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 Wistar Institute

2. **Reporting Period (start and end date of grant award period):** 01/01/2012 – 06/30/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Russel E. Kaufman, M.D.

4. **Grant Contact Person's Telephone Number:** 215-898-3926

5. **Grant SAP Number:** 4100057690

6. **Project Number and Title of Research Project:** 2: Characterizing Mechanisms of Transcriptional Activation Using Live Cell Imaging

7. **Start and End Date of Research Project:** January 1, 2012 – June 30, 2013

8. **Name of Principal Investigator for the Research Project:** Susan M. Janicki, 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:

   $ $330,933.44

   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
Janicki, Susan	Investigator	25%	56,705

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
Newhart, Alyshia	Research Assistant	25%

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
Leica TCS SP5 II laser scanning confocal microscope	This state-of-the-art instrument will help advance cancer research at the Institute by allowing investigators to carry out high-resolution, single cell observations, spectral separations, thick specimen analysis and co-localization studies in both fixed and dynamically interacting cell populations. It will support studies designed for both single time point as well as multi-dimensional time-lapse observations. This equipment will allow long-term, environmentally controlled, low photo-toxic, reduced noise, high resolution microscopy with an increased number of laser options, and the ability to carry out FRET, FRAP and FLIM analysis, 3D and 4D tracking, and temporal, spectral and spatial analysis	$224,046.50

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

Yes_____ No _____x____

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

11. Leveraging of Additional Funds

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

Yes_____ No _____x____

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

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

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐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:

I plan to apply for an R01 grant from NIH.

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

We will focus on identifying the genetic elements which regulate the recruitment of Sp100 to chromatin sites.

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.

The project resulted in the acquisition of a new Leica confocal microscope, which enhanced

the research infrastructure at the Wistar Institute.

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 agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). 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.

Specific Aim 1. Interrogate by shRNA depletion the requirement of known regulatory factors on (i) chromatin decondensation and (ii) RNA synthesis using kinetic, live cell imaging of single cells.

We use a system which allows the visualization of a transcription site in single living cells (Figure 1A), to track the recruitment of regulatory factors and determine their spatial organization at chromatin. Sp100 is a protein that we found to be recruited to the transcription site. Sp100 is a core PML nuclear body (PML-NB) protein linked to viral gene regulation. It is a single copy gene, which gives rise to four alternatively spliced isoforms, three of which, Sp100B, C and HMG (Figure 2A), contain the SAND domain (named after, Sp100, AIRE-1, NucP41/45, and DEAF-1), which preferentially binds DNA composed of unmethylated CpGs. These isoforms repress transcription from viral promoters. In addition to the SAND domain, Sp100C contains a bromodomain, which binds acetylated lysines, and a Plant Homeodomain (PHD), which binds methylated lysines in histones. Sp100 high mobility group (Sp100HMG) contains a domain homologous to the HMG-1/2 family of non-histone chromosomal DNA-binding proteins. Taken together, the domains of Sp100B, C and HMG suggest that these isoforms interact with DNA and chromatin.

In contrast to the repressive effects of the SAND-domain isoforms, Sp100A, which shares 477 of its 480 amino acids with Sp100B, C and HMG, promotes transcription. Interestingly, Sp100B, with a point mutation in the SAND domain (W655Q) predicted to disrupt its interaction with DNA, has also been reported to promote transcription. This suggests that the SAND domain masks a transcriptional promoting activity in the Sp100 N-terminus. It also highlights the importance of alternative splicing in regulating the differential functions of Sp100 in transcription.

Sp100 is enriched at the CMV promoter.

We used an antibody that recognizes all four Sp100 isoforms to evaluate its recruitment to the transgene array in the single cell imaging system. As Sp100 co-localizes with YFP-lac repressor, which marks the inactive site, in both the U2OS and HeLa cell lines, this indicates that Sp100 is also recruited to the inactive transcription site (Figure 1B). The intensity profiles (Figure 1B, panels d and h) graph the pixel intensity measurements along the line drawn through the array (note the yellow line in enlarged insets in the merged images, panels c and g). The DAPI staining of the DNA is represented by the blue line in the graph, which is uniform across the region. The significant overlap between the red (Sp100) and green (YFP-lac repressor) signals indicates the significant enrichment of Sp100 at the site in relation to YFP-lac repressor, which binds to the 10 kb of lac operator repeats upstream of the transcription unit (Figure 1A). The lack of complete overlap between the red and green signals suggests that sub-structural features of the chromatinized array can be detected.

