Please describe your significant research experiences. In your statement, please specify your research supervisor's name and affiliation, the duration of the experience, the nature of the problem studied, and your contributions to the project. The available space is 10,000 characters.

During the summer following my freshman year (2005), I worked full-time in Dr. Saraswati Sukumar's laboratory at Johns Hopkins Medical Institute. I studied the regulatory role of HOXB7 in the expression of parathyroid hormone-related protein (PTHrP) in breast carcinoma cells. HOXB7 is a newly defined oncogene that is highly expressed in breast tumors. My supervisor, postdoctoral fellow Dr. Xinyan Wu, found that HOXB7 levels increase in a step-wise manner from normal epithelial cells, to primary breast tumor cells, to bone metastatic breast tumor cells. Preliminary microarray data showed that PTHrP expression was upregulated in MCF10A cells, a non-tumorigenic breast epithelial cell line, 6 hours after transfection with an adenoviral vector containing a HOXB7 expression cassette. Since PTHrP plays a role in the malignancy of tumor cells, particularly in promoting severe osteolytic lesions in metastasis to bone, we sought to define the physiological and molecular relationship between HOXB7 and PTHrP.

To confirm the microarray expression data, I performed quantitative real-time PCR on several breast adenocarcinoma cell lines that were stably transformed with a retroviral vector that overexpressed HOXB7. I confirmed that increased HOXB7 expression resulted in increased PTHrP expression, which suggested that HOXB7 upregulated PTHrP.

I then examined the osteolytic effect of HOXB7 overexpression in tumors. I injected breast adenocarcinoma cells that expressed either wild type or elevated levels of HOXB7 into the tibia of SCID mice. Bone density was measured following tumor formation, which did not occur until 2 months after I returned to school. Dr. Wu informed me that the tumors generated from breast cancer cells expressing high levels of HOXB7 induced more bone resorption than tumors generated from control cells. This data demonstrated that HOXB7 directly or indirectly promoted osteolysis in breast cancer metastasis.

To test whether HOXB7 directly regulates PTHrP expression, I cloned several PTHrP promoter fragments into the pGL3 luciferase reporter plasmid. I cotransfected the reporter plasmids with either a HOXB7 or negative control plasmid into breast adenocarcinoma cells. HOXB7 did not drive increased luciferase expression through any of the PTHrP promoter fragments I tested. This suggested that either PTHrP was not directly regulated by HOXB7, or I failed to clone the regulatory region that responds to HOXB7. To further address the latter possibility, Dr. Wu and I planned to clone other possible regulatory regions for use in the luciferase assay and considered chromatin immunoprecipitation. Unfortunately, I was unable to pursue these options before the end of the summer. However, I learned many valuable techniques such as cell culturing, cell transfection, gene cloning, and PCR. More importantly, I learned how to generate a hypothesis, design and perform experiments, interpret results, and design subsequent experiments.

In the spring of 2006, I began a research project in Dr. David Hyde's lab at the University of Notre Dame. The Hyde lab studies mechanisms of retinal degeneration and regeneration in zebrafish. Unlike mammals, adult zebrafish regenerate retinal cells subsequent to cell death. Although retinal regeneration does not naturally occur in mammals, it may be possible to stimulate its occurrence using molecular signals that are identified to play a role in zebrafish retinal regeneration.

We expose adult zebrafish to high intensities of constant light to induce apoptosis of photoreceptors, which is detectable in TUNEL assays after 16 hours in constant light. By 31 hours of constant light, histology and immunolocalization of opsin proteins reveal an obvious loss of rod photoreceptors. After 96 hours, most of the rods and many of the cones are absent. During light treatment, we monitor the cell regeneration response by immunolocalizing proliferating cell nuclear antigen (PCNA). While there are few PCNA-labeled nuclei in the non-light treated control retina and in the first 16 hours of constant light treatment, there is a robust and significant increase in PCNA expression in the Muller glia by 31 hours. Between 51-96 hours of light, multiple neuronal progenitor cells, produced from the proliferating Muller glia, express PCNA and migrate to the outer nuclear layer (ONL), where they regenerate the lost photoreceptor cells.

