

**Response Form for the Final Performance Review Report—
Bryn Mawr College 2008F***

1. Name of Grantee: Bryn Mawr College
2. Year of Grant: 2008 Formula Grant

A. For the overall grant, 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.

Response: All expenditures are monitored and approved by the Director of Sponsored Research prior to purchase. Upon notice of reports due, the PI is notified and a follow-up is done to ensure prompt reporting.

For each research project contained in the grant, please provide a response to items B-D as listed on the following page(s). When submitting your response please include the responses for all projects in one document. The report cannot be submitted as a ZIP file, because the Department’s exchange server will remove it from the email. If the report exceeds 2MB, please contact the Health Research Program for transmittal procedures: 717-783-2548.

* 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: 0862601

Project Title: Analysis of Epigenetic Modifications at Imprinted Loci in Mouse

Investigator: Davis, Tamara

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

Reviewer 1:

1. The principal investigator should consider comparing a range of antisera for the initial fractionation of chromatin into active and inactive groups. The source and catalog number of the acH3 antisera employed was not given, but I suspect the antisera used may have been one raised against multiple sites. If this is indeed the case, it may precipitate chromatin based on the cell cycle dynamics of H3 acetylation in addition to transcriptional activity.

Moreover, the principal investigator should explore whether sequential ChIP with two different histone modification antisera can provide more stringent fractionations into active vs. active fractions. Alternatively, they could ChIP for the elongating form of RNAPII using antisera specific for CTD phosphorylations.

2. I am concerned that the methods used to dissociate tissues (mincing and pushing the suspension through a 20-gauge needle) may not have been adequate. One could mince, add HCHO to the suspension and then dounce with a loose fitting pestle.

Reviewer 2:

None.

Reviewer 3:

1. The benefit to advancement of the field is negligible considering the results. The specific aims of a grant should be commensurate with the level of funding provided. Given the level of funding provided, the investigator should have concentrated on providing a first-rate learning experience for the undergraduates or producing preliminary data for submission of a grant application for further funding.
2. The benefit to teaching has no measurable outcomes. It seems the applicant was concentrating on increasing undergraduate exposure to research. If this is true, it should have included dissemination of research results at local or state levels, perhaps with the dedication of part of the funding to sponsoring a local meeting.
3. It is unlikely the data generated can be used to leverage additional funding. Given that the data were inconclusive, it is unlikely to be compelling for a grant review by other funding agencies. The applicant should have used several experimental techniques rather than one to illustrate the points. This would provide reviewers with the impression that the applicant is examining the experimental question from multiple angles. For example, there are no

quantitative RT-PCR results showing differential expression of the paternal and maternal alleles.

4. It is a weakness that no collaboration existed in this project. The principal investigator would have benefited from a mentor in the field to assist with technical difficulties and experimental design.
5. The funds allocated to the proposal were too modest. I would advise allocating at least \$20,000 per year for a project. The modest level of funding provided made the planned experiments infeasible. The applicant was using a mouse-based experimental approach which is cost-intensive. Molecular biology reagents also tend to be expensive. These cost variables greatly contributed to the feasibility of this project.

Response:

Reviewer 1:

1. We appreciate the suggestion made by Reviewer 1 that we utilize additional, site-specific antisera for our ChIP assays. Indeed, we have already expanded our analyses to include chromatin immunoprecipitation using antisera directed against the following histone modifications: H3K4me2, H3K27me3 and H4K20me3. In addition, we are aware that acetylation of histone H3K14 is associated with actively transcribed genes, and have determined that there are commercially available antibodies that are specific to this particular acetylated form of histone H3 in mouse that can be used in future experiments.

We have also considered the use of sequential ChIP, and outlined this approach as part of our experimental design in a grant proposal submitted to the National Science Foundation. [We would like to note that we have recently been informed that this proposal is being recommended for funding.] As we are currently working with very small amounts of material collected from neonatal mice, we have not yet taken this step, but plan to assess the feasibility of sequential ChIP in the near future.

2. As stated in our final progress report, we do use a dounce homogenizer to dissociate tissue following chemical cross-linking, therefore we feel that this valid concern of the reviewer has already been addressed.

