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:** The Institute for Cancer Research

2. **Reporting Period (start and end date of grant award period):** 1/1/2011 – 6/30/2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Maria Minko Gill

4. **Grant Contact Person's Telephone Number:** 215-728-2659

5. **Grant SAP Number:** 4100054848

6. **Project Number and Title of Research Project:** 01-The Role of Histone-Complexes in Residue Specificity of Post-Translational Modifications

7. **Start and End Date of Research Project:** 1/1/2011 – 12/31/2012

8. **Name of Principal Investigator for the Research Project:** Andrew J. Andrews, Ph.D.

9. **Research Project Expenses**.

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

   $242,983

   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, First Name	Position Title	% of Effort on Project	Cost
Andrews, Andrew	PI	21% years 1-2	$53,377.76
Li, Shan	Scientific Technician III	14% year 1	$6,802.28

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, First Name	Position Title	% of Effort on Project
None		

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
TSQ Quantum Access Triple Stage Quadruple MS/MS S	The mass spectrometer is critical for all of our experiments in my lab. It critical for all of the work in my lab, allowing us to measure the acetylation of each residue in histone H3 and H4. This equipment was also critical for us to publish the four papers and obtain an R01.	$100,725

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 awarded:
R01 - The Role of Histone Chaperones in Histone Acetylation and Nucleosome Dynamics	☒ NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	March 2013	$2,231,250 anticipated start date 9/15/14	estimated award $1,356,600
Specificity and Selectivity of Histone Acetylation in DNA Damage	☐NIH ☐ Other federal (specify:_____) ☒ Nonfederal source (specify: WW Smith)	June 2012	$88,000	$88,000

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:

An additional R01 will be submitted in November on another enzyme using methodology from this grant.

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

The technology optimized in this grant will be used for a number of projects in my lab as well as collaborations.

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.

This allowed us to get the equipment we needed to start my lab. We could not have obtained funding from the NIH without it.

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 assay developed in this project allowed us to work with Dr. T. Gilmore at Boston University. We published the following paper "Histone acetyltransferase-deficient p300 mutants in diffuse large B cell lymphoma have altered transcriptional regulatory activities and are required for optimal cell growth" in Mol. Cancer. 2014

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 agreement). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u> 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. <u>Provide detailed results of the project.</u> 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.

Specific Aims.

I. Determine the impact of histone chaperones Nap1, Vps75, and Asf1 on the rate and location of histone modification by recombinant/purified PTM enzymes.

II. Identify factors that influence the location of histone modifications in various histone complexes.

Histone acetylation is involved in gene regulation and, most importantly, aberrant regulation of histone acetylation is correlated with major human diseases. Although many lysine acetyltransferases (KATs) have been characterized as being capable of acetylating multiple lysine residues on histones, how different factors such as enzyme complexes or external stimuli (e.g. KAT activators or inhibitors) alter KAT specificity remains elusive. In order to understand how the homeostasis of histone acetylation is maintained, a method that can quantitate acetylation levels of individual lysines on histones is needed. In this project we have demonstrated that our mass spectrometry (MS)-based method accomplishes this goal. In addition, the high throughput, high sensitivity, and high dynamic range of this method allows for effectively and accurately studying steady-state kinetics. Based on the kinetic parameters from in vitro enzymatic assays, we can determine the specificity and selectivity of a KAT and use this information to understand what factors influence histone acetylation. We have used these approaches to study the enzymatic mechanisms of histone acetylation and predict how changes in the cellular environment can alter the acetylation pattern in cells. Understanding the post-translational modification of individual residues within the histones will provide a better picture of chromatin regulation in the cell.

Rtt109 and Histone Chaperones

Rtt109-Vps75 targets lysines on histones that are involved in genome stability and nucleosome assembly. In order to understand the extent to which Rtt109-Vps75 targets specific lysines on histone, we used a label-free quantitative MS-based assay to determine the specificity and selectivity of Rtt109-Vps75 on individual lysines of different histone substrates. In doing so, we have expanded our understanding of how chaperones help to facilitate Rtt109 dependent acetylation.

