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: Bryn Mawr College**

2. **Reporting Period (start and end date of grant award period): 01/01/09-12/31/10**

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees): Nona C. Smith, Ph.D.**

4. **Grant Contact Person's Telephone Number: 610-526-5298**

5. **Grant SAP Number: 4100047626**

6. **Project Number and Title of Research Project: 1- Analysis of Epigenetic Modifications at Imprinted Loci in Mouse**

7. **Start and End Date of Research Project: 01/01/09-12/31/10**

8. **Name of Principal Investigator for the Research Project: Tamara L. Davis**

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:

   $ $12,629.14

   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
Davis	Principal Investigator	15%	$4000

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
Marlow	Undergraduate Assistant	40%
Morris	Undergraduate Assistant	25% Yr 1
Khaselev	Undergraduate Assistant	15% Yr 1

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

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____X____ No_____

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:
Analysis of epigenetic modifications at imprinted loci in mouse.	☒NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	May 2010	$330,000	unknown
	☐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: If the proposal submitted to the NIH in May 2010 is not funded, it will be revised and submitted it to the National Science Foundation.

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

This research project has several future directions. First, we plan to perform additional experiments to verify that the chromatin we are using in our ChIP experiments is sufficiently fragmented to ensure that we are not co-precipitating DNA that is distant from the modified histones we are examining. We also plan to examine the distribution of modified histones in at least two other tissues: brain, in which *Rasgrf1* expression is imprinted, and lung, in which *Rasgrf1* expression is biallelic. We will then continue our research by examining the distribution of additional modified histones that serve as markers for transcriptionally permissive and repressive chromatin. We also plan to examine the distribution of each modified histone on tissues isolated from F_1 hybrid mice derived from reciprocal crosses in order to confirm that any unique associations we detect between specific parental alleles and modified histones is parent of origin-specific rather than strain-specific. The results we obtain from these experiments will guide our further research directions.

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___X_____ No_____

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

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female	3			
Unknown				
Total	**3**			

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	3			
Unknown				
Total	**3**			

	Undergraduate	Masters	Pre-doc	Post-doc
White	3			
Black				
Asian				
Other				
Unknown				
Total	**3**			

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.

The funds provided by this health research project were used to purchase reagents and other supplies utilized by three undergraduate research assistants who participated in this research. Research opportunities for undergraduate students are an extremely important educational experience, enabling students to learn about cutting edge research and develop both technical and analytical skills. Without these funds, opportunities for students to perform research would have been more limited.

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_____ No_____X____

If yes, please describe the collaborations:

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.

Final Report: January 1, 2009 – December 31, 2010

Genomic imprinting is a mammalian-specific form of gene regulation whereby only one of the two inherited alleles is expressed. The expressed allele is determined based on parental origin; for some imprinted genes, only the maternally inherited allele is expressed, while for other imprinted genes the paternal allele is expressed. Parental origin of the two alleles is distinguished based on epigenetic differences in chromatin structure that are inherited at the time of fertilization, such as differential DNA methylation or differential histone modification. In either

case, one parental allele exhibits a modification that the other allele lacks, and the resulting differences appear to not only mark the parental alleles but also to play a role in the establishment and maintenance of the parent of origin-specific monoallelic expression unique to imprinted genes.

The goal of this research project was to investigate the epigenetic modifications associated with the maternal and paternal alleles of the imprinted gene *Rasgrf1* in mouse. *Rasgrf1* is a tissue-specific imprinted gene that is expressed solely from the paternal allele in brain and liver tissue, but is expressed from both the paternal and maternal alleles in lung and kidney. The parental alleles are distinguished from each other based on the DNA methylation status of the *Rasgrf1* differentially methylated region (DMR). Paternal allele-specific methylation of the *Ras*-DMR is inherited directly from sperm during fertilization, and is maintained specifically on the paternal allele in all tissues examined, regardless of their expression status. While methylation of this region clearly identifies the paternal allele and has been shown to be important for its expression in brain, methylation of this region cannot be the only factor responsible for *Rasgrf1* expression, since the maternal allele remains unmethylated in tissues exhibiting biallelic expression, such as lung and kidney. Therefore, our research has been designed to determine if there is a correlation between histone modification patterns and *Rasgrf1* expression. Specific Aim 1 was to characterize the distribution of modified histones on the parental alleles of *Rasgrf1* in an imprinted tissue (brain or liver). Specific Aim 2 was to characterize the distribution of modified histones on the parental alleles of *Rasgrf1* in a non-imprinted, biallelic tissue (lung, thymus or kidney).

