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Jessica Houtz: A Beckman Research Experience

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When I entered college I had already acquired a keen interest in science and mathematics, as well as a desire to be challenged with new information. So, naturally, I gravitated toward the Agricultural Biotechnology program (ABT) upon discovering its reputation as one of the most demanding majors at the University of Kentucky. The curriculum focuses strongly on genetics, biochemistry, and a research experience, which leads to a difficult but unique undergraduate program. During my first semester, I heard truly amazing scientists speak about their lives and career experiences, as well as their research projects. It was motivating to meet academic professionals with so much dedication and passion for their work, and it certainly contributed to my eagerness to become involved in research. I wanted the same level of enthusiasm for my investigations as I had witnessed in the speakers, so I took my time, and explored the activities of several laboratories before finally settling on the research project to which I felt the most committed.

Although my 2009-2010 Beckman project engaged me in questions regarding the relationship between circadian rhythms and the structure and function of skeletal muscle, I had also participated in several other fields of research. My first laboratory experience occurred two years earlier in a Plant Natural Products and Gene Silencing lab. The principle investigator, Dr. Guiliang Tang, had been a speaker for one of my classes, and stimulated my interest in the generation and function of RNA interference. It was in this lab that I learned basic biotechnology skills and techniques such as handling a pipette, making solutions, running DNA gels, and performing PCR. As I became comfortable with these processes, I was gradually involved in investigations designed to evaluate the roles of various microRNAs in plant regeneration and development.

The following summer I worked in an Equine Musculoskeletal lab, under the direction of Dr. James MacLeod. This group was conducting extremely intriguing work on cartilage regeneration and repair in the axolotl, a Mexican salamander capable of regenerating limbs. The axolotl is unique due to its neotenous existence; these salamanders can live their entire lives (reaching sexual maturity) in the aquatic, larval form, or they may metamorph into terrestrial individuals. This makes the axolotl an ideal model for studying differences in cartilage structure between non-weight bearing (aquatic) and weight bearing (terrestrial) animals. The developmental biology and histological techniques involved in this study remain prominent in my research interests and contributed to my decision to take classes in developmental biology, regeneration, and histology.

During the same summer, I began work in Physiology with Dr. Karyn Esser's laboratory, which focuses on skeletal muscle and circadian rhythms. Over the course of the summer and through the spring of 2009, I was involved in a project that combined my earlier interest in RNA interference with my desire to learn more about the function and physiological effects of circadian rhythms. I investigated the circadian regulation by Bmal1 of a microRNA (miRNA-206) that is highly and exclusively expressed in skeletal muscle. For my Beckman research project, I shifted my research focus upstream, from potential targets of Bmal1, to its direct influences on the structure and function of skeletal muscle.

Dr. Esser's lab has noted an interesting physical effect in muscle resulting from the disruption of the core clock. Evidence of deterioration of sarcomere structure in skeletal muscle is clear in electron micrographs (EMs) of muscle cross sections in Clock mutant and Bmal1 knock-out mice. Electron microscopy can be used to visualize the ultrastructure of muscle cells and reveal the organization of actin and myosin myofilaments that make up the sarcomere. In cross section, myofilaments appear as a repeating hexagonal pattern of dots: six large dots (myosin) around one large dot, and six small dots (actin) around each large dot. EMs show a loss of this typical hexagonal arrangement of thick and thin myofilaments in the skeletal muscle of mutant mice as compared with wildtype mice. The heads from the myosin molecule interact with α-actin to create cross-bridges. Cross-bridges between thick and thin myofilaments allow for the shortening of the sarcomere, and the generation of force by moving actin filaments past the myosin filaments. Maximum force is generated by a muscle when the overlap between thick and thin myofilaments provides the most possible cross-bridge interaction. If the arrangement of thin filaments surrounding thick filaments is disrupted, then the ability of the muscle to

generate force will be severely affected. Indeed, skeletal muscle from the arrhythmic germline Bmal1 knock out mouse exhibits significant weakness defined by a reduction in muscle specific tension.

Ubiquitous elimination of Bmal-1 or mutation of Clock in the entire mouse genome results in alterations in muscle structure and function. However, it is unclear whether or not the observed phenotype of muscle is due to the disruption of the core clock in skeletal muscle, or the disruption of a particular developmental pathway in the embryonic mouse. It is possible that the maintenance of a healthy muscle phenotype relies on the proper functioning of the local molecular clock within skeletal muscle. In order to determine the role of peripheral clock in maintaining the health and function of skeletal muscle, I generated a muscle specific Bmal1 knock-out mouse using cre-lox recombination technology. This technique utilizes loxP sites which are recognized by the bacterial enzyme Cre recombinase. When Cre is introduced into cells that have DNA with loxP sites flanking the gene (floxed gene) of interest, this gene will be selectively spliced from the genome. I used the muscle creatine kinase (MCK) promoter to drive Cre expression in striated muscle, while leaving Bmal1 expression intact in the cells of the central pacemaker in the SCN, and maintaining the rhythmic behavior of muscle specific Bmal1 knockout (MCK-Bmal1-/-) mice.

