

# Salus University

## Annual Progress Report: 2008 Formula Grant

### Reporting Period

July 1, 2009 – April 30, 2010

### Formula Grant Overview

Salus University received \$60,332 in formula funds for the grant award period January 1, 2009 through April 30, 2010. Accomplishments for the reporting period are described below.

### Research Project 1: Project Title and Purpose

*Molecular Mechanisms of Leber's Congenital Amaurosis* - Leber's congenital amaurosis (LCA) is an early-onset loss of vision that constitutes a group of genetically diverse inherited retinal disorders, often resulting in complete blindness. Severe forms of LCA result from mutations in an enzyme called retinal guanylyl cyclase 1 (RetGC1). Two new mutations in RetGC1 were recently found in patients diagnosed with LCA, and the preliminary biochemical characterization of the mutant enzyme revealed strong abnormalities in its activity and regulation. The purpose of this project is to conduct a pilot short-term study of the possible physiological implications of these two mutations using transgenic mice in order to establish their relevance to the onset of LCA.

### Duration of Project

5/1/2009 - 4/30/2010

### Project Overview

The broad research objectives related to this pilot project is to establish molecular and genetic mechanisms linking signal transduction abnormalities and photoreceptor cell death in the retina that results in blinding congenital disorders. The specific aim of this pilot study is to investigate physiological consequences of two new mutations found in patients diagnosed with Leber's congenital amaurosis (LCA), an early-onset severe form of retinal degeneration. One of the frequent targets for LCA-linked mutations is a photoreceptor-specific protein, called retinal guanylyl cyclase 1 (retGC1). The two mutations found in retGC1 of LCA patients and called D639Y and R768W alter biochemical properties of retGC1 in vitro, but their physiological consequences and the role in etiology of the LCA remain unclear. We, therefore, plan to express the mutant retGC1 in retinas of transgenic mice and determine physiological function, viability of photoreceptors and morphological organization of the retina in transgenic mice in order to establish a possible mechanistic link between the mutations and the photoreceptor cell death and/or signal transduction abnormalities they may cause in vivo.

## **Principal Investigator**

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## **Other Participating Researchers**

Andrey Savchenko, PhD - employed by Salus University

## **Expected Research Outcomes and Benefits**

Congenital blinding disorders have diverse genetic links within various individual groups of the genetic retinal diseases, and Leber's congenital amaurosis (LCA) is one of such diseases. Progress in understanding some of the LCA-linked mutations recently led to the first clinical trials abroad attempting gene therapy of retinal disorders. The outcome from these early attempts supported the general feasibility of genetic therapy as a way of treating LCA and similar disorders, but also underscored the importance of better understanding the fundamental mechanisms behind the LCA-associated photoreceptor cell death in the retina. Some particularly severe forms of LCA have been linked to mutations affecting guanylyl cyclase, RetGC1. Characterization of new mutations found in the RetGC1 gene of LCA patients can increase the general knowledge of the LCA mechanisms and visual signal transduction abnormalities and specifically evaluate possible contribution from these particular mutations in photoreceptor cell death. Establishing such information is expected to benefit future studies designed to find approaches to treating the inherited blinding diseases by means of genetic therapy.

## **Summary of Research Completed**

The purpose of this short pilot exploratory project during this reporting period was to develop the means for the subsequent in-depth long-term study of the mutations in retinal guanylyl cyclase (RetGC1) found in patients with Leber Congenital Amaurosis, an early-childhood onset of blindness.