To address this question, we utilized chromatin immunoprecipitation (ChIP) to precipitate specific modified histones and their associated DNA followed by allele-specific PCR analysis to determine which parental alleles were associated with specific histone modifications. All of our experiments were conducted using F_1hybrid tissues derived from crosses between two subspecies of mice, C57BL/6 (B or BL/6) and *Mus musculus castaneus* (C or *castaneus*), which enabled us to distinguish the parental origin of the genomic DNA fragments obtained via ChIP. Finally, we initiated experiments using two tissues exhibiting monoallelic expression of *Rasgrf1*, brain and liver, and two tissues exhibiting biallelic expression, lung and kidney. However, due to technical difficulties we encountered in working with brain and lung, for the studies completed during the course of this project we utilized liver and kidney, representing tissues exhibiting monoallelic and biallelic expression respectively.

The first step in performing these experiments was the preparation of fragmented chromatin. For ChIP, the chromatin must be fragmented into 200-1000 bp fragments to ensure that the co-precipitated DNA is in close proximity to the modified histone of interest in the original chromatin sample. We encountered technical difficulties associated with chromatin fragmentation, and ultimately utilized two different approaches. Initially, we followed standard ChIP protocols and used sonication to fragment the chromatin. However, due to difficulty achieving consistency using this method of chromatin fragmentation, we eventually switched to an enzymatic method to fragment the chromatin. The basic protocol we used for each method follows.

Chromatin fragmentation using sonication:

We first obtained relevant tissues (liver and kidney) from adult mice via dissection. The tissues were rinsed in 1x phosphate buffered saline (PBS), weighed, and either processed immediately or snap frozen in liquid nitrogen and stored at -80°C. To prepare chromatin, tissues were minced with razor blades into 1-3 mm fragments in 2 ml 1x PBS. The minced tissue was drawn into a 5 ml syringe, and pushed through a 20-gauge needle to further dissociate the tissue. PBS was added to a final volume of 10 ml, and the suspension was transferred to a 50 ml polypropylene Oak Ridge tube. To chemically crosslink the DNA and histones, formaldehyde was added to a final concentration of 1%, and crosslinking was allowed to proceed for 10 minutes at room temperature, with constant rotation. Crosslinking was stopped and unreacted formaldehyde was quenched by the addition of glycine to a final concentration of 0.125 M; this reaction proceeded for 5 minutes at room temperature, with rotation. Cells were pelleted via centrifugation at 2500 rpm for 4 minutes at 4°C, and the supernatant decanted. The cells were washed by resuspending in 5 ml cold 1x PBS, and recovered via centrifugation as described above. The cell pellet was resuspended in 1 ml cold ChIP cell lysis buffer (10 mM Tris-HCl pH 8.0, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% NP-40, 1x protease inhibitors). The cell suspension was transferred to a dounce homogenizer and cell lysis was achieved with 10-20 strokes of the dounce on ice. The cell lysate was transferred to a 1.5 ml microfuge tube, and incubated for 5-10 minutes at 4°C to release nuclei. Nuclei were pelleted by spinning the lysate in a microfuge at maximum speed for 5 minutes at 4°C. The supernatant was removed, and the nuclear pellet was resuspended in 700 µl of ChIP nuclear lysis buffer (1% SDS, 5 mM EDTA, 50 mM Tris-HCl pH 8.1, 1x protease inhibitors). Nuclear lysis and chromatin fragmentation were achieved via sonication, which was performed at 4 watts on ice, with 1-2 minutes on ice between sonication pulses to prevent the lysate from becoming too warm. The number of sonication pulses required to achieve optimal fragmentation varied for different tissues. We successfully fragmented liver chromatin into 200-1000 bp fragments using five to eight 20-second sonication pulses, while kidney chromatin require seven to ten 20-second pulses (Figure 1).

