Response Form for the Final Performance Review Report*

1. Name of Grantee: Salus University

2. Year of Grant: 2010 Formula Grant

A. For the <u>overall grant</u>, briefly describe your grant oversight process. How will you ensure that future health research grants and projects are completed and required reports (Annual Reports, Final Progress Reports, Audit Reports, etc.) are submitted to the Department in accordance with Grant Agreements? If any of the research projects contained in the grant received an "unfavorable" rating, please describe how you will ensure the Principal Investigator is more closely monitored (or not funded) when conducting future formula funded health research.

All the required reports on performance of the Formula grants at Salus University have always been submitted in a regular and timely manner, and this trend is not anticipated to change in the future. The grant performance evaluation by the external reviewers always received the ratings of "outstanding" or " favorable." This 2010 Formula grant performance received the rating of "outstanding." The results from the 2010 Formula grant study were published in two papers in professionally acclaimed peer-reviewed journal, Biochemistry.

* Please note that for grants ending on or after July 1, 2007, grantees' Final Performance Review Reports, Response Forms, and Final Progress Reports ***will be made publicly available on the CURE Program's Web site.***

Project Number: 1087001
Project Title: Role of RD3 Protein in Leber Congenital Amaurosis LCA12
Investigator: Dizhoor, Alexander

B. Briefly describe your plans to address <u>each</u> specific weakness and recommendation in <u>Section B</u> of the Final Performance Summary Report using the following format. As you prepare your response please be aware that the Final Performance Review Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.

Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations)*:

Response (*Describe your plan to address each specific weakness and recommendation to ensure the feedback provided is utilized to improve ongoing or future research efforts*):

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

<u>Reviewer 1:</u>
Only negligible weaknesses were found. The PI and institute are strongly encouraged to go to greater lengths to include undergraduate, graduate or post-doctoral trainees in the research. Though this cannot be remedied for the current project, the depth of the studies conducted in the PI's laboratory and medical relevance make it ideally suited for training and would provide excellent experience for talented young trainees.

<u>Response:</u>
We agree, and the PI laboratory would, of course, always welcome trainees and graduate students to be involved in the program of the research.

<u>Reviewer 2:</u>
1. The applicant is an accomplished biochemist, but he should place greater efforts into stepping out of his comfort zone to address and test molecular events of direct biological and pathological relevance.

2. The applicant should directly test the hypothesis that RD3 mice present increased cGMP levels in the inner segments. This will go a long way in designing (or excluding) rationale approaches to address the function of RD3 in photoreceptor function and disease. For example, what difference does it make how RD3 regulates GC1 and its interaction with GCAP in the OS of photoreceptors, if mutations in RD3 prevent the targeting of GC1 to the OS? Hence, the biological relevance of RD3 function in the OS of photoreceptors is unclear. In fact, some of the applicant's data support such a possibility. The native RD3 mutation (F100ter) has no biochemical impact on GC activity, yet it is known to be pathologically relevant. And why should the biochemical effect of this mutation (truncation) differ from K130M?

3. The third aim is problematic, and it is unclear what will be learned from rescuing mutant RD3 mice by expressing the WT RD3 protein, unless it is done in parallel with mutant RD3 constructs. Also, it would be better to use at least the regulatory sequences of RD3 to mimic its own expression. In addition, it is unclear whether the mutated (truncated) RD3 protein is still expressed in RD3 mice. Much more would be learned from probing the physiological role(s) and mechanism(s) of disease mutations in RD3 and those the applicant has already tested biochemically.

Response:
Thanks to the questions raised by the Reviewer. We would like to take this opportunity to better clarify the actual intent and perspectives of the study as follows. The continuation of this project includes plans for the study in transgenic mouse models, capable of mimicking general physiological effects of RD3 mutations in living photoreceptors.

Our data rather argue that RD3 is unlikely involved in the dynamic regulation of the cyclase in the OS. According to the biochemical model that we advocate, RD3 is more likely involved in silencing cGMP production by the nascent guanylyl cyclase in the inner, rather than outer segment (IS rather than OS). Therefore, mutations in RD3 that reduce its inhibitory capacity, in our opinion, could become detrimental for the biology of the photoreceptor in the IS, by unleashing cGMP synthesis by the cyclase in the wrong place at the wrong time. Both F100ter and, to a lesser extent, such mutations as K130M, whose inhibitory capacity is declined, could, according to our current hypothesis, contribute to the photoreceptor degeneration by failing to restrain synthesis of cGMP in the inner segment. We have started a project (now part of a funded NIH grant) to delineate the role of the recessive mutations in RD3, by distinguishing two possibilities – (a) RD3 is required solely for normal synthesis of RetGC cyclase versus (b) RD3 is necessary, solely or additionally to other possible function, for silencing the newly formed cyclase in the inner segment. Evidently, in both cases, mutations disabling RD3 function would lead to photoreceptor death, but the rate of degeneration should be different between the mice homozygous for F100ter (*RD3/RD3*) genotype and those completely lacking RetGC1 and RetGC2. This is now a subject of a long-term study, which includes morphological and physiological characterization of mouse retinas.

Reviewer 3:
All objectives were met. The results were published in two papers of high quality. Significantly, the PI has been able to identify a new, interesting, and testable hypothesis concerning the function of RD3.

C. If the research project received an "unfavorable" rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive "unfavorable" ratings.

Response: None required; the rating was "outstanding."

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: We greatly appreciate the critiques from all Reviewers and their highly positive evaluation of the performance for this pilot short-term project.

We appreciate the continuing support from the Pennsylvania Department of Health and the rigorous review process for the Formula grant.