Reviewer 3:

1. The funds awarded via the Pennsylvania Department of Health CURE award were minimal, as noted by each of the three reviewers. That said, these funds were sufficient to purchase reagents necessary for the PI and three undergraduate students to work on developing the ChIP methodology in our lab at Bryn Mawr College. [Note: two additional students have been involved in this research during the current academic year, after termination of the award.] The preliminary data obtained during the period of the Pennsylvania Department of Health CURE grant, and additional data that has been acquired subsequent to the termination of this award, were disseminated by the PI at both the 42nd Annual Meeting of the Society for the Study of Reproduction in 2009 and the

2011 Gordon Research Conference on Epigenetics; this work will also be presented at the 2012 MidAtlantic Society for Developmental Biology Meeting in May 2012. In addition, this work was presented by undergraduate students at undergraduate research symposia held at Bryn Mawr College as well as at the 2011 Sigma Xi Student Research Day at Thomas Jefferson University on April 27, 2011 and at the 13th Annual Undergraduate Research Symposium in Chemical and Biological Sciences at the University of Maryland, Baltimore County on October 30, 2010, where it was awarded a second place prize in the Biological Sciences category. I apologize that the dissemination of these data was not made clear in the final progress report.

2. To address the second issue made by reviewer 3, point 1, at the time this award was terminated, a decision on an R15 grant proposal I submitted to the National Institutes of Health in May 2010 to support future research on this project was still pending. Despite very positive reviews, NICHD only funded 7% of the R15 proposals submitted in fiscal year 2011, significantly lower than the funding rate for R15 applications across the NIH (15%), or for R01 applications across the NIH that year (20%). During the summer of 2011, I reworked the proposal and submitted it to the National Science Foundation, where it has recently been recommended for funding.

In conclusion, we feel that we achieved both goals reviewer 3 lays out in point 1: multiple undergraduate students were given high-quality research experiences and were able to disseminate their data on campus as well as at regional undergraduate research symposia and the preliminary data we obtained as a result of support from the Pennsylvania Department of Health CURE award has developed into a substantial three year award from the National Science Foundation to support our future work on this project.

3. As described in our response to reviewer 3, point 1, both the PI and the undergraduate students involved in this research project disseminated the results of this work at local, regional, and international meetings.

Although reviewer 3 states: "It is unlikely the data generated can be used to leverage additional funding", this has not proved to be the case. As described in our response to reviewer 3, point 1, a grant proposal to further this research has been recently recommended for funding by the National Science Foundation.

4. To address the second issue reviewer 3 mentions in this point, the primary mechanism for analyzing the distribution of modified histones on the parental alleles of imprinted loci is chromatin immunoprecipitation. As this technique was new to our lab, we felt that we needed to invest our time and the limited amount of resources into developing this methodology in our lab. We did not perform quantitative RT-PCR to examine the expression levels of the maternal and paternal *Rasgrf1* alleles during the period of this award for two reasons: first, we and other research groups have already examined the expression pattern of *Rasgrf1*; second, as the reviewer is no doubt aware, real-time PCR equipment and reagents are costly. At the time of the award, the equipment necessary for performing such experiments was not available at Bryn Mawr College, and the cost of

acquiring such equipment was well beyond the scope of the award. However, I would like to mention that funds to purchase a real-time PCR system from Applied Biosystems were requested and approved in my recent NSF proposal and that we look forward to acquiring this equipment so that we can perform quantitative analysis of our results.

5. The PI would like to clarify that while on-going collaborations are not part of our current research program, mentorship is. The PI's post-doctoral advisor, Dr. Marisa Bartolomei, resides at the University of Pennsylvania, and has continued to serve as a mentor to the PI. The PI maintains regular communication with Dr. Bartolomei, and has sought advice on several occasions from both graduate students and post-doctoral fellows in the Bartolomei lab who have experience with chromatin immunoprecipitation assays.

We absolutely agree with the reviewer on this point: it is expensive to conduct these experiments. We are therefore very happy that the small award from the Pennsylvania Department of Health allowed us to conduct pilot experiments and generate a sufficient amount of preliminary data for a more substantial grant proposal. As mentioned above, our recent grant proposal to the National Science Foundation has been recommended for funding, and will provide significantly more financial support for our continued investigations.

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: N/A

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

Response: N/A.