Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution: Salus University**

2. **Reporting Period (start and end date of grant award period): 01/01/10-04/30/11**

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees): Alexander M. Dizhoor, PhD**

4. **Grant Contact Person's Telephone Number: 215-780-1468**

5. **Grant SAP Number: 4100050908**

6. **Project Number and Title of Research Project: 1 – Mechanisms of Signal Transduction and Degenerative Diseases in the Retina**

7. **Start and End Date of Research Project: May 1, 2010 – April 30, 2011**

8. **Name of Principal Investigator for the Research Project: Alexander M. Dizhoor, PhD**

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $ 59,882.10

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Savchenko	Research Associate	100%	$46,666.72

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Dizhoor	Professor	10%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None	None	None

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No__X__

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No__X___

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify:		$	$

	_____)			

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_X__ No_____

If yes, please describe your plans:

This was a pilot low-budget short-term project designed to obtain the means for subsequent in-depth research. The goal was to create a mouse model that harbors a mutation in retinal guanylyl cyclase with altered enzymatic properties - guanylyl cyclase to adenylyl cyclase. We have successfully generated 9 transgenic F0 founders (see PCR genotyping attached) and began breeding them into a genetic background lacking the endogenous guanylyl cyclase. Once we have the best expressing model selected, we will conduct full-scale investigation of the impact on biochemistry and morphology of the affected retinas and publish the findings in a reputable peer-review journal, as we did before following the Formula Grant supported projects.

12. Future of Research Project. What are the future plans for this research project?

The genetic model we create will offer unique possibility to place cyclic AMP production in photoreceptors under control of light and calcium concentration changing as a result of light versus dark exposure. This will allow us to evaluate what cyclic AMP-dependent processes specifically relate to photoreceptor biology and survival. This may open a new direction of study down the road. After obtaining compelling preliminary data, we will apply for federal funding to continue this research.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No__X___

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				

Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No__X____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes__X___ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

We have developed a unique genetic model with altered cyclic nucleotide metabolism in the retina. We will use it for a new line of studies that otherwise would be impossible to conduct.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_X__ No_____

If yes, please describe the collaborations:

The genetic model created in this project will result in collaborative study using single-cell physiology conducted in Harvard Medical School

16(B) Did the research project result in commercial development of any research products?

Yes_____ No__X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No__X___

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

A variety of congenital blinding disorders arise from or are accompanied by the abnormalities in metabolism of cyclic nucleotides in retinal photoreceptors (Perrault et al., 2000, 1998; Travreso et al., 2002; Weiss et al., 1995; Woodruff et al., 2007). The main long-term purpose of this project is to study the role of different cyclic nucleotides in survival of mammalian photoreceptors. Specifically, we want to introduce a light-regulated adenylyl cyclase in mouse rods in order to assess the role of cAMP in photoreceptor survival and function. The main strategy to achieve that goal is to turn a photoreceptor membrane guanylyl cyclase (RetGC) into adenylyl cyclase with mutations described in the literature that are capable of changing substrate specificity from GTP to ATP (Tucker et al., 1999; Ramamurthy et al., 2001). RetGC in photoreceptors is accelerated by Ca2+-sensitive proteins, GCAPs, in response to light and decelerated by GCAPs in the dark. Therefore, we plan to change the specificity of the cyclase reaction while still retaining normal physiological regulation of it by light and calcium via GCAPs (Burns et al., 2002; Mendez et al., 2001; Makino et al., 2008). The present work supported by the PDH Formula grant was designed as a short-term pilot project to become the first step in that direction – to provide the necessary genetic construct and generate mouse model.

This short-term pilot project was conducted in 2010-2011, effectively starting on May 1, 2010 (total effective duration-14 months). We had two specific aims for that short period:

Aim 1. To make a genetic construct coding for a mutated guanylyl cyclase (retGC1) turned into adenylate cyclase (RetGC1AC) and target its expression to mouse rods.
Aim 2. To produce transgenic RetGC1AC mouse line and breed it into a genetic background lacking the endogenous photoreceptor guanylyl cyclase, such that it would specifically allow regulation of cAMP synthesis by the light- and calcium-sensitive mechanisms that normally control cGMP synthesis in rods.

