

UNDERGRADUATE RESEARCH AWARD APPLICATION

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Title of Proposed Project Pax6 and Neuron Differentiation
in the Developing Retina

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Department(s) Biology

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Enclosed with this application form:

Project description, Budget, Copy of your official transcripts, including a list of courses taken during Spring 2005 and Support letter(s).

Electronic submission is required. Submit via email as a single attached file in PDF format to the address shown below. Send the official transcripts by campus mail to UG Research (MS 435).

Dr. M. Saiidi, Director, Undergraduate Research Programs
Email: ugresearch@unr.edu

Applications must be received by 5:00 pm on Friday, March 04, 2005.

Pax6 and Neuron Differentiation in the Developing Retina

Michael Berberoglu

Department of Biology, Dr. Grant Mastick, mentor

Abstract:

This research focuses on the development of the retina and the specific genes involved in the timing and differentiation of retinal neurons. My focus is on the Pax6 gene, which plays a vital role in the development of the vertebrate eye. We must first show that Pax6 is necessary for the proper development of the eye. It will then be possible to study the function of other genes downstream of Pax6 that are involved in retinal neuron differentiation. This research will greatly expand the knowledge in the field of developmental neuroscience and will hopefully open the door to new treatments for nervous system-related diseases.

Introduction:

The eye is a very fascinating and intricate structure that allows for the successful visualization of the environment around us. The complex interactions between the visual system and the brain are mediated by the central nervous system, which makes the eye virtually a part of the brain. The research that I am currently performing in the field of developmental neurobiology deals largely with the timing and differentiation of neurons in the retina. The various neuronal types and their interactions with one another in the retina is a well defined system which we are thus using to further understand the timing and differentiation of neurons as mediated through particular sets of genes. The knowledge gained in this retinal system can thus be applied on a larger scale to the field of neuroscience and the current knowledge in that field.

The particular gene of interest is Pax6, a member of the Pax family of transcription factors that plays a vital role in the development of the brain, eye, and other organs in vertebrates. It has been shown that Pax6 is present in the developing mouse retina, and Pax6 knockout mice show significant defects in eye development (Ashery-Padan et al. 2001). Though Pax6 is necessary for proper neuron differentiation, it is not sufficient, as there are many other transcription factors and genes downstream of Pax6 (such as Hes1, Mash1, and NeuroD) that also play an important role in the process. Though Marquardt et al. 2001 proposed that there is no neuron differentiation at all in the Pax6 knockout embryo, it has recently been shown that this is not the case, as neurons in the developing retina express indicative neuronal markers in Pax6 knockout mice (Philips et al. 2005). In addition, Philips et al. 2005 were able to show that Pax6 regulates the timing of neuron differentiation as well. Turning off Pax6 at different stages of development results in differences in the timing and differentiation of neurons that are still not fully understood.

By further understanding the specific genes involved in retinal development and neuron differentiation, we will hopefully open the door to new treatments for nervous system-related diseases. Once the mechanisms of neuronal precursor cell differentiation are deduced, it will be possible to use stem cells, along with the upregulation of specific genes, to treat various neural degenerative disorders and allow the field of developmental neuroscience to progress further into the future.

Objectives:

To better understand the function of Pax6 and other genes in eye development, we propose to develop a specific loss-of-function strategy to knock down gene expression in chick

embryos. Since the development of the chick embryo is similar to the development of the human embryo, I will be using the chick embryo as a model system (since the results obtained can be applied to humans as well).

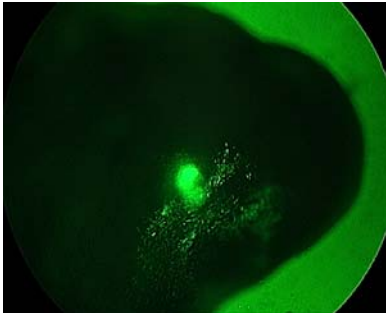
I will take two approaches to knock down Pax6 in chick embryos. It will be necessary to first show that knocking down the function of Pax6 produces the same phenotypic defects in chick eye development that have been previously shown in mouse.

Objective 1) I will attempt to show this by using an RNA interference approach to knock down the function of Pax6. RNA interference (RNAi) is an approach in which short double-stranded RNAs complimentary to a specific gene sequence are targeted to the desired cells in which the Pax6 loss-of function is being tested (Ambion, Inc. 2004).

Objective 2) I will also perform loss-of-function experiments using a dominant negative form of Pax6, Pax6/Engrailed repressor fusion, designed to directly interfere with the function of the Pax6 protein and thus block the subsequent transcription of genes.

Research Plans:

Objective 1) I will be using an RNAi Pax6 plasmid DNA construct that has been engineered with a specific sequence from the chick Pax6 gene. This plasmid will be microinjected and electroporated, along with a GFP plasmid, into the optic vesicles of chick embryos. The chick will then be allowed to grow another one to three days following electroporation in order for the RNAi construct to have time to knock down the function of Pax6.