Chromatin fragmentation using micrococcal nuclease:

The initial steps used to obtain and dissociate tissue and to crosslink the DNA and histones were identical to those described above. Following the quenching step, cells were pelleted and washed once with 10 ml of cold 1x PBS. Cell pellets were resuspended in 1 ml cold 1x PBS, transferred to a 2 ml dounce homogenizer and homogenized with 10-20 strokes on ice. Cells were washed once more with 10 ml 1x PBS, followed by two washes with 1 ml 1x PBS + protease inhibitors. A cell volume equivalent to 50 mg of tissue was lysed in 500 µl membrane extraction buffer (Chromatin Preparation Module, Pierce Thermo Scientific). The resulting nuclei were collected by centrifugation at 9000 g for 3 minutes. Nuclei were resuspended in micrococcal nuclease digestion buffer, and incubated in the presence of 2.5 units of micrococcal nuclease for 15 minutes at 37°C. The micrococcal nuclease was inactivated via the addition of 20 mM EGTA, and the fragmented chromatin was released by incubating the nuclei in nuclear extraction buffer for 15 minutes on ice. Nuclear extracts were centrifuged at 9000 g for 5 minutes, and the supernatant containing fragmented chromatin was transferred to a fresh tube. An image illustrating the optimization of micrococcal nuclease digestion can be found in Figure 2.

Since the goal of this work was to determine which parental alleles of the *Rasgrf1* gene are associated with specific histone variants, we utilized the fragmented liver and kidney chromatin

to perform chromatin immunoprecipitation (ChIP), then amplified co-precipitated DNA products via the polymerase chain reaction (PCR). To achieve immunoprecipitation, liver and kidney chromatin were diluted in 900 µl ChIP Dilution Buffer + protease inhibitors (EZ-ChIP kit, Upstate). The chromatin was pre-cleared by incubating with 60 µl protein G agarose for 1 hour at 4°C, with rotation. The agarose was pelleted by centrifugation at 4000 g for 1 minute at 4°C. 10 µl of supernatant was removed and saved as the "input" fraction at 4°C. The remainder of the supernatant was transferred to a fresh tube and incubated with 5 µg of antibodies directed against either anti-acetylated histone H3 antibody (H3ac) or anti-trimethylated histone H3 lysine 9 (H3K9me3) overnight at 4°C with rotation. The following day, DNA-protein-antibody complexes were incubated with 60 µl protein G agarose for 1 hour at 4°C with rotation. The complexes were precipitated via centrifugation at 4000 g at 4°C for 1 minute. The supernatant, consisting of the unbound fraction, was removed and stored at 4°C. The agarose-antibody-protein-DNA complexes were washed in the following buffers for 3-5 minutes at 4°C with rotation, and complexes were precipitated via centrifugation as described above after each wash: low salt immune complex wash buffer, high salt immune complex wash buffer, LiCl immune complex wash buffer, two washes in Tris-EDTA. The protein-DNA complexes were eluted by incubating in 100 µl Elution Buffer (1% SDS, 0.1 M $NaHCO_3$) for 15 minutes at room temperature; 10 µl of the input fraction was incubated in 200 µl of Elution Buffer. The eluted complexes were separated from the protein G agarose by centrifugation at 4000 g for 1 minute, and the supernatant containing the protein-DNA complexes was transferred to a fresh tube. To maximize yield, the elution step was repeated, and the eluates were combined. Crosslink reversal was achieved by adding NaCl to a final concentration of 0.2 M, and incubating at 65°C overnight. The following day, 1 µl 10 mg/ml RNase was added, and the reaction proceeded for 30 minutes at 37°C. To degrade the protein, 10 mM EDTA, 40 mM Tris-HCl and 1 µl proteinase K were added, and incubation proceeded at 45°C for 1-2 hours. To purify the DNA, 1 ml of Bind Reagent A was added to each sample. 600 µl of this solution was transferred to a spin filter collection tube, and centrifuged for 30 seconds at 12,000 g. The flow through was discarded, and the remaining solution was centrifuged through the same column. The column was washed with 500 µl Wash Reagent B three times. The spin column was transferred to a fresh collection tube, and the DNA was eluted with 50 µl Elution Reagent C by spinning for 30 seconds at 12,000 g. The eluted DNA was stored at -20°C.