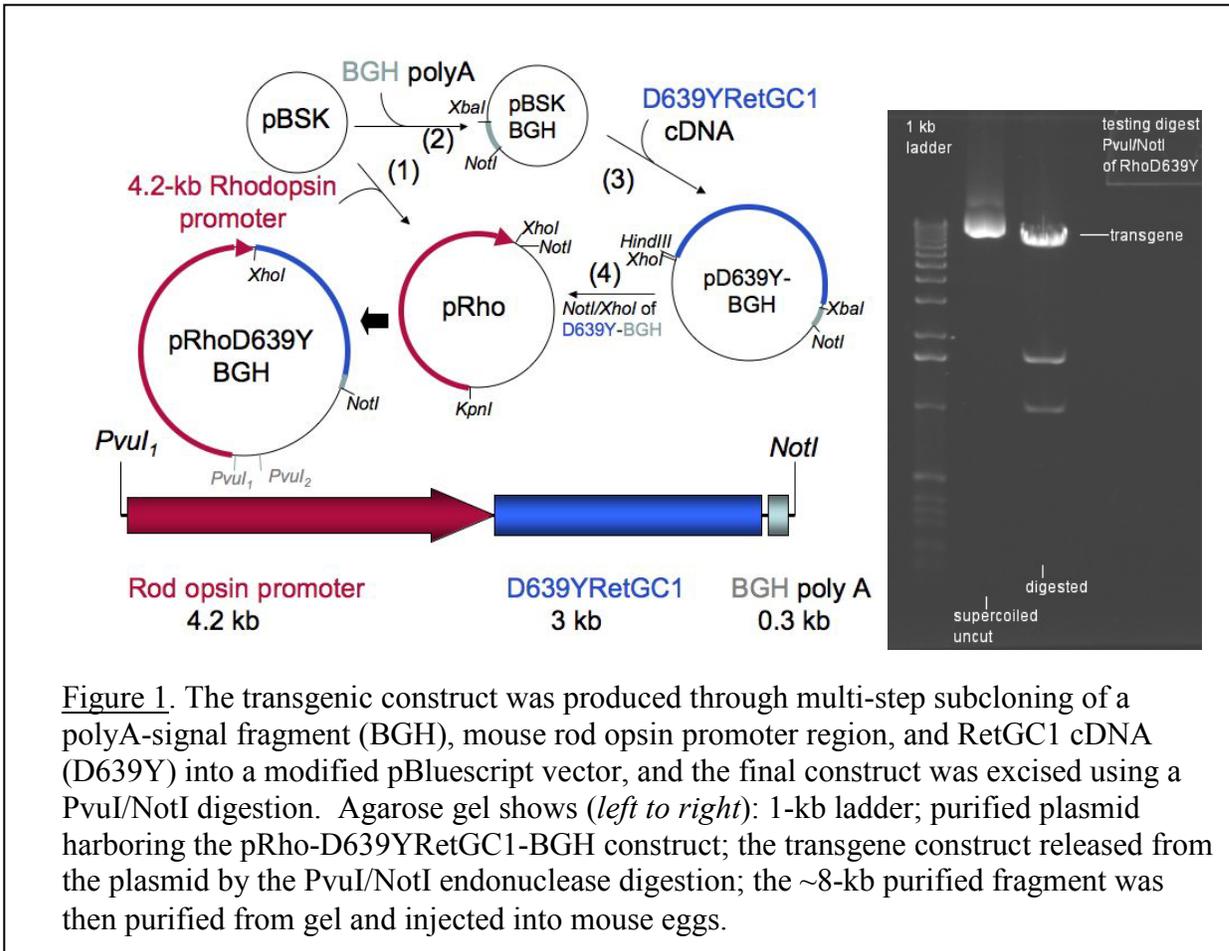
- We have completed biochemical characterization of the D639Y and R768W mutations of RetGC1 in vitro and described it in a peer-review publication demonstrating the lack of activation of the mutant RetGC1 and its failure to bind GCAP1.
- We produced genetic constructs for expression of the modified RetGC1 carrying these mutations in mouse retinas under control of rod opsin promoter (Figure 1).
- We have started injections of a construct coding for the D639Y RetGC1 mutant in mouse eggs. The injections are to be completed by the end of June 2010. The first litter of the F0 pups from the injections is due July 7, 2010.