Another aim of my Beckman project was designed to eliminate personal errors and increase statistical power during data collection by turning the qualitative assessments of myofilament arrangement into a quantitative analysis. The degree of organization of myofilaments in EMs of muscle cross sections is difficult to define for images containing thousands of dots in which just one pixel is equivalent to many nanometers. Thus, manually demarking filaments in an image, and measuring the distances and angles between filaments is an inefficient and inaccurate method for determining myofilament structure.

The human eye is remarkably capable of detecting divergences in patterns, but there are many additional layers of information in an image that are not visually discernable. An image is actually a matrix of data that is divided into pixels with intensity values ranging from 0 to 255. There are three layers of the matrix corresponding to the red, green, and blue components of the picture. While this quantity of information is dauntingly vast to a person, a computer can be instructed to process this data with great speed. Of course, such an endeavor requires the knowledge to program a computer how to perform a particular task, and more importantly, the ability to plan series of tasks for optimum performance and limited personal bias. As a biotechnology major faced with this challenge, my skills in computer programming were somewhat lacking. I spent the first summer of my Beckman experience pouring over programming threads and posts and debugging my rough compositions of MATLAB code. Fortunately, I had the instruction of Dr. Kenneth Campbell in the Department of Physiology to help guide my efforts to organize and communicate my ideas into functional MATLAB code. Dr. Campbell has computational biology and striated muscle expertise, and provided me with numerous applications of math, especially matrices, to molecular biology. Through my collaboration with Dr. Campbell's laboratory, I learned new ways to approach not only data analysis but also data collection. In this way, I was able to merge my aptitude for mathematics with my interest in biology to develop a mathematical imaging tool for the high-throughput analysis of myofilament architecture. I am excited by the knowledge that my work will contribute to the emerging field of computational biology and enhance the availability of technological tools for the promotion of novel research and scientific understanding. It is also my hope that the applications of this software will extend beyond evaluation of muscle in circadianly disrupted models to address the myofilament structure in other conditions affecting muscle health.

Looking back on the events leading up to my application to the Beckman Scholars Program, my junior year seems like a summit of synergy in my education. I pooled information from my courses in matrix algebra, techniques in biotechnology, and the biology of circadian rhythms to arrange a fascinating and intricate mosaic of intellectual pursuit. In the process of obtaining a minor in mathematics, my courses in calculus and matrix algebra provided me with the fundamental concepts necessary for grasping image analysis. Matrices were also the topic of conversation that led to my acquaintance and subsequent collaboration with Dr. Campbell. My knowledge of circadian rhythms was greatly enhanced by a course in the biology of sleep and circadian rhythms taught by Dr. Bruce O'Hara who became my sponsoring Beckman mentor. Additionally, my biotechnology courses focused on the development of research projects and set the stage for the composition of my proposal.

I have been very fortunate to have the guidance of so many wonderful mentors and instructors as an undergraduate at UK. Without the patience and dedication of these and other generous individuals, undergraduate research would not be possible. I also attribute much of my own success and interest in research to membership in the UK Appalachian and Minority Science, Technology, Engineering, and Mathematics Majors (AMSTEMM) program sponsored by NSF. AMSTEMM is a paradigm for engaging students in science and research. This amazing group of people has provided me with support, resources, and advice to aid in my research career while attending UK. In the Spring of 2009, I received a fellowship from AMSTEMM supporting my research on the circadian

regulation of microRNA-206 in skeletal muscle. AMSTEMM was also influential in my decision to apply for the prestigious Beckman Scholar Award, which I was honored to receive for my proposed research on the computational modeling of myofialments in muscle-specific Bmal knock-out.

The Beckman Scholars Program is a remarkable organization. Everything from the application process and panel interview to the responsibilities as a scholar has strengthened my abilities as a student and a scientist. Being named a Beckman Scholar provided me with the financial support to participate in some incredible experiences. I was able to travel to national conferences including the Biophysical Society, and Experimental Biology Meetings in California, where I had the opportunity to present my research and participate in several poster competitions. My interactions with other scientists during these meetings challenged me to think critically about my project and experimental methods. Additionally, the immense gatherings of intellect often resulted in valuable networking potentials where I met some of the same people I interviewed with for graduate schools.

My undergraduate research experiences in a variety of fields have factored strongly in my pursuit of a Ph.D. in Cellular, Molecular and Developmental Biology at Johns Hopkins University. I aspire to use my growing skill set to make discoveries that contribute to the expansion of knowledge for the benefit of society as a whole. Furthermore, I hope to give back to the community as a university instructor who nurtures the academic and research aspirations of future generations of curious students.