Chromatin immunoprecipitation (ChIP) analyses of the inactive transgene from both the U2OS and HeLa cell lines indicate that Sp100 is also enriched at the CMV promoter (Figure 1C). Taken together, these results suggest that sequences in the CMV promoter seed the accumulation of Sp100 at the array and that this system can be used to study how it regulates transcription.

Sp100A is the isoform most strongly recruited to the transgene array.

To evaluate the effects of the Sp100 isoforms on the chromatinized CMV-promoter regulated reporter, we first examined the recruitment of YFP-tagged constructs (Figure 2A) to arrays in the U2OS cell line (Figure 2B). Sp100A was the most strongly recruited isoform at both the inactive site, marked by Cherry-lac repressor (Figure 2B, panels a-d), and the activated site, marked by Cherry-tTA-ER (Figure 2B, panels e-h). To determine whether sumoylation is required for Sp100A recruitment, we examined the localization of Sp100A(K297R), in which the sumoylated lysine is converted to an arginine (Figure 2B, panels i-p). The enrichment of Sp100A(K297R) at both inactive and activated arrays indicates that sumoylation is not required for Sp100A recruitment. This is consistent with a report showing that sumoylation is not required for Sp100A targeting to PML-NBs/ND10s. In contrast to Sp100A, only minimal accumulation of Sp100B was seen (Figure 2B, panels q-x). This suggests that there may be a correlation between the localization patterns of the Sp100 isoforms and their effects on transcription.

Sp100A promotes decondensation of the transgene array chromatin but not transcription.

The strong accumulation of Sp100A at activated arrays in both U2OS and HeLa cells correlates with increased chromatin decondensation. This suggests that Sp100A promotes chromatin decondensation during transcriptional activation. However, from this analysis, it is unclear whether Sp100A does so by increasing transcription or boosting a mechanism which unwinds activated chromatin. Although Sp100A promotes chromatin decondensation in activated U2OS cells, this cell line cannot be used to answer this question because the activator, on its own, is able to induce transcription due to its ATRX-null status. In contrast,

the CMV-promoter regulated array in the HeLa cell line is refractory to activation and, therefore, can be used to address this question

To determine whether the Sp100A-induced increase in chromatin decondensation at the activated array in HeLa cells is accompanied by transcription, we used the Cherry-MS2 binding protein to measure RNA accumulation at the transcription site (Figure 3A and 3B). In control cells co-expressing YFP-tTA-ER (the activator) and CFP-lac repressor (to identify the array), Cherry-MS2 does not significantly accumulate (Figure 3A, panels a-d; 3B). Interestingly, Cherry-MS2 also does not accumulate at activated arrays in YFP-Sp100A-expressing cells (Figure 3A, panels e-h), which indicates that, despite its ability to promote chromatin decondensation (Figure 3A, note the accumulation of CFP-tTA-ER at the activated array; panel g, enlarged inset), Sp100A cannot overcome Daxx and ATRX mediated transcriptional repression (Figure 3B). Consistent with our previous report that ICP0 permits transcriptional activation in HeLa cells, Cherry-MS2 accumulates strongly at the activated site in YFP-ICP0-expressing cells (Figure 3A, panels i-l; 3B).

To measure the effects of Sp100A and ICP0 on the levels of total mRNA expressed in HeLa cells, we used quantitative RT-PCR (qRT-PCR) (Figure 3C). Because this analysis was done on RNA isolated from cell populations, we expressed these factors and the activator using lentiviruses in order to achieve high expression efficiency (>90% of cells). Consistent with the single-cell analysis (Figure 3A and 3B), the increase in transcription induced by Sp100A was small compared to ICP0 (Figure 3C). Interestingly, co-expression of Sp100A and ICP0 did not significantly increase transcription above the level induced by ICP0 alone, which indicates that, in this assay, their effects are not additive (Figure 3C). It is possible that transcription plateaus in ICP0-expressing cells because RNA pol II saturates the gene template or another limiting factor is exhausted. Taken together, these results indicated that Sp100A does not promote transcription in HeLa cells, which have a functional Daxx and ATRX pathway.

Although we proposed to use shRNA knockdowns to analyze the effects of regulatory factors on transcription, we were able to conduct these studies on Sp100 using two different cell lines, U2OS and HeLa, with different genetic backgrounds and over-expression of the Sp100 isoforms.