Much of the previously published work on Rtt109 has been done using classical techniques that either quantitate the total amount of acetylation or identify the locations of acetylation, but not both. Because of this limitation, the investigation of the specificity/selectivity of an enzyme is incomplete. To overcome this problem, the ability of targeted MS allows us to accurately measure the increases in activity and/or the changes in selectivity for individual sites.
As we have previously shown with p300, the histone substrate (whether it is H3 or H3/H4) can affect the specificity of a KAT. Here we observed that H3 was a much better substrate for Rtt109-Vps75 than H3/H4. We also observed a higher specificity for K9 and K23 on histone H3 alone than on H3/H4. Although the high acetylation preference of Rtt109-Vps75 for K9 and K23 over any other potential lysine sites remains the same on both substrates, the higher specificity and selectivity quantitated for H3 alone demonstrates that monomeric H3 is the preferred substrate *in vitro*. While *in vivo* a majority of H3 is bound to H4 to form either a histone-histone chaperone complex or a nucleosome, it cannot be ruled out that H3 is a possible substrate *in vivo*. Furthermore, the role of H3K9 acetylation in newly produced histones and their deposition in yeast, suggests that Rtt109-Vps75 plays an important role in histone deposition of newly synthesized histone H3. While H3 exhibits selectivity for K9 over K23 and higher specificity than H3/H4 tetramer, there is no site selectivity for acetyl-CoA turnover. This data suggests that unlike p300 the residue specificity for Rtt109 is determined by histone interactions and not acetyl-CoA concentrations. This predicts that reduced levels of acetyl-CoA in the cell will equally affect all sites acetylated by Rtt109-Vps75. The specificity of acetyl-CoA for specific sites differed between H3 alone and H3/H4 tetramer in that we observed cooperativity (with a Hill coefficient, nH=2) in the presence of saturating H3/H4, but not when titrating H3/H4 in saturating acetyl-CoA. One possible explanation for this could stem from the fact that the Rtt109-Vps75 complex is reported to be a 2:2 complex. It is also known that one acetyl-CoA binds to one Rtt109, which then allows Rtt109 to acetylate histones. At low levels of acetyl-CoA, it is more likely that only one of the two Rtt109 molecules in a complex with Vps75 dimer may have an acetyl-CoA molecule bound, and Vps75 dimer will bind one H3/H4 tetramer. This will result in only one of the two H3 molecules being acetylated. As the acetyl-CoA concentration increases, the probability of both Rtt109 molecules in complex having an acetyl-CoA molecule bound increases, and thus there is a non-hyperbolic increase in v/E as a function of acetyl-CoA. This model also explains why cooperativity is not observed in H3 monomer titrations.

In addition to Vps75, Asf1 has been suggested to alter the residues selectivity of Rtt109, Asf1 interacts with H3/H4 dimer instead of $(H3/H4)_2$ tetramer. In the present study, we followed up on the possibility that Asf1 binds H3/H4 dimer and functions as a substrate for Rtt109-Vps75. We observed a 1:1 stoichiometry of Asf1 to H3/H4 dimer and 4~6-fold increases in the apparent k_{cat}. Further supporting a model where H3/H4-Asf1 is acetylated by Rtt109-Vps75 is the fact that

when we titrated acetyl-CoA under saturating H3/H4-Asf1, we lost the cooperativity observed in the H3/H4 alone reactions. Given the current structural model of 2:2 Rtt109:Vps75 and this loss of cooperativity, it suggests that H3/H4-Asf1 could position itself, so that both Rtt109 molecules could acetylate both H3 molecules in H3/H4 tetramer. Therefore, Asf1 may enhance Rtt109-Vps75 activity by correctly positioning the histone substrate in the primed Rtt109 active site with acetyl-CoA.

From the results of the H3/H4-Asf1 steady-state assay, we noted that H3K9 and H3K23 were still the targeted sites of acetylation. Our data also demonstrate that Asf1 enhances Rtt109-Vps75 acetylation on H3K9 and HK23 without altering the selectivity or preference between possible sites. This would mean that even if K56 acetylation has been increased, since it is still a secondary site we would not detect significant acetylation on K56 prior to 10% acetylation of the primary sites. We also conclude that an increase in specificity due to additional Asf1 is primarily from an increase in k_{cat} and to a lesser degree K_m. Thus, we suggest that Vps75 and Asf1 work together to promote histone acetylation in the cell. Consistent with this hypothesis is that we observed the same amount of decrease in K9 acetylation *in vivo* with the loss of either Asf1 or Rtt109, suggesting that you need both for proper acetylation levels.