During the 14-months period of the project, we accomplished the main portion of the research plan (the entire Aim 1 and most of the Aim 2): we constructed the transgenic vector, verified the biochemical phenotype of the cyclase mutant encoded by this construct in a series of transfection experiments that involved other mutants of the same cyclase as controls for specificity, injected the transgene in mouse eggs and selected 10 positive F0 founders out of 53 mice born from the injected eggs. We are currently already breeding them into RetGC1 knockout background. The breeding has not reached the point when the transgene is in the background of a complete null of RetGC1, but this step can be realistically achieved within following months. At which point we will be able to quantify the expression of the transgene in different lines and select the best expressing line(s) for the subsequent in-depth study. The detailed description of the results follows.

i) Progress toward Aim. 1. DNA construct and adenylyl cyclase activity verification.

For the construction of RetGC1AC cDNA, we incorporated mutations described by Tucker et al., 1999, E925K and C995D, into human RetGC1 cDNA using a "splicing by overlap extension" technique. The sequence of the mutated cDNA fragment was verified by automated DNA sequencing and then DNA was subcloned into the NarI/NheI restriction sites of the human RetGC1 cDNA-containing modified pRCCMV plasmid (Laura et al., 1996) thus replacing the portion of the wild type human RetGC1 coding for catalytic domain of the cyclase. The resultant RetGC1AC in pRCCMV vector was verified by DNA sequence of the entire cDNA on both strands. We then subcloned the cDNA from that vector to assemble the transgene construct in a pBluescript vector placing RetGC1AC cDNA under control of a 4.2-kb rhodopsin promoter that we previously used for targeting protein expression to mouse rods. The only difference from the original plan was the use of polyadenylation signal – we chose to replace protamine 1 gene fragment by a standard BGH polyadenylation signal from pQBI plasmid, as most of our expression verification experiments conducted in HEK293 cell utilized that control element. The construct was verified by restriction nuclease digest and sequencing, amplified in E. coli, purified, and the transgenic insert was excised from the plasmid by NotI/PvuI digest as shown in the Fig. 1, after which the transgene fragment was gel purified for injection into mouse eggs.

Before we proceeded with the injections, we needed to verify the biochemical phenotype of the RetGC1AC mutant in our hands. The same cDNA fragment coding for RetGC1AC was expressed from pRCCMV vector under control of CMV promoter using a Promega Profection protocol for transfection, and the expression of the modified cyclase was confirmed by immunoblotting

We then established that the mutant RetGC1AC binds GCAP in the same manner as the wild type RetGC1 does (Fig. 2), causing GFP-labeled GCAP1 in living cells to associate with the membranes expressing RetGC1 and RetGC1AC cyclases alike.

The RetGC1AC activity was assayed in membrane fraction of HEK293 cells with [α-^{32}P]ATP as a substrate instead of [α-^{32}P]GTP that we normally use for wild type RetGC assays (Makino et al., 2008; Peshenko et al 2010, 2011). The products of the reaction were separated by thin-layer chromatography on fluorescent plastic-backed polyethyleneimine cellulose plates and [^{32}P]cAMP was quantified from the excised spots on chromatogram by radioactive scintillation counting (Fig. 3).

We have confirmed that the mutant RetGC1AC encoded by the transgene construct indeed demonstrated specific GCAP-dependent stimulation of cAMP synthesis instead of cGMP. The assay was conducted in the presence of EGTA at 10 mM free Mg concentrations, conditions optimized previously for studying wild type recombinant RetGC activation by GCAPs. While there was little adenylyl cyclase activity detected in the absence of GCAP1, in the presence of 10 µM GCAP1 cAMP activity in the same membrane preparation was sharply increased and reached over 7 pmol cAMP/mg protein/min (Fig. 3 panel a). We did not observed this effect in several control preparations expressing RetGC1AC (panels b-d): when the catalytic activity of the recombinant cyclase was inactivated by additional mutation that blocked Mg^{2+} binding in the active site (Tucker et al., 1999), which completely prevents the binding of the MgATP as a substrate (Fig. 3b); when RetGC1AC

contained a mutation, D639Y (Fig 3 c) which blocks GCAP1 binding to RetGC1 (Peshenko et al., 2010); when we expressed wild type RetGC1, Fig. 3 d (the wild type RetGC possesses only guanylyl cyclase activity and does not utilize ATP as a substrate, Tucker et al., 1999). Making these additional mutants consumed substantial effort, but we wanted to ensure that the transgene we were going to deliver to the retina would impart the exact activity we expected. We can rely on the results shown in Fig. 3 that the RetGC1AC in the transgenic construct would impart to mouse rods the unique ability to accelerate cAMP synthesis in the presence of GCAP, which would normally regulate only cGMP synthesis in photoreceptors (Dizhoor et al., 2010).