<u>Specific Aim 2. Determine the kinetic and spatial dynamics of post-translational modifications and their binding proteins during transcription activation and mitosis.</u>

In our efforts to determine the mechanism through which Sp100A promotes chromatin decondensation, we examined mechanisms of lysine acetylation because this is a post translational modification (PTM) associated with transcriptionally active chromatin. We examined both the enzymes that acetylate proteins (histone acetyltransferases) and proteins that bind to acetylated lysines, those that contain bromo domains.

Sp100A promotes lysine acetylation at the activated transgene array.

The increase in chromatin decondensation induced by Sp100A in HeLa cells is not

accompanied by increased transcription (Figure 3), which indicates that they are separable events. This, therefore, led us to speculate that Sp100A may promote decondensation by recruiting proteins, which unwind activated chromatin, such as acetyl-lysine regulatory factors. To test this hypothesis, we measured YFP-tagged histone acetyltransferases (GCN5, PCAF and p300) and acetyl-lysine-binding proteins (Brd2 and Brd4) at activated arrays in U2OS and HeLa cells co-expressing Cherry, Cherry-Sp100A or Cherry-Sp100B (Figure 4A-B). All of the factors were enriched at activated arrays in U2OS cells (Figure 4A), consistent with our previous report that they accumulate during activation. Interestingly, Sp100B did not prevent their accumulation at activated arrays in U2OS cells (Figure 4A) despite its inhibitory effects on chromatin decondensation. In fact, the concentration of Brd2 and Brd4 was the highest in Sp100B-expressing U2OS cells (Figure 4A) due to the compaction of the arrays.

In HeLa cells, in contrast, the acetyl-lysine regulatory factors were only highly enriched at activated arrays in Sp100A-expressing cells (Figure 4B). This result indicates that, in the presence of a functional Daxx and ATRX pathway, only Sp100A is able to promote acetyl-lysine regulatory factor recruitment and chromatin decondensation during activation. Interestingly, Sp100B prevented their accumulation in HeLa cells (Figure 4B). The higher and more variable increases in acetyl-lysine regulatory factor levels at arrays in HeLa control cells suggests that the SAND-domain containing isoforms may function to stabilize transcriptionally silent expression states by preventing lysine acetylation. The fact that Sp100B fails to inhibit the accumulation of these factors at activated arrays in U2OS cells (Figure 4A) suggests that it requires the Daxx and ATRX pathway to do so.

Taken together, these results suggest that Sp100A promotes chromatin decondensation at transcription sites by increasing lysine acetylation. In order to determine whether increasing the concentration of Sp100A at the site is sufficient for this effect, we measured acetyl lysine regulatory factor levels at inactive arrays (marked by CFP-lac repressor) in Cherry-Sp100A expressing cells. The low accumulation of these factors at inactive arrays in both Sp100A-expressing U2OS and HeLa cells indicates that Sp100A only promotes their recruitment in conjunction with the activator (Figure 4A and 4B). This suggests that Sp100A functions to accelerate transcription by promoting lysine acetylation and chromatin decondensation during activation.

Using antibodies against endogenous Brd4 and histone H4 lysine 5 acetylation (H4acK5), we show that their levels also increase at activated arrays in Sp100A-expressing cells (Figure 4C-E), further supporting the conclusion that Sp100A promotes chromatin decondensation by increasing lysine acetylation at activated transcription sites. This result also indicates that YFP-tagged acetyl lysine regulatory factor accumulation at the activated arrays (Figure 4A-4B) is not due to over expression.