Studies that have reported higher levels of K56 acetylation were using chicken erythrocytes histones, which already contain modifications, and/or stoichiometric amounts of enzyme. While, both stoichiometric amounts of enzyme and preexisting modification make determining specificity impossible, a preexisting modification is likely capable of altering selectivity. For this reason, we hypothesized that preexisting modification(s) would likely increase the acetylation of K56 as compared to K9 and/or K23. In fact when we utilized chicken erythrocyte histones, we found that both K9 and K23 acetylation were reduced but K56 was not. This suggests that any potential modifications that exist on chicken erythrocytes histones are responsible for Rtt109 favoring K56 acetylation over K9 and K23, and histone chaperone might not be the only one factor influencing acetylation specificity. While K56 acetylation is likely to occur secondarily to K9 and K23 acetylation, K56 acetylation may not be dependent on K9 and K23 acetylation, but instead is simply less favorable. This idea is supported by the fact that stoichiometric amounts of enzyme and long time points, even on recombinant histones, enhance K56 acetylation. However, under steady-state conditions using histones with no preexisting marks, Rtt109-Vps75 is not capable of acetylating K56. These data suggest that an initial post-translational modification(s) alters the selectivity of Rtt109-Vps75 to favor K56 acetylation.

These data highlight the impact of histone conformations: dimer, tetramer, or bound to a chaperone has significant effects on residue specificity for both enzymatic and nonenzymatic acetylation. This suggests that chromatin dynamics may play as big a role in the specificity of KATs as KATs play in chromatin dynamics. Linking both aspects is the role of histone chaperones, which can both alter chromatin dynamics and histone conformation, which in turn influences acetylation. In this research, we present evidence of increased specificity by two different chaperones interacting with different proteins: Rtt109-Vps75 and H3/H4-Asf1 working together for optimal acetylation. Finally, together these data demonstrate that a label-free quantitative mass spectrometry-based assay is a useful tool to facilitate the understanding of enzyme kinetics, which have provided a better understanding of protein-chaperone interactions and their role in histone acetylation.

P300 and the impact of histone complex formation and acetyl-CoA concentration

We have observed significant differences in the specificity of CBP and p300 histone acetylation. We have shown that although both preferentially acetylate similar residues they have very different specificities. Using our label free, quantitative method, we were also able to determine the kinetics for these proteins at several other sites of histone H3 and H4. We have also shown that these selectivities are affected by acetyl-CoA levels and can be altered by the formation of histone complexes. Understanding how the activities of these two enzymes differ is the first step in understanding why they cannot compensate for each other in an organism deficient in either protein, while determining how these specificities can be altered is important in deciphering how the histone epigenetic code is written.

These steady state experiments reveal several differences in specificity between p300 and CBP. The large increases in specificity (up to 10^{31}-fold) are mostly a result of an increase in the apparent cooperativity, which results in a much larger denominator when calculating the specificity constant ($k_{cat}/K_{1/2}^{nH}$). The origins of cooperativity in this system are likely complicated but some possibilities are precluded by our data: if cooperativity were truly a function of either histone or acetyl-CoA binding in a specific complex, resulting in a specific residue being acetylated, then one would expect that one particular site would begin to out compete others. This would result in what would appear to be product inhibition at certain residues, or in other words as the v/E increased for the site with the higher Hill coefficient, the v/E would begin to decrease for sites with a smaller Hill coefficient. This is not what we observe under conditions where we detect cooperativity for one site and not another site; we see no signs of the major v/E decrease that we would expect from this type of mechanism. Another possibility is that we are observing the dimerization of H3 or H3/H4, resulting in the appearance of cooperativity. This has been seen before with Nap1 binding H3/H4, but if this was the case we would expect to see a similar Hill coefficient for all sites where the catalytic efficiency is enhanced by dimerization. However, it is possible that certain sites are less or more sensitive to the dimer form of the substrate, which we cannot rule out. Another interesting possibility is that the enzyme is in multiple conformations or isomerization states, all of which are catalytically active at different rates but are slow compared to the catalytic reaction, with the rate of isomerization being influenced by substrate. This model would make biological sense in the fact that proteins could bind p300 or CBP to act as allosteric regulators, altering their specificity for particular lysines. Together, these observations may suggest mechanisms by which both the chromatin conformation and factors interacting with p300 or CBP could alter the residues acetylated, opening a wide field of investigation into factors that influence enzyme specificity. Regardless of the mechanism behind this observed cooperativity, we believe that it is an important factor in determining the specificity of p300 and CBP, and as such will focus our discussion on our calculations that take into consideration the Hill coefficient.