ii) Progress toward Aim 2. Production of transgenic mice.

From the eggs injected with the purified transgene fragment, there were 53 mice born, which we screened by PCR of their tail DNA samples using specific primers designed for the unique human RetGC1 DNA sequence, not present in mouse RetGC1 gene (in addition to that, the transgen is a cDNA, without multiple introns present in the endogenous RetGC1 gene). The specificity of the screening is shown in Fig. 4 (upper panel), where littermates either did or did not carry the transgene. We have identified 11 positive mice (one is not shown in Fig. 3), which we consider a reasonably confident number for selection of proper expressor line. However, in order to compare the expressing lines by immunoblot of retinal proteins, we need first to transfer the transgene into a genetic background lacking the endogenous RetGC1 expression. We receive such a line from Dr. Garbers laboratory in U. of Texas (Yang et al., 1999) several years ago and maintain it since that time in our vivarium at Salus University (we have just published in the Biochemistry a study using that strain, Peshenko et al., 2011). We already set up the breeding of the F0 RetGC1AC mice with the RetGC1 knockout line and should be able to accomplish this process within several months, at which point we expect to select at least one mouse from each line for initial comparison of the transgene expression levels.

REFERENCES

Burns ME, Mendez A, Chen J, Baylor DA (2002) Dynamics of cyclic GMP synthesis in retinal rods. Neuron 36: 81-91

Makino CL, Peshenko IV, Wen XH, Olshevskaya EV, Barrett R, Dizhoor AM (2008) A role for GCAP2 in regulating the photoresponse. Guanylyl cyclase activation and rod electrophysiology in GUCA1B knock-out mice. J Biol Chem 283: 29135-29143

Mendez A, Burns ME, Sokal I, Dizhoor AM, Baehr W, Palczewski K, Baylor DA, Chen J (2001) Role of guanylate cyclase-activating proteins (GCAPs) in setting the flash sensitivity of rod photoreceptors. Proc Natl Acad Sci U S A 98: 9948-9953

Dizhoor A.M., Olshevskaya E.V., Peshenko I.V. (2010) Mg2+/Ca2+ cation binding cycle of guanylyl cyclase activating proteins (GCAPs): role in regulation of photoreceptor guanylyl cyclase. Molecular and Cellular Biochemistry 334:117–124

Perrault, I., Rozet, J. M., Gerber, S., Ghazi, I., Ducroq, D., Souied, E., Leowski, C., Bonnemaison, M., Dufier, J. L., Munnich, A., and Kaplan, J. (2000) Spectrum of retGC1 mutations in Leber's congenital amaurosis. *Eur J Hum Genet 8*, 578-82.

Perrault, I., Rozet, J. M., Gerber, S., Kelsell, R. E., Souied, E., Cabot, A., Hunt, D. M., Munnich, A., and Kaplan, J. (1998) A retGC-1 mutation in autosomal dominant cone-rod dystrophy. *Am J Hum Genet 63*, 651-4.

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*)*.

Peshenko, I.V., Olshevskaya E.V., Savchenko, A.B., Karan, S., Palczewski, K., Baehr, W., Dizhoor, A.M. (2011) Enzymatic Properties and Regulation of the Native Isozymes of Retinal Membrane Guanylyl Cyclase (RetGC) from Mouse Photoreceptors. *Biochemistry* 50(25): 5590-600.

Ramamurthy, V., Tucker, C., Wilkie, S. E., Daggett, V., Hunt, D. M., and Hurley, J. B. (2001) Interactions within the coiled-coil domain of RetGC-1 guanylyl cyclase are optimized for regulation rather than for high affinity. J Biol Chem 276, 26218-26229.