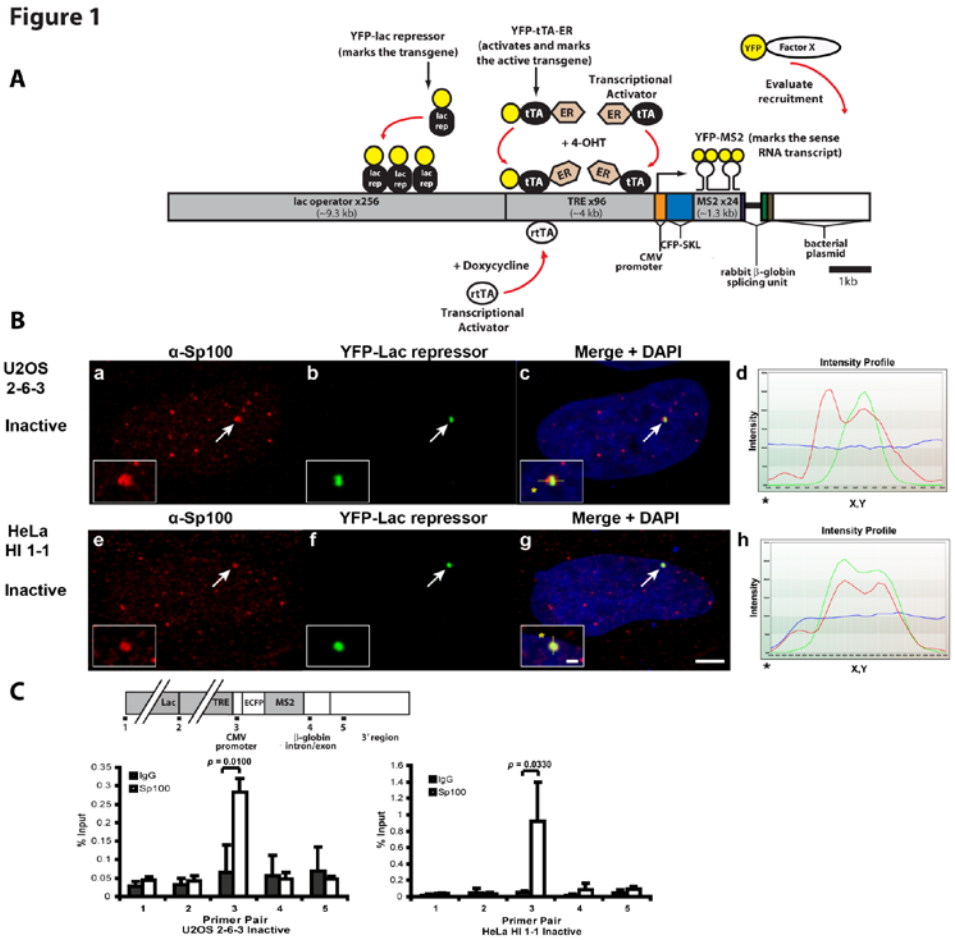

Figure 1: Sp100 is recruited to the CMV promoter of the inducible transgene in the U2OS and HeLa cell lines. (**A**) Diagram of the inducible transgene drawn to scale. Expression of YFP-lac repressor allows the transgene to be visualized in both the inactive and active state. Transcription is induced from the minimal CMV promoter by the activators, YFP-tTA-ER or ER-tTA, in the presence of 4-hydroxytamoxifen (4-OHT) and rtTA in the presence of Doxycycline (Dox). The transcribed RNA encodes CFP fused to a peroxisomal targeting signal (SKL). The RNA is visualized by YFP-MS2, which binds to the stem loops in the transcript. The 3' end of the transcription unit is composed of the intron 2 splicing unit of the rabbit β-globin gene. YFP-tagged regulatory factors can be monitored for recruitment to the array by co-expression with the fluorescently-tagged lac repressor and/or activator proteins. All of the factors are shown in YFP-tagged form. However, Cherry and CFP-tagged versions are also used in different combinations in the assays as described in figures and legends. (**B**) Immunofluorescence localization of Sp100 at the inactive array, marked by YFP-lac repressor, in (**a-d**) U2OS (2-6-3), and (**e-h**) HeLa (HI 1-1) cells. Arrows indicate the location of the transgene array. Yellow lines in enlarged merge insets show the path through which the red, green and blue intensities were measured in the intensity profiles (**d and h**). Asterisks mark the start of the measured lines. Scale bar=5 µm. Scale bars in enlarged inset=1 µm. (**C**) Diagram of the transgene showing the location of the primer pairs used for real-time PCR in the Sp100 ChIP assays with chromatin lysates prepared from the inactive U2OS (2-6-3) and HeLa (HI 1-1) cell lines. Results are the average of at least 3 independent experiments. Standard deviations, in the form of error bars, and P values, calculated using unpaired t-test, are presented in the graphs.