In analyzing the specificity data, it is important to first draw attention to the magnitude of the differences in selectivity between CBP and p300. As we mentioned previously, different diseases arise due to mutations in either CBP or p300, suggesting that one protein is incapable of fully substituting for the other. As we see that both proteins target the same residues, it is likely that it is the ratio of acetylation that is the important marker of these proteins' activities. For CBP, acetylation of H3K18 is much greater than any other site on H3, with a specificity that is a

factor of 10^{14} greater than the second most abundant site (H4K5). For p300, though, the specificity for each site is much closer, with only a tiny ~1.05-fold difference between K18 and the next most abundant site (H3K14). Additionally, the acetylation of K9 is much higher for p300, with K9 not detectable for CBP before 10% of the histone is acetylated. All of these factors combined mean that p300 and CBP, despite targeting the same sites, will do so to different degrees of efficacy; while p300 will acetylate K9, 14, 18, and 23 in a more evenly distributed fashion, CBP will heavily favor K18 acetylation, to the detriment of the other sites, just as we see with H3K9. Though weak, it is likely that the acetylation of H3K9 is still an important part of the activity of p300 and potentially CBP. We also observe that the level of acetylation of H3K27 was significantly lower than the other sites we have detected and under steady-state conditions, no acetylation is detected at this site before 10% of the histone is acetylated. It is likely that some co-factor (possibly a histone chaperone) is influencing p300 acetylation of this site.

When comparing the steady-state experiments where either H3/H4 is limiting or acetyl-CoA is limiting, it is worth noting that the specificity of both CBP and p300 changes. For CBP, when titrating H3/H4 the order of specificity for H3 acetylation is K18>K23>K14. Meanwhile, for p300, titrating H3/H4 leads to almost identical specificities for K18, K23, and K14. When titrating acetyl-CoA, however, a clear order emerges of K18>K14>K23 for both KATs. Similarly, we see a change in the acetylation order of H4 both with CBP and p300 when comparing H3/H4 titrations versus acetyl-CoA titrations. In addition, the difference in specificity from the highest to the lowest residue decreases when acetyl-CoA is limited, compared to when histone is limited. For CBP, specificity varies by a factor of 10^{32} when titrating H3/H4 compared to a factor of 10^{11} when titrating acetyl-CoA. These changes that are observed when acetyl-CoA are limited could be indicative of a mechanism for altering histone acetylation patterns when, for example, nutrient intake is limited. Such a change could potentially correlate with the upregulation/downregulation of certain genes in response to limited energetic intake.

Histone complexes/conformation can also influence CBP and p300 specificity; we observe changes to the specificity of p300 and CBP depending on whether the substrate is the H3/H4 tetramer or just H3 alone. Under conditions of limiting histone and p300, specificity for H3K18 decreases slightly (by 1.4-fold) when the tetramer is formed, but the $k_{cat}/K_{1/2}$ for H3K14 and H3K23 both increase on the tetramer (~4.5-fold for each). Observing a higher specificity for a site when H3 is alone is not necessarily surprising, as without the additional H4 sites for p300 to target, we would expect to see an increase in acetylation of the H3 sites. Seeing a higher specificity for a site on the H3/H4 tetramer (as is the case for K14 and K23), is less expected. While it is unclear why there would be this drop on H3, it is possible that confirmation changes as a result of the formation of the H3/H4 tetramer could make K14 a sterically more viable target. This study, as well as previous work from our lab, noted that H3K4 was rarely targeted for acetylation, possibly due to its ability to more readily sample different conformations. In other words, while it is important for a residue to be accessible to the KAT, too much conformational freedom could be detrimental to KAT binding (and therefore acetylation). This could also explain the change in behavior of p300 for K14: it is possible that formation of the tetramer limits the conformational freedom of K14, which could account for the increase in acetylation of this site on the H3/H4 tetramer.

With the information currently available in the field, it is difficult to know exactly what the biological reason is for the change in specificity of CBP to be more uniform on histone H3 alone when substrate is limited. We believe that the increase in specificity that we observe for H3K14 and H3K23 are important to newly synthesized histones, or that they could be important to histone assembly. The reason for this is that, in the cell, the most readily available free H3 would be from newly synthesized histone that has yet to be assembled into a nucleosome. Therefore, it is under these conditions that an increase of H3K14 and H3K23 acetylation would be the most relevant. An alternative explanation is that instead of high levels of K14 and K23 acetylation being an important epigenetic marker, it could be key to not have disproportionally high levels of K18 during histone assembly, and that is why the acetylation pattern of CBP is more evenly distributed on H3 alone, although this has yet to be seen.