Tucker, C. L., Woodcock, S. C., Kelsell, R. E., Ramamurthy, V., Hunt, D. M., and Hurley, J. B. (1999) Biochemical analysis of a dimerization domain mutation in RetGC-1 associated with dominant cone-rod dystrophy. Proc Natl Acad Sci U S A 96, 9039-9044.

Traverso V, Bush RA, Sieving PA, Deretic D. 2002. Retinal cAMP levels during the progression of retinal degeneration in rhodopsin P23H and S334ter transgenic rats. Invest Ophthalmol Vis Sci 43(5):1655-1661

Weiss ER, Hao Y, Dickerson CD, Osawa S, Shi W, Zhang L, Wong F. 1995. Altered cAMP levels in retinas from transgenic mice expressing a rhodopsin mutant. Biochem Biophys Res Commun 216(3):755-761.

Woodruff ML, Olshevskaya EV, Savchenko AB, Peshenko IV, Barrett R, Bush RA, Sieving PA, Fain GL, Dizhoor AM (2007) Constitutive excitation by Gly90Asp rhodopsin rescues rods from degeneration caused by elevated production of cGMP in the dark. J Neurosci 27: 8805-8815

Yang RB, Robinson SW, Xiong WH, Yau KW, Birch DG, Garbers DL (1999) Disruption of a retinal guanylyl cyclase gene leads to cone-specific dystrophy and paradoxical rod behavior. J Neurosci 19: 5889-5897

