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

Dear Members of the Search Committee:

I am writing to apply for the tenure-track Assistant Professor position advertised in *Science*. I have been trained in the lab of Dr. Victor Ambros at Dartmouth Medical School as a postdoctoral fellow to study the function of microRNAs using *Drosophila* genetics. My goal is to understand both the molecular mechanisms by which evolutionarily conserved microRNAs regulate gene expression and also the specific biological contexts in which such regulation is required. Towards this end, I have initiated three main projects. First, using the detailed genetic analysis of *miRNA-1* as a case study, I am exploring the role of microRNAs in regulating muscle cell identity. Second, I am investigating how three microRNAs (*miRNA-100*, *-125* and *let-7*) that form a co-transcribed cluster function independently and collectively during *Drosophila* metamorphosis. Finally, I have developed a sensitive genetic system in the fly eye that indicates *let-7* activity and am using this system to identify proteins that serve as effectors and regulators of microRNA activity in general and *let-7* activity specifically, as a step towards delineating the molecular mechanisms of microRNA function. These studies will illuminate the critical role of microRNAs for normal development and also demonstrate how disruption of microRNA activity can lead to human disease, including cancer.

My doctoral studies with Dr. Lynn Cooley at Yale University focused on genetic analysis of the actin cytoskeleton during *Drosophila* oogenesis. This rigorous training in cell biology and genetics has enabled me to successfully pursue a completely independent project using *Drosophila* in the Ambros Lab, which has heretofore exclusively studied *C. elegans*.

Please find enclosed my curriculum vitae, statement of research and teaching interests, and the names and addresses of references from whom I have requested letters of recommendation to be sent directly to your address. Please do not hesitate to contact me with any further questions. Yours sincerely,

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My goal is to use *Drosophila* to analyze the functions of conserved microRNAs (miRNAs), a recently discovered class of small RNAs that regulate gene expression. MiRNAs play an essential role during vertebrate development and their misregulation causes human disease, including cancer. As a principal investigator, I will analyze the specific biological roles of individual miRNAs, identify the biologically relevant mRNA targets of these miRNAs, and determine the ways in which the genetic pathways involving miRNAs are integrated with broader networks regulating cell behavior and development. Towards these ends, my lab will 1) use molecular, genetic and cell biological techniques to analyze the *in vivo* functions of four highly conserved miRNAs (*DmiR-1*, *Dlet-7*, *DmiR-100*, *DmiR-125*) and 2) carry out large-scale genetic screens to delineate the broader genetic networks within which these miRNAs function.

These studies will address many pressing needs in the field of miRNA research. *Drosophila*, with its complex cell biology and sophisticated genetics, offers an exemplary system not only to dissect the roles of miRNAs at the subcellular, cellular, tissue and organismal levels but also to identify the functional relationships between miRNAs and interacting genes, both upstream and downstream. Findings made about evolutionarily-conserved miRNAs in *Drosophila* will inform our knowledge about human disease processes. Where possible, I anticipate establishing collaborations with scientists working in mammalian systems to explore how the functions of these miRNAs are conserved between flies and humans.

Aim 1: Determine the function of *DmiR-1* during muscle growth.

MiR-1 sequence and muscle-specific expression pattern is conserved from flies to humans. I found that *DmiR-1* expression in muscles is regulated by two transcription factors: Twist and Mef2. My work revealed that *DmiR-1*'s critical role is to maintain, not establish, muscle cell fate: muscles form and function normally in *DmiR-1* mutant embryos but degrade dramatically during the rapid cellular growth that takes place during larval development. From these results, and considering the identity of computationally-predicted *DmiR-1* target sequences, I proposed that *DmiR-1* maintains muscle identity by repressing muscle-toxic gene expression, particularly at times of rapid growth.

The analysis of DmiR-1 will serve as a case study to understand the molecular mechanisms by which miRNAs regulate cell and tissue identity. My lab will employ cell biological and molecular genetic approaches to characterize both the previously unstudied process of larval muscle-cell growth in general as well as the roles of *DmiR-1* and computationally-predicted *DmiR-1* target genes during this process. In addition, the *DmiR-1* mutant phenotype will be described within specific muscle subsets (somatic muscles, visceral muscles, dorsal vessel, etc) using tissue-specific Gal80 lines. Genetic suppressor screens of tissue-specific phenotypes also will be performed to identify the functionally relevant mRNA targets. Finally, the *DmiR-1* mutant background will be used as a starting point to knockout two nearby muscle-specific and evolutionarily conserved miRNAs, *DmiR-133* and *DmiR-288*. Combined genetic and cell biological analysis of these three miRNAs will lead to a comprehensive understanding of miRNA function in muscle biology.

Aim 2: Determine the function of *DmiR-100*, *-125* and *Dlet-7* during metamorphosis.