Figure 2

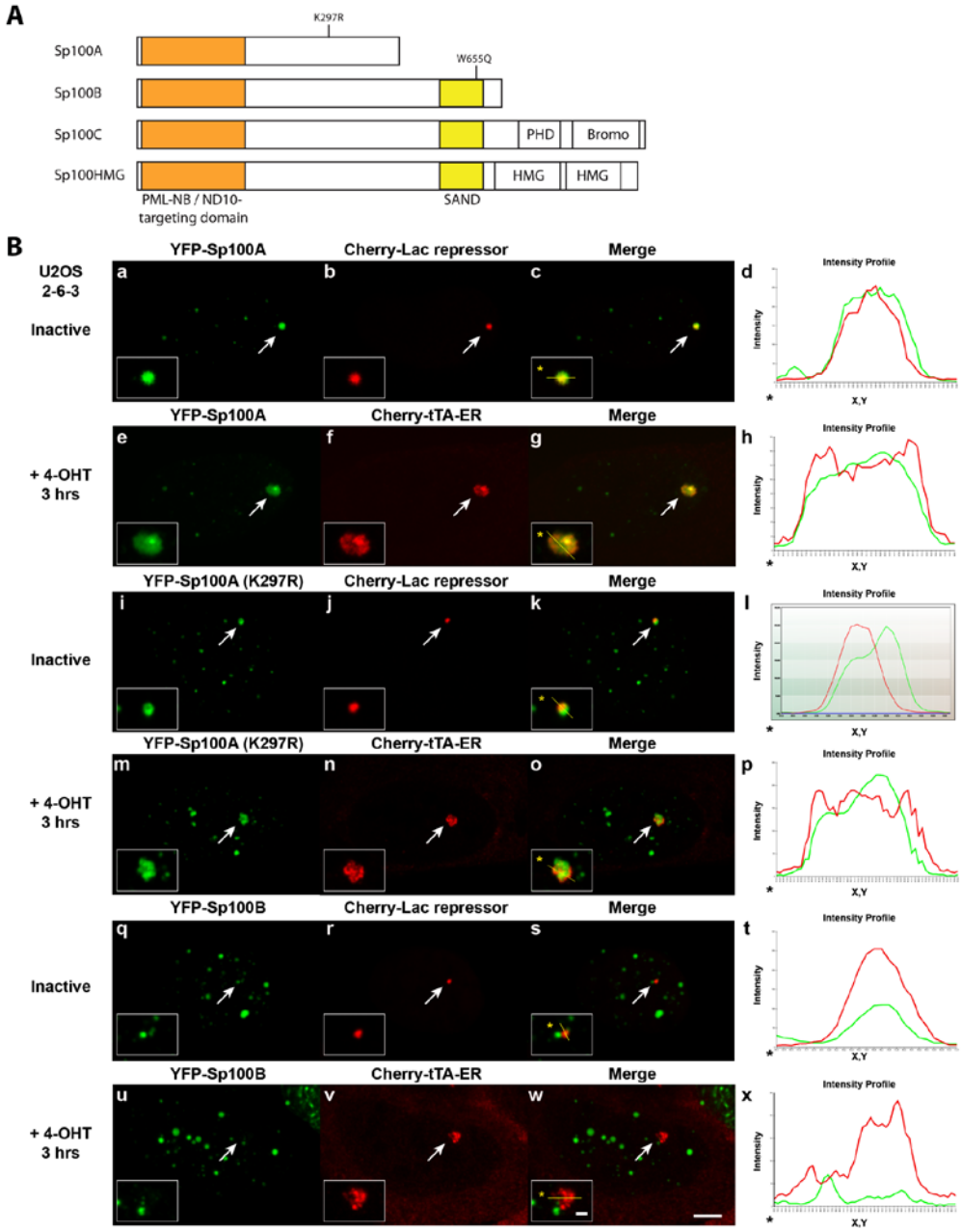

Figure 2: Analysis of Sp100 isoform recruitment to inactive and activated transgene arrays in U2OS cells. (**A**) Diagram of the domain organization of the Sp100 isoforms and the locations of the K297R sumoylation mutation in Sp100A and the W655Q mutation in the SAND domains of Sp100B, C and HMG. (**B**) Localization of (**a-h**) YFP-Sp100A, (**i-p**) YFP-Sp100A(K297R) and (**q-x**) YFP-Sp100B at inactive arrays, marked by Cherry-lac repressor, and activated arrays, marked by Cherry-tTA-ER, in U2OS (2-6-3) cells. Arrows indicate the transgene array locations. Yellow lines in enlarged merge insets show the path through which the red and green intensities were measured in the intensity profiles (**d, h, l, p, t and x**). Asterisks mark the start of the measured lines. Scale bar=5 µm. Scale bars in enlarged inset=1 µm.