FIGURES

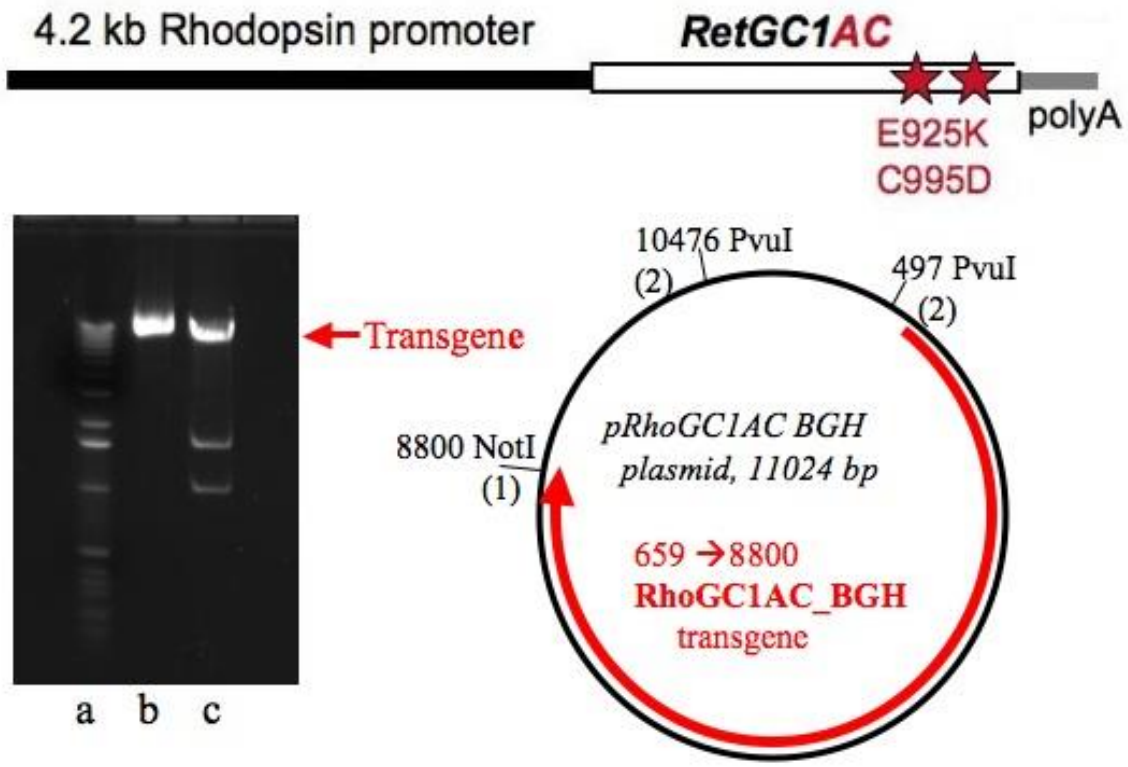

Fig. 1. The schematics of the transgene construct for RetGC1AC expression in mouse rods. The transgenic construct containing a 4.2 kb 5'-upstream fragment of mouse rod opsin gene (Rho), 3-kb RetGC1AC cDNA, and BGH polyadenylation fragment was assembled in a modified pBluescript vector and excised using NotI/PvuI digest releasing 8.2 transgene insert (gel: a – 1-kb ladder marker; b- uncut plasmid; c – digested plasmid). The insert purified from gel was injected in fertilized mouse eggs.

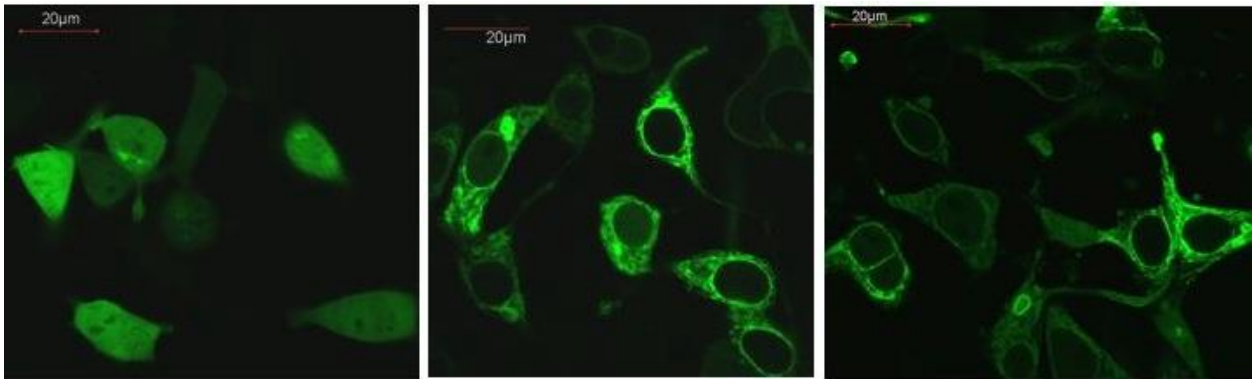

Fig. 2. GCAP1-GFP fluorescence is distributed diffusely throughout the transfected HEK293 cells when co-expressed with a negative-control RetGC1 construct truncated at kinase homology doman (*left panel*), but changes its localization to the endoplasmic reticulum containing the membrane RetGC1 when co-expressed with the wild type RetGC1 (*middle panel*). It maintains the same membrane-associated pattern when co-expressed with the RetGC1AC mutant (*right panel*) The cell-based assay was conducted as described in Peshenko et al., 2010.

