

Dean's Scholar Summer 2008 Research Proposal

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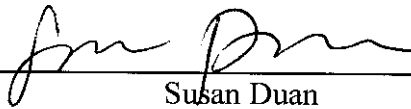
Major

Biological Sciences, Neurobiology and Behavior

Faculty Advisor

Ronald Booker, Project Advisor
Laurel Southard, Major Advisor

I hereby waive access to my faculty recommendation letter:



Susan Duan

Abstract

In an average eukaryotic cell, one out of three proteins undergo reversible phosphorylation. While there has been a major focus on the role of kinases in the regulation of signaling pathways, it is now apparent that protein phosphatases are highly regulated enzymes that play a key role in the control of protein phosphorylation and of signaling activity. Based on *in vitro* biochemical studies, Protein Phosphatase 2AM (PP2A) is predicted to play a key role in almost all cellular processes. However, it is puzzling to reconcile the broad range of possible PP2A substrates *in vitro* with the exquisite temporal and spatial specificity required of PP2A-dependent dephosphorylation events *in vivo*. One solution to this puzzle is offered by variation in the availability of the regulatory subunits that associate with the PP2A catalytic subunit. PP2A is a highly conserved holoenzyme consisting of three subunits: A, B, and C. Subunits A and B are regulatory while C is a catalytic subunit. The role of PP2A in the EGFR (epidermal growth factor receptor) pathway of early eye specification is what I will be looking at. There has also been some evidence that PP2A has a role in two different pathway locations. I hope to elucidate both those roles through manipulation of the genes that code for the B subunit. I will use *Drosophila* as my research organism and cross different flies in order to produce genotypes of interest; focusing on the genes *Twins23*, *widerborst*, and *well-rounded*, all B subunit genes. After varying the level of gene expression for the B subunit, I will look at the resultant phenotypes, including transformation of the eye into a second antenna, an enlarged rough eye, and a reduced eye. I will also be looking at the larval eyes discs, staining them with specific eye and antennal markers in order to track the process of eye development. I will photograph the staining and evaluate the different genotypes by comparing them to the staining of the wild type fly eye disc. Adult flies will also be photographed to view the various phenotypes and comparisons will be made with the wild type. My final objective is to create a collection of photos and descriptions that will give a greater understanding of the regulatory roles played by the different B subunit genes of PP2A. EGFR and many of the genes in the signaling pathway are oncogenes and thus have implications for cancer. PP2A is conserved in humans along with the EGFR signaling pathway and it has a diverse set of functions. Thus understanding the effects PP2A has in one fly pathway will be useful for discovering its various roles in other species as well.

Biographical Sketch

I was born in Changchun, China but immigrated to the United States at a young age. My family moved frequently while I was in elementary school, but we finally settled in Thousand Oaks, CA. I attended Thousand Oaks High School, where I graduated as valedictorian in 2005. In high school, I ran on the cross-country team, played violin in the local orchestra and volunteered at the Westminster Free Clinic. The clinic is where my interest in biology and more specifically, medicine, developed. I am now a junior in the College of Arts and Sciences, majoring in Biology and concentrating in Neurobiology and Behavior.

My interests in healthcare and public service have found great outlets here at Cornell. I am very involved with the Public Service Center, working as a student manager, but also as a participant in many of the student-run service programs. I am a

lead tutor for the Raising Education Attainment Challenge program (REACH), that works to mobilize college students to go out into the local community and act as both tutors and mentors for younger students. Last year, when the new Human Papilloma Virus vaccine was released, my friend and I realized how important the issue of HPV and sexual health are, especially to college students. We founded the Society for HPV Education and Prevention (SHEP) and we work to spread accurate information on the risks of HPV, its connection to cervical cancer, and we emphasize the importance of regular check ups. We are currently planning an educational spa event for this spring. I will also be going on an alternative spring break trip this year to the Kensington Welfare Right Union in Philadelphia. The alternative spring break program emphasizes not only volunteerism, but provides students with an opportunity to learn about the issues of poverty, homelessness and the struggle many face to earn a living wage. I hope to take the knowledge I gain from these experiences and become an effective advocate for change.

The past two summers, I have participated in research projects at two different colleges, and through those experiences, I decided to become more involved with research here at Cornell. The project I am working on and hope to continue this summer is related to the eye development pathway in *Drosophila*. I hope to be able to apply what I learn through my research to a larger picture of health in humans

Statement of Purpose

Egfr signaling plays a key role in regulating many fundamental cellular processes including cell proliferation, survival, differentiation, motility and metabolism (reviewed in Shilo, 2003). Egfr uses the Ras/Erk pathway, one of the most important and well-studied pathways that generate signals which find their way to the nucleus and alter the pattern of gene expression and ultimately the behavior and fate of a cell. While the essential biochemistry of the pathway is now established, our understanding of how the Ras/Erk pathway can process a broad range of specific inputs into diverse biological output is far from complete. Recent results in my laboratory reveal that the heterotrimeric Protein Phosphatase 2A (PP2A) plays a key role in regulating neurogenesis by targeting of the Ras/Erk signaling pathway. Based on this work it is hypothesized that altering the B regulatory subunits will allow the PP2A holoenzyme to target different components of the Ras/Erk pathway. To better define the role of PP2A in regulating neurogenesis, the following specific aims are proposed:

The molecular mechanisms involved in the regulation of *Drosophila*'s adult are known in detail. Seven genes, including *eyeless*, have been found to act in the early specification of the *Drosophila* compound eye. Loss of function of any of these genes results in a failure of the eye to form while ectopic expression of these genes results in ectopic eyes. The EGFR (epidermal growth factor receptor) signaling pathway has been shown to function in the specification of the eye and *Egfr* acts upstream of the eye specification genes. This function is dependent on Ras/Erk or mitogen activated protein (MAP) kinase signaling pathways (Kumar and Moses, 2001). Two phenotypes have been seen as a result of activation of the Ras pathway: homeotic transformation of the eye into a morphologically complete antenna (Kumar and Moses, 2001) and hyperplastic growth of the eye (Karim and Rubin, 1998). *Egfr* and its downstream components Ras and Raf have been found induce this eye to antenna transformation.

The protein serine/threonine phosphatase 2A (PP2A) controls phosphorylation of many proteins involved in cell signaling and it is also an important regulator of cell growth (Virshup, 2000). The PP2A holoenzyme is a heterotrimer that consists of a core dimer, a scaffold (A) and catalytic (C) subunit that associated with various regulatory (B) subunits. One function of PP2A is the regulation of Ras/Erk signaling. Our understanding of the role PP2A plays in the regulation of the Ras/Erk signaling is far from complete. In the literature there are reports that PP2A is both a positive and negative regulator of Ras/Erk signaling. During *Drosophila*'s adult eye development pathway, PP2A has been shown to have a negative effect upstream of Raf but a positive effect downstream of Raf (Wasserman et. al., 1996). It is possible that the different effects of PP2A in the EGFR signaling pathway are mediated by distinct PP2A holoenzymes. I will be looking at the

genes that encode 3 out of the 4 *Drosophila* B regulatory subunits (*twins*, *widerborst*, *B''* and *well-rounded*) to define the role each plays in regulating the early specification of the fly eye. I hope to discover which B regulatory is responsible for the targeting of PP2A activity during eye development. I will also carry out a series of gene-gene interaction experiments to determine whether PP2A is involved in regulating Ras/Erk. I will use immunocytochemistry and confocal microscopy to track the expression of a series of protein known to play a critical role in regulating eye development. I hope to verify the previously mentioned transformation of the eye into an antenna. I also expect to see rough and reduced eyes. I hope to create a collection of photos and descriptions that will give a greater understanding of the regulatory roles played by the different B subunit genes of PP2A in the eye development pathway of *Drosophila*.

I will use an *ey*-GAL4 driver to express the genes in the eye region. This drives expression first in the eye and antenna anlagen in the embryo and then in regions before the furrow in the eye imaginal disc (Gehring, 1998) To remove Egfr function in the eye domain I will express a dominant negative form of the receptor. I will also be using the catalytic subunit of PP2A, which is encoded by *microtubule star* (MTS), the dominant negative form of MTS, and the three genes I am interested in, *Twins23*, *widerborst*, and *well-rounded*. To study the specific effects of the three B subunits of PP2A, I will be crossing flies and forming genotypes of interest with three proteins in the pathway, Egfr, Ras, and Raf. Thus I hope to narrow down the place within the pathway that each B subunit functions.

The EGFR signaling pathway and PP2A are highly conserved even in higher organisms. PP2A has been shown to promote cell survival indirectly by inhibiting

apoptosis. The depletion of PP2A causes apoptosis by increasing caspase activity. This activity has been documented in many cell types (Silverstein et. al., 2002). High levels of EGFR promote solid tumor growth and have been identified as a common component of many cancer types. Aberrant EGFR activation, which is mediated by changes in gene amplification, is an important factor in tumorigenesis and is also a critical driving force for the aggressive growth of cancer cells (Salomon et. al., 1995). The study of such a common pathway and an understanding of the role each specific gene product encoded by *Twins23*, *widerborst*, and *well rounded* will hopefully contribute to a greater knowledge base for cancer as well as cell apoptosis.

I am continuing the research done in this field of *Drosophila* eye development by searching for the specific role of each B subunit gene and where it functions along the EGFR signaling pathway. I will be pursuing this project at Cornell in my current lab where I will have access to the materials of my professor as well as Cornell's resources. I hope to complete this project and/or continue it next year in the fall and use the data I collect for my senior honors thesis.

References

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Proposed Budget

I will be staying in Ithaca for my summer research project, but I will be flying home to California to see my family at the start of the summer. Thus I will need to fly back to Ithaca. I estimate a plane ticket from Los Angeles to Ithaca to be approximately \$300. I will be staying in my current apartment until the end of July when my lease ends and then I will be moving into my apartment for next semester. My current rent is \$380 a month and my rent for next year will be a bit more at \$595 since I am moving to Collegetown. I estimate approximately \$60 for utilities each month and \$50 a month for local travel expenses including the bus and gas for my car. I anticipate needing approximately \$60 a week for food, which amounts to \$240 a month. I know that Dean's Scholar funding is up to \$2500 and I hope to apply for the remaining funds through Cornell Tradition or to take a weekend job for the summer.

Calculation:

300	300
380 x 2	760
595	595
60 x 3	180
50 x 3	150
<u>240 x 3</u>	<u>720</u>
Total	\$2705