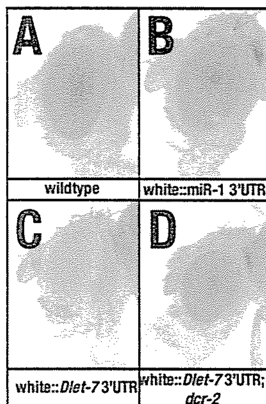
Although *C. elegans let-7* and *lin-4,* the first miRNAs discovered, play critical roles in controlling a decision between cell proliferation and terminal differentiation, they are expressed and function at two different developmental stages. I found that the *Drosophila* homologue of *lin-4* (*DmiR-125*) are co-regulated and co-transcribed not only with *Dlet-7* but also with a third miRNA (*DmiR-100*) and that the expression of these three miRNAs begins at the onset of metamorphosis. *MiR-100*, *-125,* and *let-7* are conserved in sequence and genomic organization between flies and humans, suggesting that aspects of their regulation are also conserved. Importantly, misregulation of all three miRNAs has been linked to cancer in humans. Therefore, molecular and genetic analysis of the *DmiR-100*, *-125* and *Dlet-7* miRNA cluster will determine the *in vivo* function of three critical miRNAs. Furthermore,

such an analysis provides an exceptional opportunity to explore how three sequence-unrelated miRNAs collaboratively regulate interdependent biological processes.

I have generated a deletion mutation that removes the entire *DmiR-100*, *-125* and *Dlet-7* miRNA cluster. Homozygous mutants are lethal and die during metamorphosis. Phenotypic analysis of this mutation is currently underway to determine the precise development defects of the mutant flies. Furthermore, having defined a genomic fragment capable of rescuing this lethality, I have developed tools for analyzing the role of each miRNA individually and in combination with one another using transgenic flies. Finally, using an assay system (described below), I have validated a number of computationally predicted *DmiR-100*, *-125* and *Dlet-7* targets and am currently using cell biological and genetic approaches to further investigate the relationship between *DmiR-100*, *-125* and *Dlet-7* and these targets.

Aim 3: Use a novel genetic system to identify genes that functionally interact with *Dlet-7*.

Simple but powerful screens in the fly eye have allowed *Drosophila* geneticists to delineate conserved developmental pathways that, when misregulated, can cause human disease. I have developed such a screen specifically to identify genes that are required for or that regulate *Dlet-7* activity. This system is based on an insulated copy of the eye pigmentation gene, *white*, which causes wildtype fly eyes to be red (Fig 1A). Insertion of synthetic *Dlet-7* (or *miR-100* or *-125*) binding sites into the *white* 3'UTR causes a decrease in eye pigmentation (Fig. 1C) whereas the insertion of other miRNA binding sites (for example, *DmiR-1*) does not (Fig. 1B). The repression of *white* is miRNA dependent because it can be suppressed by a mutation in the miRNA-processing enzyme called Dicer (Fig. 1D). Thus, easily visualized eye pigment levels are a readout for *Dlet-7* activity.



Large-scale F_1 screens for mutations that modify *Dlet-7* (or *miR-100* or *-125*) activity in the eye will be a major focus of my research program. Expected modifier loci would include genes encoding transcription factors that regulate *Dlet-7* expression, processing components involved in the production of the mature miRNA, components that mediate miRNA::mRNA interactions, and effectors of mRNA silencing. I have generated reporter constructs for secondary screens to determine whether modifier loci affect *Dlet-7* function specifically, also affect *DmiR-100* and *DmiR-125* function, or miRNA function more generally. Genes identified in this screen will be cloned by standard techniques, and characterized by molecular and genetic methods to establish their roles in the context of *Dlet-7* function *in vivo*. Particular emphasis will be placed on the characterization of genes and gene products conserved in mammals. As validation of this approach, I performed a pilot screen from which I isolated four new mutations that appear to specifically affect *Dlet-7* activity. In addition, this same genetic system will be applied to a second project: to quickly assay the many computationally-predicted *Dlet-7* target mRNAs for functional *Dlet-7* binding sites by fusing their 3' UTRs with the coding region of the *white* transcript. The functional relationship between *Dlet-7* and gene products that test positive in this assay will be explored using cell biological and genetic techniques. As validation of this approach, I have already found that three of five predicted *Dlet-7* targets mediate the downregulation of *white*.

Currently, most approaches to identifying components that regulate miRNA activity are biochemical in nature. The above screen offers a complementary genetic approach that will identify miRNA regulatory molecules based on their function rather than on their presence in a protein complex. This screen will reveal the specific regulatory inputs that control the function of *DmiR-100*, *Dlet-7* and *DmiR-125*, evolutionarily conserved miRNAs whose human homologues have been implicated in caner. My approach also will delineate more general mechanisms by which the miRNA regulatory pathway functions to control gene expression and may, ultimately, establish the miRNA pathway as a tractable therapeutic target.

Teaching Interests

The broad interdisciplinary scope of my training enables me to teach a wide variety of courses, depending on the needs of the department. At Yale, I have taught introductory molecular/cellular and reproductive biology courses. From my postdoctoral training, I have experience in mentoring high school, undergraduate, and graduate students in a variety of genetic, molecular and cell biology techniques. I am deeply committed to teaching and greatly enjoy interchange with students of diverse talents, experiences, and backgrounds. Although my primary research focus is on microRNAs, I am interested in teaching classes that cover any aspect of developmental biology as well as eukaryotic cell biology and molecular biology.