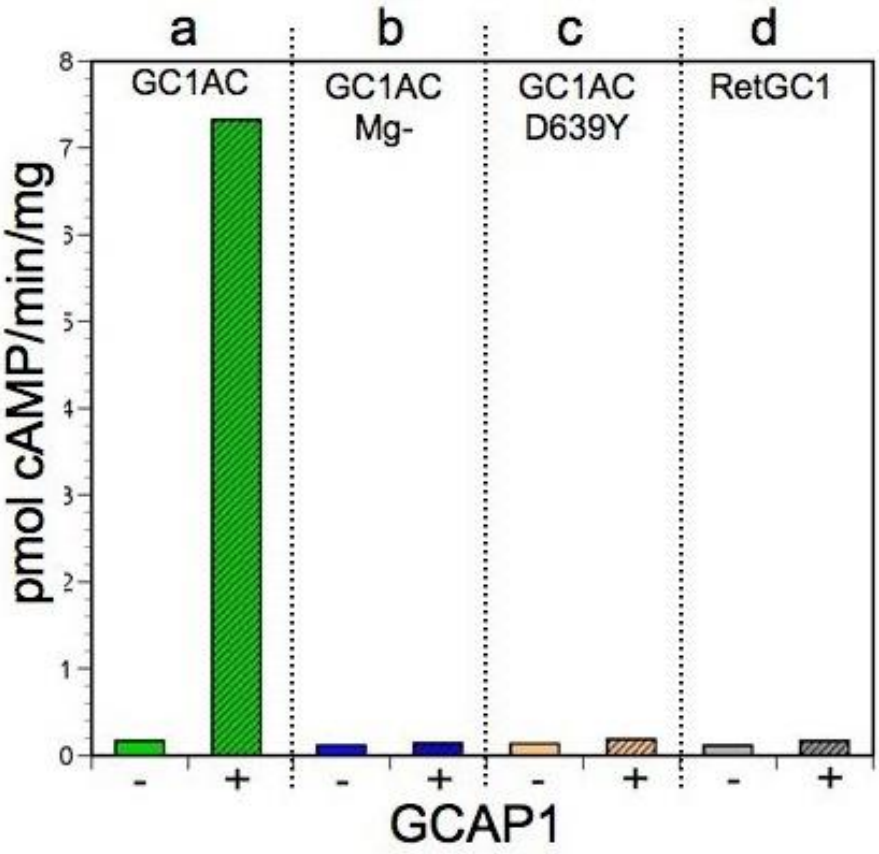

Fig. 3. RetGC1 mutants expressed in HEK 293 cells were assayed in the presence of 2 mM EGTA and 10 mM $MgCl_2$ in the absence (-) or in the presence (+) of 10 µM purified recombinant GCAP1; a- RetGC1AC, b-RetGC1AC with the Mg2+-binding site of the catalytic domain inactivated, c – RetGC1AC with the GCAP1 binding domain inactivated, d

- wild type RetGC1. The assay was conducted as descried in (Makino et al., 2008; Peshenko et al 2010, 2011), except that ATP was used as a substrate.

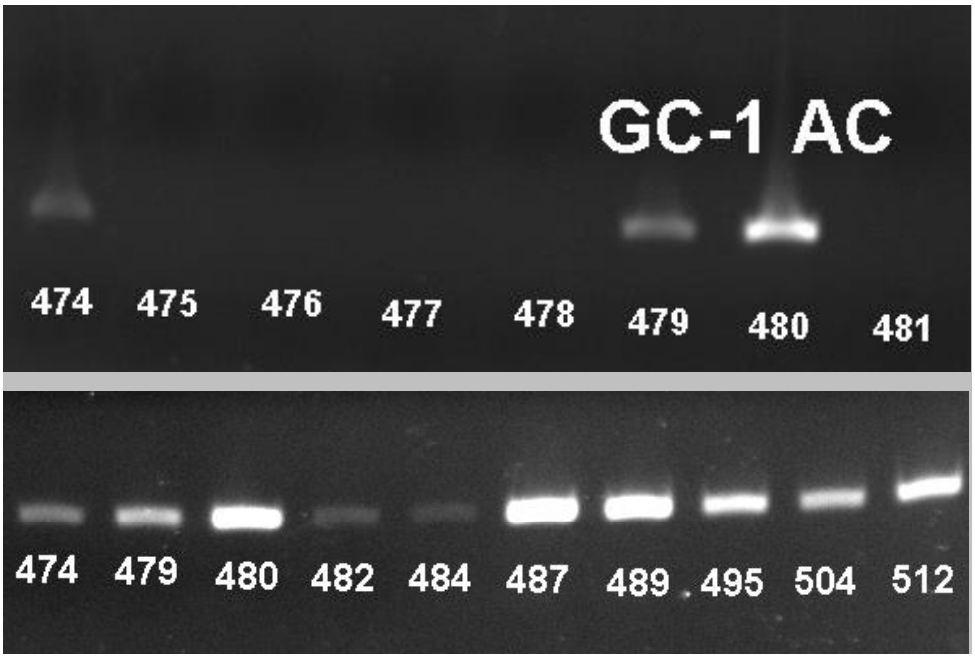

Fig. 4. PCR detection of the RetGC1AC transgene in F0 mouse tail DNA samples screened by PCR using primers specific for the human RetGC1AC mutant. Top: an example of the transgene detection in a single litter. Bottom: re-testing of all positives selected from screening of 52 F0 mice.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
_X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
_X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_X____ No_____

If yes, please describe your plans:

This was a very short-term project designed to create a particular genetic model. We will now conduct full-scale investigation of the impact of the light- and Ca^{2+}-controlled cAMP synthesis in photoreceptors on the biochemistry, morphology, and physiology of the retina and will then publish the findings in a reputable peer-review journal acknowledging the PDH support, as we did before following the Formula Grant supported projects.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.
None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.
None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No_____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

(NIH Biocketch format, short)

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Alexander M. Dizhoor	Professor of Pharmacology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, c*

INSTITUTION AND LOCATION	DEGRE	YEAR(	FIELD OF STUDY
Moscow University, Moscow, Russia (USSR)	BS	1977	Biochemistry, Virology
Moscow University, Moscow, Russia (USSR)	PhD	1985	Molecular Biology

A. Personal Statement.

I received my PhD in molecular biology from Moscow University, USSR, and went on my subsequent postdoctoral and junior faculty training in biochemistry of phototransduction at Moscow University, at CNRS in France (with Mark Chabre), and at University of Washington (with James Hurley and Ken Walsh). While working in Hurley's lab in HHMI, and subsequently as a junior faculty at University of Washington, I focused on Ca2+ binding proteins and cGMP synthesis in mammalian rods and identified and characterized several new proteins in photoreceptors - recoverin, GCAP2, and retinal guanylyl cyclases (RetGC1 and -2). Since having established my independent laboratory in 1997 at Wayne State University, I focus primarily on molecular mechanisms of calcium feedback in photoreceptors and genetic models related to human retinal diseases caused by its abnormalities. I presently continue the same line of study in Pennsylvania College of Optometry (since 2008 - at the fiunded by PCO Salus University), where I established my laboratory after having received the endowed Hafter Chair in Pharmacology. My laboratory, independently or through multiple collaborations, made the following contribution to the field. We established the molecular mechanisms underlying dominant-negative retinal degeneration caused by mutations in GCAP1 found in human patients. By creating transgenic mouse models expressing GCAP1 mutants in vivo, we demonstrated that photoreceptor cell death correlates with accumulation of cGMP and Ca2+ in outer segment in the dark and it can be averted by activation of the opposing reaction, cGMP hydrolysis. Using new combinations of transgenic models, we identified the molecular mechanism of spontaneously elevated activity of the G90D rhodopsin leading to stationary night blindness in humans. We established three-dimensional structure of GCAP2 (with Dr. J. Ames); demonstrated that the exchange of Ca2+ for Mg2+ in GCAPs is a required step in activation of retinal guanylyl cyclase; established the regulatory role of cation binding in individual domains of GCAP1 and GCAP2; mapped major functional domains in GCAPs; identified mutations in RetGC1 found in patients with LCA that disrupt interaction with GCAP1; developed a method for detection of GCAP/RetGC binding in living cultured cells and determined the stoichiometry of the binding between GCAP1 and RetGC1; obtained evidence that activation of RetGC1 by GCAPs involves dimerization of GCAP/RetGC complex. In a joined effort with the group of C. Makino at MEEI, we have demonstrated, by using new GCAP knockout mouse models developed in my laboratory, that different phases of rod recovery recruit different GCAPs based on their Ca2+ sensitivity.

The last but not least, in collaboration with Dr. Pan from Wayne State University we pioneered the use of light-sensitive microbial channel for restoration of light sensitivity in mouse retinas lacking photoreceptors. Over these years, I have published 65 papers, most of them as a senior author. As a Principal Investigator, I continuously maintain my R01 grant from NIH (since 1996) and Pennsylvania Department of Health Formula grant (since 2004) and have also administered grants from other state and private foundations, such as Human Frontier Science Program Organization (1991-1993) or Research to Prevent Blindness (1997-2001).