The presence of GFP (green fluorescent protein) seen within the retina of these chick embryos (as shown in the picture) indicates that the GFP plasmid and the RNAi Pax6 plasmid were successfully taken up by those cells (and the protein is being expressed). After sectioning and antibody labeling these retinal tissue samples with the appropriate Pax6 antibody, we will be able to determine if there is a decrease in the level of Pax6 protein in these GFP-labeled cells. If a sufficient area of the retina obtains the plasmid constructs, a phenotypic defect will be

expected if the RNAi approach is properly working.

Upon successfully showing that knocking down the function of Pax6 does in fact produce defects in chick eye development, it will be possible to use RNAi to successfully knock down Pax6 expression in chick embryos at various stages of development in order to better understand the mechanisms of timing and differentiation of neurons in the developing retina. It will also be possible to use Pax6 to study the functions of other genes downstream of Pax6 that also play a role in the differentiation of neurons into various cell types.

If injection of the RNAi Pax6 plasmid construct does not successfully knock down the expression of Pax6, we will attempt to achieve RNA interference by constructing a double-stranded RNA that is complimentary to a specific sequence in the Pax6 gene. This double-stranded construct will be microinjected and electroporated into the cells, and the effects on Pax6 function will be determined in the same way. We are currently working on making the dsRNA construct for this part of the experiment.

Objective 2) If the above methods of RNA interference do not work for our purposes, it will be necessary to knock down the expression of these genes through other mechanisms such

as dominant negative mutations in chick. The dominant negative approach is based on the idea that a mutant protein will prevent the Pax6 transcription factor from successfully initiating transcription of the target gene. This may prove useful in further understanding the mechanisms by which Pax6 and other genes work in embryonic development.

Timetable:

Summer/Fall Semester: May 2005 – December 2005

- Continue microinjection and electroporation of Pax6 constructs in order to perform the necessary loss-of-function experiments showing that Pax6 is necessary for proper eye development in the chick embryo.
- I will be performing many experiments through injection and electroporation of various RNAi and dominant negative plasmids.
- The embryos that were successfully electroporated will be fixed and embedded.
- These embryos will then be sectioned using the chriostat and antibody-labeled using various antibodies to show that Pax6 is essential for proper retinal development.

Spring Semester: January 2006 – May 2006

- Once the Pax6 knock-down experiments are successful, I will be able to investigate the role that other genes play in the timing and differentiation of neurons in the retina.
- This will also be done in chick through the injection and electroporation of various RNAi and dominant negative constructs in chick that are specific for the known downstream genes.
- The downstream genes of interest are Hes1, Mash1, and NeuroD to name a few.

Dissemination of Results:

The results obtained in this researcher endeavor will be used in the writing of a research article that will hopefully be published in a major scientific journal. I would also like to have the experience of presenting my findings at a conference or scientific meeting once I obtain the results that I am now working on. The research that I am performing will thus add to and expand the current knowledge in the field of neuroscience.

Qualifications:

I am currently a fourth year Biology major with a 3.98 cumulative GPA (4.0 in Biology). I began working in Dr. Mastick's lab during the summer of 2004 and continued my research through the fall of 2004 and now the spring of 2005. By the end of the Spring 2005 semester, I will have completed my senior thesis in Biology and will have fulfilled the requirements for the UNR Honors Program as well as the Biology Degree with Distinction. In the preparation for my graduate studies in Cell and Molecular Biology, I will be spending one more year at UNR as a full-time student and will also continue to work on my research project in Dr. Mastick's lab during that year. The experience that I have obtained in the laboratory over the past year has allowed me to work single-handedly on this research project with the guidance of my instructor and mentor, Dr. Grant Mastick. In addition, I just recently became an author of a paper that has been published which includes some of the experiments that I performed (Philips et al. 2005). I thus feel very well qualified to continue with my research project during the 2005-2006 academic year.

Budget:

Performing the experiments needed to fulfill the goals of this research project will require the following supplies.

Item	Quantity	Price per Unit	Total
Eggs (in dozens)	35	\$7.50	\$262.50
Antibodies	2	\$200.00	\$400.00
Platinum wire (for electrodes)	1	\$175.00	\$175.00
DNA preparation (Maxiprep Kit)	1	\$150.00	\$150.00

Request: \$987.50

I also hope to present my findings at a conference during the Spring 2006 semester. Any of the remaining funds that are not used for laboratory supplies will thus go toward the travel expenses.

References:

- Ambion's Online Appendix. 2004. The Mechanism of RNA Interference (RNAi).
www.ambion.com/techlib/append/RNAi_mechanism.html.
- Ashery-Padan, R. and Gruss, P. 2001. Pax6 lights-up the way for eye development.
Current Opinion in Cell Biology 13:706-714.
- Marquardt, T. et al. 2001. Pax6 is required for the multipotent state of retinal progenitor cells.
Cell 105:43-55.
- Philips, G. et al. 2005. Precocious retinal neurons: Pax6 controls timing of differentiation and determination of cell type. *Dev. Biol.* 279:308-321.