Figure 3

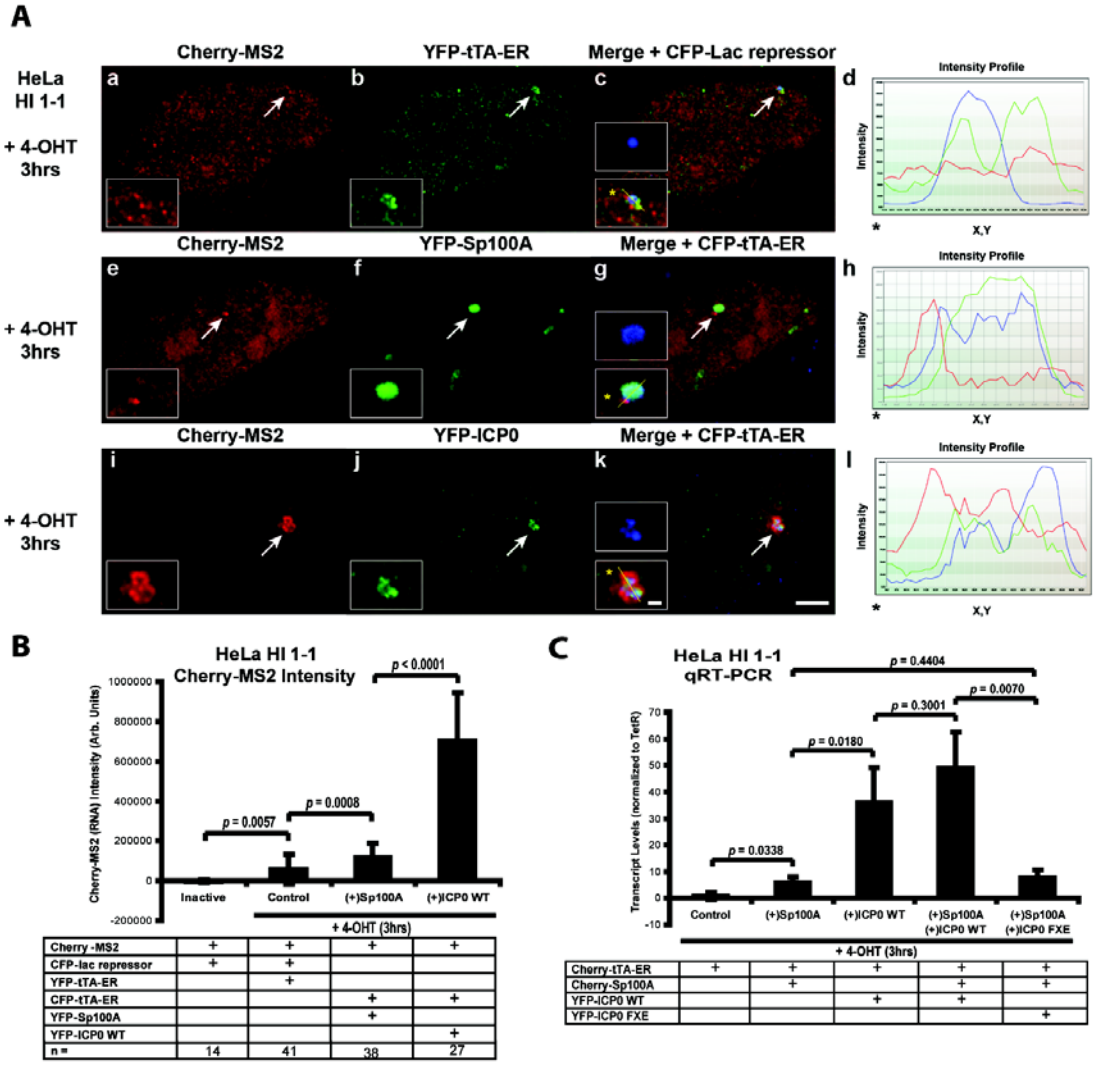

Figure 3: Sp100A cannot overcome Daxx and ATRX-mediated transcriptional repression. **(A)** Images of Cherry-MS2 accumulation at activated transgene arrays in (**a-h**) control, (**e-h**) YFP-Sp100A- and (**i-l**) YFP-ICP0-expressing HeLa (HI 1-1) cells. Arrows indicate the locations of the transgene arrays. Yellow lines in enlarged merge insets show the path through which the red, green and blue intensities were measured in the intensity profiles (**d, h** and **l**). Asterisks mark the start of the measured lines. Scale bar=5 µm. Scale bars in enlarged inset=1 µm. (**B**) Single-cell analysis of Cherry-MS2 intensity levels at inactive and activated transgene arrays in HeLa (HI 1-1) cells. Factors were transiently expressed from plasmids. Constructs expressed and N values are shown in the chart below the graph. (**C**) qRT-PCR analysis of mRNA levels in activated HeLa (HI 1-1) cells after lentiviral transduction of Sp100A alone or in combination with ICP0 or ICP0-FxE, deleted of the RING finger zinc-binding motif, which is required for activity. Constructs expressed are shown in the chart below the graph. Results are the average of at least 3 independent experiments. Standard deviations, in the form of error bars and P values, calculated using the unpaired t-test, are presented in the graphs.