To determine whether the maternal and/or paternal alleles of *Rasgrf1* were associated with H3ac and H3K9me3, we performed the polymerase chain reaction (PCR) on the eluted co-precipitated DNA samples. We chose to analyze two distinct regions associated with *Rasgrf1*. The first region we analyzed was the *Ras*-DMR, which is located approximately 30 kb upstream of the transcriptional start site, is methylated solely on the paternal allele in both imprinted and biallelic tissues and is known to play a role in regulating the monoallelic expression of *Rasgrf1* in brain. The second region we analyzed was the *Rasgrf1* promoter, where we predicted chromatin structure to correlate directly with expression, regardless of parental origin. If we observed both maternal and paternal products following PCR, then we would conclude that the modified histone was associated with both maternal and paternal DNA. In contrast, if we amplified only paternally-derived DNA, we would conclude that the modified histone was only associated with paternal DNA. We hypothesized that we would find permissive histone modifications, such as H3ac, associated with expressed *Rasgrf1* alleles, such as the paternal allele in liver and both the maternal and paternal alleles in kidney. In contrast, we hypothesized that a repressive histone

modification, such as H3K9me3, would be associated with silent *Rasgrf1* alleles, such as the silent maternal allele in liver. PCR primers were designed to flank regions where we had identified strain-specific DNA sequence polymorphisms that would allow us to distinguish between DNA originating from the maternal (BL/6) *vs.* paternal (*castaneus*) alleles. We specifically utilized polymorphisms that could be distinguished using restriction endonuclease digestion; for example, within the *Ras*-DMR, a 550 bp PCR product derived from the maternally-inherited BL/6 allele will be cleaved by *Ava*II, while the same 550 bp product derived from the paternally-inherited *castaneus* allele will remain intact.

We first examined the distribution of the permissive modification, H3ac, at the *Ras*-DMR in liver and kidney chromatin. We successfully amplified PCR product from samples precipitated with H3ac, demonstrating that acetylated histone H3 is found at the *Ras*-DMR in liver and kidney (Figure 3A). We then digested the PCR products with the restriction endonuclease *Ava*II, which cleaves the maternally-derived BL/6 products into 425 and 125 bp fragments but does not cleave paternally-derived *castaneus* products. As H3ac is a permissive modification, we predicted it would be associated with the expressed maternal and paternal alleles in kidney, but hypothesized that it would only be associated with the expressed maternal allele in liver, and would not be found on the silent paternal allele. Contrary to our expectations, we found that H3ac is associated with the *Ras*-DMR on both the maternal and paternal alleles in liver and kidney chromatin, as evidenced by the presence of both maternal (425 bp, B) and paternal (550 bp, C) products in the H3ac samples (Figure 3B). Analysis of a second, non-overlapping region contained within the *Ras*-DMR yielded similar results (data not shown).

We next examined the distribution of the repressive modification, H3K9me3, at the *Ras*-DMR in liver and kidney chromatin. *Rasgrf1* is imprinted in liver, such that the paternal allele of *Rasgrf1* is expressed and the maternal allele is silent; therefore, we hypothesized that the repressive H3K9me3 would be found on the maternal allele but not the paternal allele. In contrast, we predicted that we would not see H3K9me3 on either parental allele in kidney, a tissue with biallelic expression of *Rasgrf1*. Once again, contrary to our predictions, we found that H3K9me3 was associated with both parental alleles in both liver and kidney chromatin (Figure 3B).