In summary, this study has revealed several important facts about the specificity of p300 and CBP and how it is regulated. Although both KATs are capable of acetylating the same residues of H3 and H4, p300 and CBP both display unique specificities for each lysine residue, under a variety of conditions. Changing the histone substrate from H3 alone to the H3/H4 tetramer clearly influences this order of specificity, although the targeted sites remain the same. Limiting acetyl-CoA concentrations also affects the specificity of these proteins and also alters their order of specificity on H3 and H4. The results presented here distinguishing the targets and specificity of CBP and p300 provide valuable insight into how these enzymes differentially acetylate the histone. Such knowledge could be invaluable for treating the cancers and neurodegenerative disorders that arise from mutations in either CBP or p300. Ultimately, if we can understand how to better manipulate p300 KAT activity to mimic that of CBP and vice versa, we may be able to overcome some of the detrimental effects that result from mutations in either.

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 or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

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. Quantitating the specificity and selectivity of Gcn5-mediated acetylation of histone H3.	Kuo, Y.M., **Andrews, A.J.**	*PLoS One*	October 2012	☐Submitted ☐ Accepted ☒Published
2. Differences in Specificity and Selectivity Between CBP and p300 acetylation of histone H3 and H3/H4	Henry R.A., Kuo Y.M., **Andrews A.J.**	*Biochemistry*	May 2013	☐Submitted ☐ Accepted ☒Published
3. A quantitative multiplexed mass spectrometry assay for studying the kinetic of residue-specific histone acetylation.	Kuo Y.M., Henry R.A., **Andrews A.J.**	*Methods: a Companion to Methods in Enzymology*	August 2014	☐Submitted ☒ Accepted ☐Published
4. Histone acetyltransferase-deficient p300 mutants in diffuse large B cell lymphoma have altered transcriptional regulatory activities and are required for optimal cell growth	Haery L., Lugo-Picó J.G., Henry R.A., **Andrews A.J.**, Gilmore T.D.	*Molecular Cancer*	June 2013	☐Submitted ☐ Accepted ☒Published

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

Yes_____ No___X_____

If yes, please describe your plans:

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.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Andrew J. Andrews	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) ajandrew	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
North Carolina State University, Raleigh, NC	B.S.	06/98	Microbiology
North Carolina State University, Raleigh, NC	M.S.	06/00	Microbiology
University of Michigan, Ann Arbor, MI	Ph.D.	08/06	Biological Chemistry

A. PERSONAL STATEMENT

Prior to starting at Fox Chase Cancer Center, I was an NIH post-doctoral fellow with Karolin Luger, HHMI Investigator at Colorado State University. During my NIH fellowship, *"Modulation of Nucleosome Dynamics,"* I developed many of the assays, techniques, and models needed for studying the thermodynamics of histone-protein interactions including the ability to measure the thermodynamics of the nucleosome. Until now, it has been impossible to dissect the thermodynamic constants of the nucleosome, and therefore many hypotheses regarding the role of histone variants and post-translational modifications could not be tested. My experimental approach has allowed me to measure the thermodynamics of the nucleosome and demonstrate that Nap1 acts to remove non-nucleosomal H2A-H2B-DNA interactions both *in vitro* and *in vivo*.

The combination of what I learned during my post-doc and my mechanistic background in enzymology/chemistry (Ph.D. earned under Dr. Carol Fierke at the University of Michigan) makes me uniquely qualified to tackle the complex problem presented here. I have a diverse educational background (B.S./M.S.-Evolution/Microbiology, Ph.D.- Chemical Biology/Enzymology, Post-Doc.-Structure/Biophysics) that has broadened my view of science and provided me with many different perspectives and skill sets for approaching difficult, fundamental questions in biology. Additionally, the human and physical resources currently available to me at Fox Chase Cancer Center make it an exceptional partner in this investigation.