In the spring of 2006, I conducted experiments to determine the minimum length of light exposure that was required to induce the regeneration response. I treated adult zebrafish for 0, 1, 12, 24, and 96 hours of constant light and then immunolocalized rhodopsin to monitor rod cell loss, and PCNA to detect proliferating progenitor cells, 96 hours after starting the light treatment. I found that 24 hours of light treatment was sufficient to induce rod photoreceptor loss and a robust progenitor cell proliferation response.

In the spring of 2007 (I studied in Dublin, Ireland in the fall of 2006), I continued my studies to determine the extent and rate of retinal regeneration following different lengths of light exposure. I exposed zebrafish to either 3 or 7 days of constant light and had a non-light treated control group. Rhodopsin and PCNA immunolocalization and retinal histology were performed 0, 7, 14, 21, and 28 days after starting the constant light treatment. I found that retinas isolated 7 days after starting the constant light treatment showed significant rod and cone cell loss and an intense proliferative response, despite the extended light treatment. By 28 days after the start of either light treatment, retinas reached a maximal and nearly equivalent stage of regeneration. Thus, it appeared from these studies that once a robust regeneration response was induced, regeneration reached an equivalent recovery state.

This summer, I began focusing on the molecular mechanism underlying the regeneration response. When the Hyde lab performed a gene microarray experiment to identify genes involved in photoreceptor cell regeneration, it was found that olig2 expression began increasing after 51 hours of light treatment, and continued increasing through 96 hours. To confirm this, the lab showed that expression of an olig2:EGFP transgene, which consists of the olig2 promoter driving EGFP expression, began in the proliferating neuronal progenitor cells approximately 50-55 hours into the constant light treatment and persisted through 96 hours. Because Olig2 expression was not detected in differentiated photoreceptor cells, it may serve as one of the earliest markers for proliferating progenitor cells.

To confirm this transgene expression pattern, I am currently immunizing rabbits with conjugated peptides to produce an anti-Olig2 polyclonal antiserum for use in immunohistochemistry and immunoblots. I am also creating anti-Olig1 and anti-Olig3 antisera to localize these highly conserved proteins, which may be functionally redundant with Olig2.

Although Olig2 is known to regulate the neuronal or glial cell fate decision during CNS development in a variety of organisms, its role in retinal neurogenesis is unknown. Furthermore, no function has been assigned to Olig1 or Olig3. Thus, to study Olig function, I am knocking it down in both regeneration and development, given that regeneration may recapitulate developmental processes, and assessing a phenotypic response. To knock down Olig expression, I am using morpholinos, modified antisense oligonucleotides, which anneal to complementary

mRNA sequences and block protein translation. To study development, an anti-Olig morpholino and a control morpholino are independently injected into zebrafish embryos at the 1-4 cell stage and embryos are collected for analysis at 2, 3, and 4 days post-fertilization. I am using immunohistochemistry and histology to determine if Olig expression is reduced and if the retinal cell number or pattern is perturbed. To study retinal regeneration, an anti-Olig morpholino and a control morpholino are independently injected and electroporated into adult dark-adapted zebrafish eyes, which are then harvested following 0, 16, 31, 51, 68, and 96 hours of constant light treatment. Immunoblots are used to confirm a reduction in Olig protein expression in morphants compared to wild-type and control morphants. If the knockdown of individual Olig proteins has no effect, I will coinject two or three different anti-Olig morpholinos in case the Olig proteins possess redundant functions.

So far this summer, I conducted several trials of morpholino injections. I showed that the anti-Olig2 morpholino successfully knocks down EGFP expression in the Tg(olig2:EGFP) transgenic line and also disrupts motor neuron development, a known Olig2-mediated process. Confirming successful knockdown of Olig1 and Olig3 will require the development of the anti-Olig1 and anti-Olig3 polyclonal antisera. I am currently in the process of screening for a retinal phenotypic response when Olig is knocked down. I plan to continue this project through the coming academic school year.

When I entered Notre Dame in 2004 as a biology major, I had a general idea of the importance of scientific research, but only a vague notion of how it is conducted. Reflecting on my experiences, I learned that laboratory work is demanding. It requires careful planning, constant mastery of new skills, rigorous attention to detail, meticulous documentation, and an ability to look at results from many perspectives to interpret them. But I also learned that I truly enjoy this demanding endeavor. While the repetition of experiments or the generation of negative results can be frustrating, I love the intellectual challenge and the thrill of making a new discovery. This enjoyment makes me confident that I want to pursue a research career, and equally confident that I will succeed.