We then examined the distribution of H3ac and H3K9me3 at the *Rasgrf1* promoter in liver and kidney chromatin. PCR was performed on eluted co-precipitated DNA following ChIP using primers that flank a polymorphic *Dpn*II site, and the resulting PCR products (Figure 3C) were digested with *Dpn*II, which cleaves the paternally-derived *castaneus* product into 244 + 126 bp fragments, but leaves the 370 bp maternally-derived BL/6 products intact. We found that both the maternal and the paternal alleles of the *Rasgrf1* promoter are associated with H3ac and H3K9me3 in liver and kidney (Figure 3D). Based on the data shown, it is possible that H3ac is enriched on the expressed paternal allele in liver and that H3K9me3 is enriched on the silent maternal allele in liver; however, these experiments will need to be repeated such that a quantitative analysis can be performed before drawing such a conclusion.

To confirm that we were achieving sufficient chromatin fragmentation and could detect differential distribution of modified histones if differences exist, we analyzed another imprinted gene, *H19*, at which the expressed maternal allele is known to be enriched for H3ac and the silent paternal allele is known to be enriched for H3K9me3. While the liver chromatin data are

inconclusive, the data obtained from kidney chromatin do illustrate an enrichment of the maternal BL/6 allele in the H3ac ChIP sample, and a corresponding enrichment of the paternal *castaneus* allele in the H3K9me3 ChIP sample (Figure 4). Therefore, the methodology we have adapted for use in our laboratory is capable of detecting differential distribution of modified histones on the parental alleles of imprinted genes.

In conclusion, the overall goals of this project were achieved. We successfully adapted the methodology to perform chromatin immunoprecipitation using mouse tissues in our laboratory. We used this technology to fragment chromatin isolated from mouse liver and kidney, and perform ChIP to determine the distribution of markers of permissive (H3ac) and repressive (H3K9me3) chromatin at two distinct regions associated with the tissue-specific imprinted gene *Rasgrf1*. We initiated this investigation based on the hypothesis that permissive modifications would be associated with expressed *Rasgrf1* alleles while repressive modifications would be associated with silent *Rasgrf1* alleles, regardless of parental origin. One implication of this hypothesis is that the maternal *Rasgrf1* allele would be associated with different types of histones in tissues in which it is silent, such as liver, versus tissues in which it is expressed, such as kidney. The data we obtained regarding the distribution of modified histones at *Ras*-DMR do not completely support this hypothesis: we found that H3ac and H3K9me3 are associated with both the maternal and the paternal alleles in both liver and kidney. However, some of our data suggest that there may be a preferential association of H3ac with the promoter of the expressed *Rasgrf1* allele, and that there may a preferential association of H3K9me3 with the silent *Rasgrf1* allele. Quantitative experiments may help to resolve this question. It is also possible that differential distribution of transcriptionally permissive and repressive modified histones may not exist in tissues with low levels of *Rasgrf1* expression. *Rasgrf1* is expressed at high levels in brain, and it is possible that differential distribution of H3ac and H3K9me3 on the parental alleles of *Rasgrf1* in brain chromatin. The work we conducted during the course of this project provides a strong foundation for future experiments designed to enhance our understanding of how chromosome structure influences the expression of genes.