- We have also optimized the tail DNA PCR screening test for the presence of the transgene sensitive to ~ 1 copy per genome (Figure 2).
- We have established several breeding colonies of mouse lines (produced by the PI group or obtained from other sources) that we will use in breeding of the mutant RetGC1 transgene into various genetic backgrounds once the germ-line transmission of the transgene is confirmed:
  - GCE null (mouse line originally produced by Dr. D. Garbers) – this line has no endogenous RetGC1 isozyme expression and it will be used for testing the levels of the D639Y RetGC1 expression when transferred into wild type RetGC1<sup>-/-</sup> genotype (otherwise it would be impossible to estimate the level of the mutant cyclase expression by immunoblot) and possibly use for breeding heterozygous wild type RetGC1<sup>+/-</sup> background in order to mimic or approximate normal complement of the total RetGC1 expression levels. This line will also be used to evaluate the effect of relative levels of expression for the wt and the mutant RetGC1.
  - Gucy2F<sup>-/-</sup> (originally developed by Dr. W. Baehr) – this line is lacking the second isozyme of RetGC2 and will be used to test retGC2 as a possible modifying factor for the D639Y-related phenotype.
  - Double Gucy2f/GCE knockout
  - Guca1A<sup>-/-</sup> and Guca1B<sup>-/-</sup> (both developed in the PI's lab) – these gene knockout lines are lacking guanylyl cyclase activating proteins, GCAP1 or GCAP2, respectively, and they will be used for studying possible modifying effects of the guanylyl cyclase activating proteins on the D639Y phenotype.

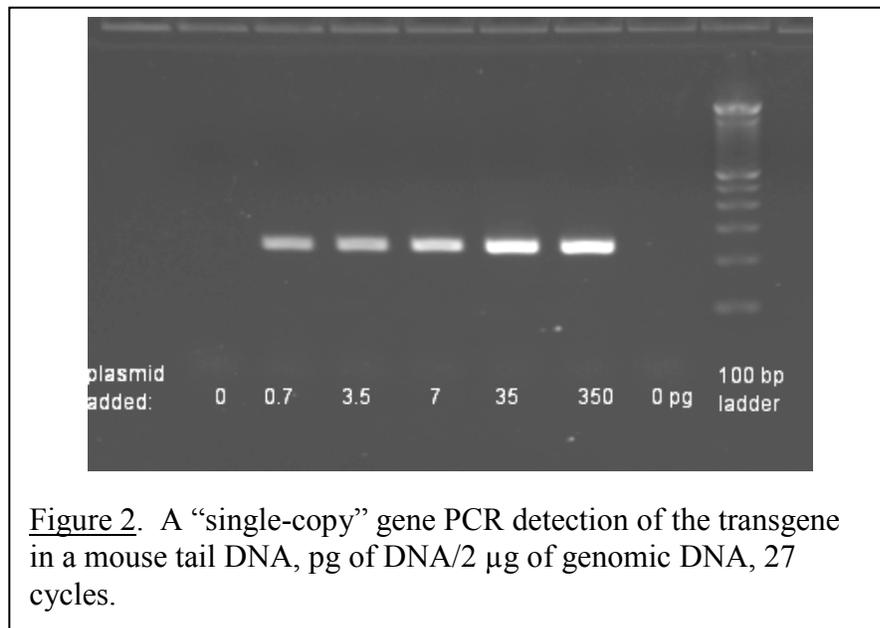
*Publication:*

Peshenko IV, Olshevskaya EV, Yao S, Ezzeldin HH, Pittler SJ, Dizhoor AM (2010) Activation of retinal guanylyl cyclase RetGC1 by GCAP1: stoichiometry of binding and the effect of new LCA-related mutations. *Biochemistry* 49(4):709-17.

Figures



**Figure 1.** The transgenic construct was produced through multi-step subcloning of a polyA-signal fragment (BGH), mouse rod opsin promoter region, and RetGC1 cDNA (D639Y) into a modified pBluescript vector, and the final construct was excised using a PvuI/NotI digestion. Agarose gel shows (left to right): 1-kb ladder; purified plasmid harboring the pRho-D639YRetGC1-BGH construct; the transgene construct released from the plasmid by the PvuI/NotI endonuclease digestion; the ~8-kb purified fragment was then purified from gel and injected into mouse eggs.



**Figure 2.** A “single-copy” gene PCR detection of the transgene in a mouse tail DNA, pg of DNA/2 μg of genomic DNA, 27 cycles.