B. *Positions and Employment*

1977-1979 Junior Researcher, Biological Faculty, Moscow State University
1979-1985 Graduate student and pre-doctoral Junior Investigator, Dept. of Virology and A.N.Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University
1985-1990 Investigator and Senior Investigator, Dept. Enzymology, A.N.Belozersky Interdepartmental Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University
1989-1990 Visiting scientist at Dept. of Biochemistry, University of Washington (with Dr. K.A. Walsh and Dr. J.B.Hurley)
1990-1992 Leading Investigator, Institute of Physico-Chemical Biology, Moscow, USSR (Russia).
1991 Visiting Scientist, Institute des Pharmacologie Moleculair et Cellulair, CNRS, Sophia Antipolice, Valbonne, France (with Dr. M. Chabre)
1991-1997 Associate, Howard Hughes Medical Institute (with Dr. J.B. Hurley), Senior Fellow, Dept. of Biochemistry, then Research Assistant Professor of Biochemistry, University of Washington, Seattle, WA.
1997-2002 Assistant Professor, then Associate Professor of Ophthalmology, Pharmacology, Anatomy and Cell Biology, Wayne State University, Detroit, Michigan.
2002-present Hafter Chair and Professor of Pharmacology, Pennsylvania College of Optometry (since 2008 – at Salus University), Elkins Park, PA

Other professional experience and memberships

Study Sections: (NIH CSR): BDPE (2005-2006, *ad hoc*; 2006 – 2010 permanent member)
CSR/ ZRG1 CB-G; CSR/ZRG1 CB-C(95)S. ARVO, Society for Neuroscience, Biophysical Society.

C. Fifteen representative peer-reviewed publications (out of 65)

1. Dizhoor, A.M., Lowe, D.G., Olshevskaya, E.V., Laura, R.P., Hurley, J.B. (1994) *Neuron* 12: 1345-1352
2. Lowe, D.G., Dizhoor, A.M., Liu, K., Gu, O., Spencer, M., Laura, R., Lu, L., Hurley., J.B. (1995) *Proc. Natl. Acad. Sci. U.S.A.*. 92: 5535-5539
3. Dizhoor, A.M., Olshevskaya, E.V., Henzel, W.J., Wong, S., Stults, J.T., Ankoudinova, I., Hurley, J.B. (1995) *J. Biol. Chem.* 270: 25200-25206
4. Mendez, A., Burns, M.E., Sokal, I., Dizhoor, A.M., Baehr, W., Palczewski, K., Baylor, D.A., Chen, J. (2001). *Proc. Natl. Acad. Sci. U.S.A.* 98: 9948-53
5. Peshenko, I.V., Dizhoor, A.M. (2004) *J. Biol. Chem. 279:* 16903-16906
6. Olshevskaya, E.V., Calvert P.D., Woodruff, M.L., Peshenko I.V., Savchenko A.B., Makino C.L., Ho Y-S., Fain G.L., Dizhoor, A.M. (2004) *Journal of Neuroscience 24*: 6078-85
7. Bi, A., Cui, J., Ma, Y.-P., Olshevskaya, E., Pu, M., Dizhoor, A.M., Pan, Z.-H. (2006) *Neuron* 50: 23-33
8. Woodruff ML, Olshevskaya EV, Savchenko AB, Peshenko IV, Barrett R, Bush RA, Sieving PA, Fain GL, Dizhoor AM. (2007) *Journal of Neuroscience* 27:8805-15.
9. Makino, C.L, Peshenko, I.V., Wen X-H., Elena V. Olshevskaya, E.V., Barrett, R, Dizhoor, A.M. (2008) *J. Biol. Chem*. 283, 29135-43.

10. Dizhoor, A.M, Woodruff, M.L, Olshevskaya, E.V, Cilluffo M.C, Carter Cornwall, M., Sieving, P.A, and Fain, G.L. (2008) *Journal of Neuroscience 28: 11662-72.*
11. Liu X, Pawlyk BS, Adamian M, Olshevskaya EV, Dizhoor AM, Makino CL, Li T. (2009) *PLos One 4* (12): e8438
12. Peshenko, I.V., Olshevskaya, E.V., Dizhoor, A.M. (2008) *J. Biol. Chem.* 283: 21747-57
13. Peshenko, I.V., Olshevskaya, E.V., Yao, S, Ezzeldin, H.H., Pittler S.J., Dizhoor AM (2010)
14. Dizhoor, A.M., Olshevskaya E.V., Peshenko I.V. (2010) *Molecular and Cellular Biochemistry* 334:117
15. Peshenko, I.V., Olshevskaya E.V., Savchenko, A.B., Karan, S., Palczewski, K., Baehr, W., Dizhoor, A.M. (2011) *Biochemistry* 50(25): 5590-600.