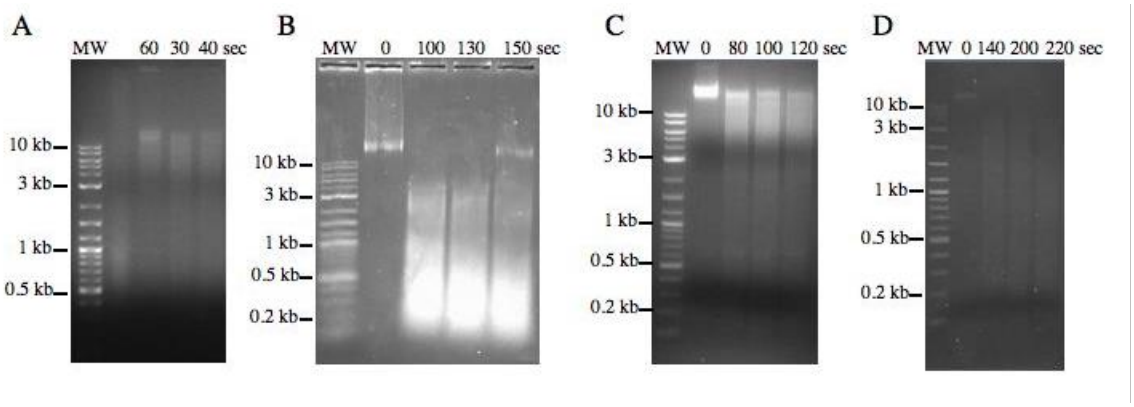

Figure 1. Optimizing sonication conditions for chromatin. (A) Sonication of liver chromatin with a 3.2 mm probe for 30-60 seconds achieved minimal fragmentation, as evidenced by the smear of DNA fragments around 10 kb. (B) Sonication of liver chromatin for 100-150 seconds sheared the bulk of the chromatin into 200-1000 bp fragments. (C) Sonication of kidney chromatin for 80-120 seconds was not optimal. (D) Sonication of kidney chromatin for 140-220 seconds sheared the chromatin into 200-3000 bp fragments. MW = molecular weight marker.

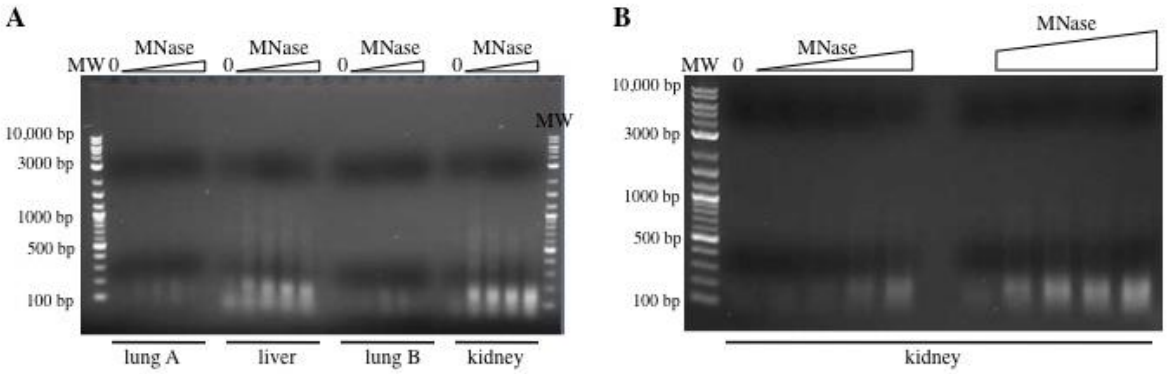

Figure 2. Optimizing chromatin fragmentation using micrococcal nuclease digestion. (A) Lung, liver and kidney chromatin were treated with increasing concentrations of micrococcal nuclease (MNase). Amounts of MNase used: 2.5, 5, 7.5 and 10 units. Mononucleosomes of 160 bp DNA are visible as bright bands just above the 100 bp marker; notice that larger fragments of DNA are visible at lower MNase concentrations. (B) Kidney chromatin was treated with increasing concentrations of MNase: 0.25, 0.5, 1, 2.5, 5, 7.5 and 10 units. Notice that fewer mononucleosomes are visible at lower MNase concentrations.