Figure 4

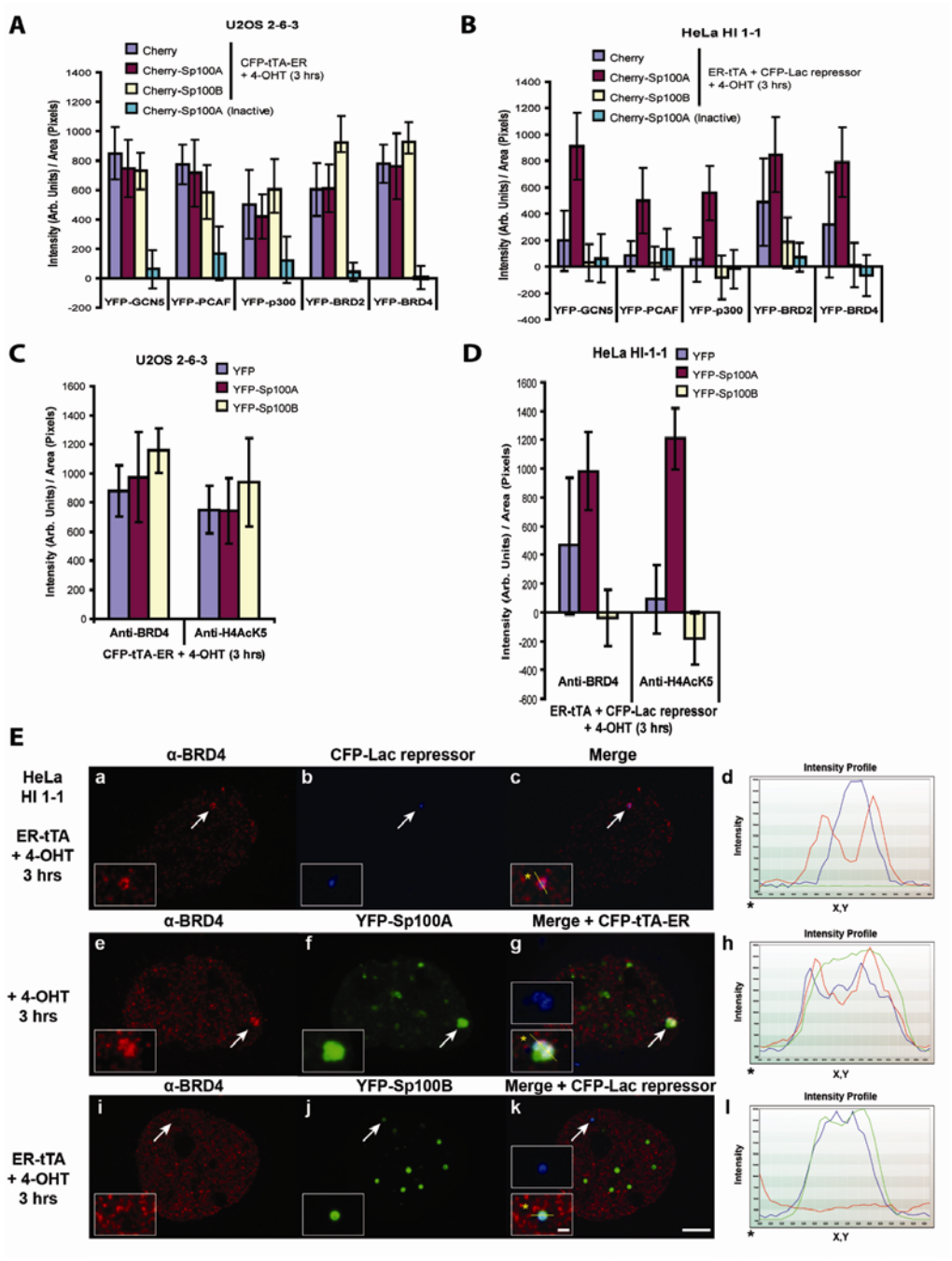

Figure 4: Sp100A promotes lysine acetylation at the activated CMV-promoter regulated transgene array. Average intensity levels of YFP-tagged acetyl-lysine regulatory factors at transgene arrays in (**A**) U2OS (2-6-3) and (**B**) HeLa (HI 1-1) cells. Factors were expressed by transient transfection. For activated U2OS (2-6-3) cells, the activator (CFP-tTA-ER) was co-expressed with Cherry, Cherry-Sp100A or Cherry-Sp100B and a YFP-tagged acetyl lysine regulatory factor. For activated HeLa (HI 1-1) cells, the activator (tTA-ER) was co-expressed with Cherry, Cherry- Sp100A or Cherry-Sp100B, a YFP-tagged acetyl lysine regulatory factor, and CFP-lac repressor (to identify the array). For analysis of the Sp100A's effects on acetyl lysine regulatory factor recruitment to the inactive array, YFP-tagged factors

were co-expressed with Cherry-Sp100A and CFP-lac repressor (to identify the array). Standard deviations, in the form of error bars, are presented in the graphs. P values, calculated using the unpaired t-test, are listed in Table 2. Average intensity levels of endogenous Brd4 and histone H4 lysine 5 acetylation (H4AcK5) levels, detected by immunofluorescence staining, at CFP-tTA-ER activated arrays in (**C**) U2OS (2-6-3) and (**D**) HeLa (HI 1-1) cells co-expressing YFP, YFP-Sp100A or YFP-Sp100B. Standard deviations, in the form of error bars, are presented in the graphs. P values, calculated using the unpaired t-test, are listed in Table 3. (**E**) Immunofluorescence localization of Brd4 at the activated array in (**a-d**) control, (**e-h**) YFP-Sp100A and (**i-l**) YFP-Sp100B-expressing HeLa, (HI 1-1) cells. YFP could not be imaged in control samples because it was extracted by the immuofluorescence staining protocol. Arrows indicate the locations of the transgene arrays. Yellow lines in enlarged merge insets show the path through which the red, green and blue intensities were measured in the intensity profiles (**d, h** and **l**). Asterisks mark the start of the measured lines. Scale bar=5 µm. Scale bars in enlarged inset=1 µm.

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. Sp100A promotes chromatin decondensation at a CMV-promoter regulated transcription site.	Newhart A, Negorev DG, Rafalska-Metcalf IU, Yang T, Maul GG and **Janicki SM**.	*Molecular Biology of the Cell*	September 2012	☐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:

