from which they originate. By analyzing double *dcl* mutant combinations, we also revealed partially redundant functions among the four DCL proteins. In addition, we are exploring the regulatory relationship between miR168 and ARGONAUTE1 (AGO1), a target of miR168-directed cleavage that encodes the key RNA SLICER protein implicated in RNA silencing, miRNA and tasiRNA pathways. We have identified an AGO1-mediated

miR168 stabilization feedforward loop, which together with miR168-directed AGO1 cleavage, maintains the miRNA homeostasis.

## Teaching Interests

My primary teaching interest is to develop a graduate level course centered around posttranscriptional gene regulation, with an emphasis on small-RNA mediated regulation. Background lectures on specific mechanisms of posttranscriptional regulation including, but not limited to, RNA silencing, splicing, RNA editing, and nonsense-mediated mRNA decay, and the roles of small RNAs during plant development and pathogen defense would mesh with weekly discussions of relevant current literature.

As a graduate student in the Department of Biological Sciences at the University of South Carolina, I was the primary instructor of both an introductory Biology Principles 2 Laboratory class, the second level of a set of two Biology Principle Laboratory classes, and an advanced Animal Physiology Laboratory class, and I frequently guest lectured in an introductory Biology Principles 1 course. Both laboratories were designed to apply the topics discussed in the accompanying lecture classes. As an instructor, I lectured on introductory level topics including plant and animal biology, microbiology and taxonomy, and advanced level topics in animal physiology. I also designed all lectures, quizzes and exams for both classes. Because the two laboratory classes were at different academic levels, I had the chance to interact with students ranging from accounting and education majors to upper-class pre-medical majors and nursing students.