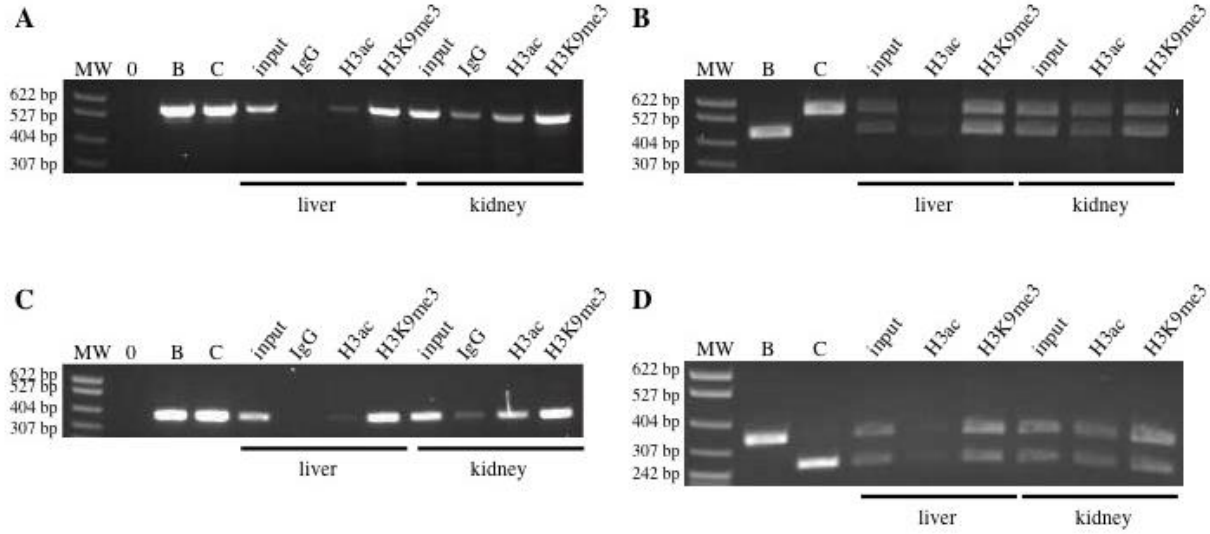

Figure 3. ChIP of liver and kidney chromatin, using anti-H3ac, a permissive histone modification, and anti-H3K9me3, a repressive histone modification. (A) PCR amplification of the *Ras*-DMR from control BL/6 and *castaneus* DNA, and ChIP input, IgG, anti-H3ac and anti-H3K9me3 from liver and kidney chromatin. The expected size of the PCR product is 550 bp. (B) Restriction digestion of PCR products from (A); both BL/6 and *castaneus* products are observed in all liver and kidney chromatin samples, illustrating that both maternal and paternal chromatin is associated with the permissive marker H3ac and the repressive marker H3K9me3 in both a tissue with imprinted expression of *Rasgrf1* (liver) and a tissue with biallelic expression of *Rasgrf1* (kidney). (C) PCR amplification of the *Rasgrf1* promoter; the expected size of the PCR product is 370 bp. (D) Restriction digestion of PCR products from (C); both BL/6 and *castaneus* products are observed in all liver and kidney chromatin samples, illustrating that both maternal

BL/6 and paternal *castaneus* chromatin is associated with the permissive marker H3ac and the repressive marker H3K9me3 in both a tissue with imprinted expression of *Rasgrf1* (liver) and a tissue with biallelic expression of *Rasgrf1* (kidney).

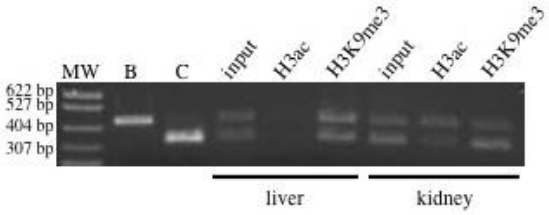

Figure 4. ChIP analysis of liver and kidney chromatin at the *H19* locus. PCR products derived from the *H19* differentially methylated region were digested with *Dpn*II, which cleaves products derived from the paternal *castaneus* chromosome into 300 + 98 bp fragments; the 398 bp maternal BL/6 product remains intact. Note a relative increase in maternally-derived BL/6 product as compared to paternally-derived *castaneus* product in the H3ac kidney sample, and an increase in the paternally-derived *castaneus* product relative to maternally-derived BL/6 product in the H3K9me3 kidney sample.

Posters presented on this work:
Davis, T., L.D. Dockery, C. Harview, R. Horton, N. Khaselev and S. Marlow, July 18-22, 2009, abstract and poster, 42nd Annual Meeting of the Society for the Study of Reproduction, Epigenetic analysis of tissue-specific imprinting of *Rasgrf1* in brain, liver and placenta.

