University of Wisconsin-Oshkosh Student/Faculty Collaborative Grant

“Possible control of mitochondrial populations by PGC-1α in the 13-lined ground squirrel retina”

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Abstract: Mitochondria are known as the “power houses” of the animal cell as they convert chemical energy from fuel into a form usable by the cell. This conversion unfortunately also creates “reactive oxygen species” (ROS) that can damage cells so that they malfunction or even die. Many diseases, including those of the eye, are caused or worsened by an accumulation of ROS. Cells, including mitochondria themselves, combat ROS by making molecules known as antioxidants. A key protein, PGC-1α, has been shown to induce production of both mitochondria and the antioxidants within them, making it a novel therapeutic target to treat diseases associated with the accumulation of ROS.

The 13-lined ground squirrel is an animal model used in my mentor’s lab for research of the retina. Research previously done here shows that the photoreceptors of the retina change the number and activity of mitochondria over an annual cycle. In the retinas of summer-active squirrels, the number of photoreceptor mitochondria is high. During winter hibernation, in contrast, the number of mitochondria is low as is their energy-generating activity.

The PGC-1α cell signal pathway is a likely mechanism by which the regulation of mitochondria is controlled. I hypothesize that the decrease in mitochondria during hibernation is associated with low levels of PGC-1α and that the increased number of mitochondria during the summer is due to high levels of PGC-1α.
**Research Problem:** Mitochondria are an essential part of most cells in the human body. The mitochondria are responsible for energy production from fuel molecules, using oxygen in this process, but also generating “reactive oxygen species” (ROS) as by-products (Barnstable, 2009). Also known as “free radicals”, ROS damage cell proteins, membranes, and even the genetic material. In excess, ROS can kill the very cells that produce them, so mitochondria produce antioxidants that serve to detoxify ROS (Barnstable, 2009).

Loss of mitochondria, or even just their function, is life-threatening due to energy starvation; hence “mitochondriopathy” is the core of many diseases (Swerdlow, 2009), including those of the retina. However, excessive mitochondrial activity is also a problem, since the normal ROS detoxification mechanisms can be swamped. For both reasons, the pharmaceutical industry is very interested in the cellular pathways that control the number and function of mitochondria in cells.

Recently, a collaborator working in muscle showed that the number of mitochondria of winter-hibernating ground squirrels skyrockets above what is seen in summer-active animals (R. Cohn, personal communication). This increase is not to avoid energy starvation, since the muscles aren’t used during hibernation; but is instead to protect the inactive animal from accumulated ROS. The research team found that a key part of the signal pathway controlling this increase in muscle mitochondria involves a protein known as PGC-1α, a “transcription factor” that controls gene function (Huiyun & Ward, 2008).

Our interests in mitochondria are two-fold. First, increasing mitochondrial numbers -- as seen in hibernating muscle -- might be a viable therapy for retinal
mitochondriopathies such as diabetic retinopathy. Second, in contrast to hibernating muscle, we’ve seen the opposite effect of hibernation on squirrel retina: in winter, mitochondria decrease in both number and function, only to recover to normal levels in spring (Figure 1) (Gruber et al., 2006). The principle of parsimony would suggest that the same PGC-1α signal pathway controls mitochondria in squirrel muscle and retina, but in opposite fashion.

Therefore, I propose to test the hypothesis that, in squirrel retina, PGC-1α controls mitochondrial number and function across the annual cycle of hibernation and activity. I predict that the amount of PGC-1α protein in the retina will decrease in winter, concurrent with lower mitochondrial numbers (Fig. 1), but will increase in spring, when mitochondrial numbers recover.

A literature search reveals that only in the last two or three years are vision scientists thinking of PGC-1α in terms of high impact diseases such as macular degeneration (e.g. Del V Cano & Gehlbach, 2008), but no one has yet examined its specific role in mitochondrial control within the retina. That will make this project novel and innovative.

**Research methodology:** We already have IACUC approval to use squirrels from the UW-Oshkosh breeding colony for this project and I am already animal certified. My
methods amount to measuring how much \( \text{PGC-1}\alpha \) is being synthesized by retinal cells at given times during the year.