B. POSITIONS AND HONORS

Graduate School, Department of Microbiology, Raleigh, N.C. 1998-2000
Project: To characterize the *Mathanococcus* RNase P holoenzyme by purification and basic physical properties - Advisor: Dr. James Brown

Graduate School, Biological Chemistry Department, University of Michigan, Ann Arbor, MI 2000-2006
Project: Further our understanding of the role of inner and outer sphere metal ions by the use of outer-sphere mimics such as cobalt hexamine in combination with metal mapping and single turn kinetics in the *B. subtilis* RNase P holoenzyme - Advisor: Dr. Carol A. Fierke

NIH Fellowship**,** Modulation of nucleosome dynamics, Colorado State University 2008-2010
Mentor: Karolin Luger, HHMI Investigator at Colorado State University

Assistant Professor, Cancer Epigenetics, Fox Chase Cancer Center 2010-date

HONORS AND AWARDS
Post-doctoral Fellowship, NIH F32 GM083532

C. SELECTED PEER-REVIEWED PUBLICATIONS

Andrews, A.J., Brown, J.W. The partial purification of Methanococcus jannaschii RNase P holoenzyme. *Nucl. Acids Symp. Ser.* **41**:60-62, 1999.

Andrews, A.J., Hall, T.A., Brown, J.W. Characterization of RNase P holoenzymes from Methanococcus jannaschii and Methanothermobacter thermoautotrophicus. *Biol. Chem.* **382**(8):1171-1177, 2001.

Hsieh, J., **Andrews, A.J.**, Fierke, C.A. Roles of protein subunits in RNA-protein complexes: lessons from ribonuclease P. *Biopolymers* **73**(1):79-89, 2004.

Andrews, A.J., Fierke, C.A., Measuring the Stoichiometry of Magnesium bound to RNA, in Handbook of RNA biochemistry., R.K. Hartmann, et al., Editors. 2005, Wiley-VCH: Weinheim. p. 250-258, 2004.

Getz, M., **Andrews, A.J.**, Fierke, C.A., Al-Hashimi, H.M. Structural plasticity and Mg^{2+} binding properties of RNase P P4 from combined analysis of NMR residual dipolar couplings and motionally decoupled spin relaxation. *RNA* **13**(2):251-266, 2006.

Park, Y., Sudhoff, K., **Andrews, A**., Stargell, L., Luger, K. Histone chaperone specificity in Rtt109 activation. *Nat. Struct. Mol. Biol.* **15**(9):957-964, 2008.

Andrews, A., Downing, G., Brown, K., Park Y., Luger, K. A thermodynamic model for Nap1-histone interactions. *J. Biol. Chem*. **283**(47):32412-32418, 2008.

Andrews, A.J., Luger, K. Histone Modifications: Chemistry and Structural Consequences. Wiley Encyclopedia of Chemical Biology, 2008.

Geiss, B.J., Thompson, A.A., **Andrews, A.**, Sons, R.L., Gari, H.H., Keenan, S.M., Peersen, O.B. Analysis of flavivirus NS5 methyltransferase cap binding. *J. Mol. Biol*. **385**(5):1643-1654, 2009.

Koutmou, K.S., Casiano-Negroni, A., Getz, M.M., Pazicni, S., **Andrews, A.J.**, Penner-Hahn, J.E., Al-Hashimi, H.M., Fierke, C.A. NMR and XAS reveal an inner-sphere metal binding site in the P4 helix of the metallo-ribozyme ribonuclease P. *Proc. Natl. Acad. Sci. USA* **107**:2479-2484, 2010.

Andrews, A.J., Chen, X., Zevin, A., Stargell, L.A., Luger, K. The histone chaperone Nap1 promotes nucleosome assembly by eliminating nonnucleosomal histone DNA interactions. *Mol. Cell* **37**:834-842, 2010.

Böhm, V., Hieb, A.R., **Andrews, A.J.**, Gansen, A., Rocker, A., Tóth, K., Luger, K., Langowski, J. Nucleosome accessibility governed by the dimer/tetramer interface. *Nucleic Acids Res*. **39**(8):3093-3102, 2011.

Andrews, A.J., Luger, K. Nucleosome structure(s) and stability: Variations on a theme. *Annu. Rev. Biophys*. **40**:99-117, 2011. Review

Kuo, Y.M., **Andrews, A.J.** Quantitating the specificity and selectivity of Gcn5-mediated acetylation of histone H3. *PLoS One* **8**:e54896, 2013.

Henry, R.A., Kuo, Y.M, **Andrews, A.J**.. Differences in specificity and selectivity between CBP and p300 acetylation of histone H3 and H3/H4. Biochemistry 52(34), 5746-59, 2013

Haery, L., Lugo-Picó, J.G., Henry, R.A., **Andrews, A.J**., and Gilmor, T.D,. Histone acetyltransferase-deficient p300 mutants in diffuse large B cell lymphoma have altered transcriptional regulatory activities and are required for optimal cell growth. Mol. Cell v13:29, 2014