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

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: Once a more comprehensive study has been completed, the work initiated during the course of this project will be incorporated into a manuscript to be submitted to a peer-reviewed journal. All undergraduate students who have contributed significantly to the study will be listed as co-authors.

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.*

A. EDUCATION

1991	B.A., Molecular Biology	University of California, San Diego
1996	Ph.D., Molecular and Cell Biology	University of California, Berkeley
1997-1999	Postdoc, Molecular Genetics	University of Pennsylvania

B. RESEARCH INTERESTS AND EXPERIENCE

Recent Research Activities

I have been an Assistant/Associate Professor of Biology at Bryn Mawr College since January 2000. Over the course of the last ten years, I have maintained a research laboratory staffed by undergraduate research assistants. Undergraduate research assistants working under my supervision at Bryn Mawr College conducted all of the work described in the Preliminary Studies section of the Research Strategy. Annually, I train and supervise approximately four undergraduate students who work in my laboratory during the summer and academic year. In addition, I am responsible for mouse breeding, I isolate the cells and tissues used in our research and I perform some of the experiments.

The overall goal of my research is to understand the establishment of parental allele-specific epigenetic modifications at imprinted genes in mouse. Specifically, I have focused on defining the time at which parent of origin-specific patterns of DNA methylation are acquired in the germline and in post-implantation embryos, and I have also investigated DNA methylation patterns at the tissue-specific imprinted gene *Rasgrf1* (14). Recent progress on these projects is detailed in the research proposal and in Dockery *et al*. (14), and a manuscript describing our work on *Gtl2* is in preparation for submission during the late spring of 2010. The current application builds logically on my previous work, and we have obtained preliminary data to support the feasibility of performing the proposed experiments at Bryn Mawr College.

Experience Supervising Student Research at Bryn Mawr College

Since May 2000, I have supervised thirty-four undergraduate students in my research lab at Bryn Mawr College, including three students who are working in my laboratory during the summer of 2010. In addition to supervising their independent research projects, I hold weekly lab meetings during which students either present their current research or discuss a paper from the current primary literature. Four Bryn Mawr undergraduates are co-authors on a paper published in *Genomics* (7), and six are co-authors on a paper published in *Epigenetics* (14); an additional seven undergraduate students are co-authors on one manuscript that is currently in preparation. In addition, twelve of my Bryn Mawr undergraduate research assistants have presented posters on their research at extramural undergraduate research symposia, and five of these students won prizes for their posters. I have also taken five students to Annual Meetings of the Society for the Study of Reproduction, where they each presented posters, and three students to the 2008 meeting of the Society for Developmental Biology.

C. PROFESSIONAL COMMITMENTS AND HONORS

Professional Employment/Experience

1989-1991 Undergraduate Assistant, lab of Donald Helinski, UC San Diego, La Jolla, CA

1991-1996 Graduate Student, laboratory of Barbara Meyer, UC Berkeley, Berkeley, CA
1997-1999 Postdoctoral Fellow, lab of Marisa Bartolomei, University of Pennsylvania, Philadelphia, PA
2000-2005 Assistant Professor, Biology Department, Bryn Mawr College, Bryn Mawr, PA
2005-present Associate Professor, Biology Department, Bryn Mawr College, Bryn Mawr, PA
2009-present Chair, Biology Department, Bryn Mawr College, Bryn Mawr, PA

Other Experience and Professional Memberships

1998-present Member, DNA Methylation Society
1999-present Member, AAAS
2001-present Member, Society for the Study of Reproduction
2002-present Member, Council on Undergraduate Research
2007 NIH Peer Review Committee: CMIS study section, ad hoc reviewer
2010 NIH Peer Review Committee: Special Emphasis Panel, ad hoc reviewer

Honors

1993 Outstanding Graduate Student Instructor, UC Berkeley, Berkeley, CA
1997-1998 Howard Hughes Medical Institute Postdoctoral Fellow
1998-1999 NIH NRSA Postdoctoral Fellowship
2005 Rosalyn Schwartz Teaching Award, Bryn Mawr College, Bryn Mawr, PA