To synthesize \( \text{PGC-1}\alpha \), its gene (made of DNA) is first transcribed into a “messenger RNA” (mRNA). Then the mRNA is translated into the \( \text{PGC-1}\alpha \) protein. To most accurately gauge the synthesis of \( \text{PGC-1}\alpha \) over the year, it is thus necessary to quantify both of these stages. I will quantify \( \text{PGC-1}\alpha \) mRNA using qPCR and \( \text{PGC-1}\alpha \) protein using Western blots.

As animals are sacrificed, I will place their retinas into an ice-cold solution specially designed to preserve both mRNA and protein so that measured levels are accurate. From here on out, I will use the identical lab procedures that were successful on squirrel muscle (Cohn, personal communication), but on my retina samples. From mRNA that is purified by a series of standard steps, I will perform qPCR using instruments in our department to measure the amounts of the \( \text{PGC-1}\alpha \) mRNA in winter, spring, and summer samples; Figure 2 shows my prediction for the data. From protein samples also purified in a standard manner, I will use Western blots to measure the amount of actual \( \text{PGC-1}\alpha \) in those samples as well; my predictions follow the same pattern shown in Figure 2.

![Graph showing the change in quantity of PGC-1α being expressed in the different physiological states of the ground squirrel](image)
Link to further educational experience: The proposed project is commensurate with the skills I have obtained during my undergraduate career at UW-Oshkosh as well as a graduate student. I have already successfully carried out qPCR and Western blots in lab classes and other research projects. Where this project has its biggest impact on my scientific development is its possibility for being “translational”, meaning “from bench to bedside”. Cell signal pathways, such as PGC-1α, are highly fundable by research agencies but are also the major lines of inquiry in drug discovery research. Completion of this project will situate me well for continuing graduate education in molecular biology and/or a position in the biotechnology industry.

Timeline for completion: There will be some work done already this winter and spring, ahead of funding, solely to collect tissue samples that I will work on during the 8 weeks of the summer grant award.

- February 2010: Collect and freeze retinas Hibernating squirrels (N = 3).
- Upon award: Order non-perishable supplies (primers, gels, antibodies.).
- April 2010: Collect and freeze retinas from Spring-Emergent animals (N=3).
- June 2010 (project formally starts): Collect and freeze retinas from Summer-Active squirrels (N = 3). Isolate mRNA, make cDNA, perform qPCR on all samples to measure mRNA of PGC-1α and a housekeeping “negative control” gene. Also contribute to animal care.
- July 2010: Isolate protein, run on gels, blot onto membranes, perform Western blots to detect PGC-1α protein. Also contribute to animal care.
- Early August 2010: Write my report in Master’s thesis and abstract submission form.
**Expected outcomes:** In addition to writing the required project report, in spring of 2011 I will present this project at Celebration of Scholarship day. In December of 2010, I will submit my results as an abstract for presentation at the May 2011 Association for Research in Vision & Ophthalmology annual meeting or, if funds for graduate student travel are no longer available due to budget cuts, at the March 2011 Chicago Society for Neuroscience annual meeting. The data collected will also be added to the research done for my Master’s thesis.

**Budget justification for supplies:**

<table>
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<th>Products</th>
<th>Price</th>
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<tr>
<td>TRIzol RNA extraction kit</td>
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<td>Turbo DNA free kit</td>
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<tr>
<td>Primers for DNA synthesis</td>
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**Total**

$415.50*

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*The requirement of shipping these reagents on ice will mean that the total cost of reagents will approximate $500. Our collaborator will gift us the antibody needed for the Western blots so no further expenses are needed there.
References:


Statement of Mentor’s Support

I am willing to serve as this student’s research mentor for the project described here. I have known this student since his junior undergraduate year; instructed him in two classes in that time frame; and he also successfully conducted research using the methods proposed here in my lab last summer. He has shown great ability to troubleshoot the procedures and knows where the pitfalls lie already. He has had several courses in advanced molecular biology that taught him about cell signal pathways, which represent a new direction for my lab that hopefully captures the reported attention of funding agencies said to currently prefer cell signaling grants.

My direct support of the project will be in terms of providing animals, arranging the gift of needed reagents from my collaborator Ronald Cohn MD at Johns Hopkins Medical Institute, and editing the final paper.