I plan to generate a publication about our studies of the mechanisms regulating Sp100 recruitment to transcription sites.

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.*

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BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
JANICKI, Susan M.	**Assistant Professor**
eRA COMMONS USER NAME **SJANICKI**	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Tufts University, Medford, Massachusetts	B.S.	1993	Biology and English
University of Maryland, Baltimore, Maryland	Ph.D.	1999	Human Genetics

A. Personal Statement

For a gene to be expressed, the functions of multiple molecular machines must be coordinated at the site of transcription. To investigate the timing and spatial organization of these regulatory events, my laboratory uses single-cell live-cell imaging. We engineer reporter constructs to include sequence elements, which permit the visualization of nucleic acids *in vivo*. Upon stable integration, these transgenes form chromatinized arrays, which can be imaged during activation to obtain high-resolution quantitative information about transcriptional dynamics. Modeling can suggest new hypotheses, which can be tested both in the single-cell imaging system and at endogenous genes. We are currently using this approach to investigate the role of RNA in mammalian chromatin regulation and the mechanisms through which mutations in chromatin regulatory factors cause cancer.

B. Positions and Honors:

1993-1995 Research Assistant in the laboratory of Dr. Mervyn J. Monteiro, University of Maryland, Baltimore
1995-1999 Graduate Research in the laboratory of Dr. Mervyn J. Monteiro, University of Maryland, Baltimore. "Understanding the Function of the Presenilins in Apoptosis and Cell Cycle Progression"
1999-2005 Postdoctoral fellow in the laboratory of Dr. David L. Spector, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. Live cell imaging of gene expression.
2005-present Assistant Professor, The Wistar Institute, Philadelphia, PA
2011-present Scientific Director, The Wistar Institute Cancer Center Shared Facility Imaging Core

Teaching experience:

2001 and 2002 Undergraduate Research Program, supervised research of summer students in the laboratory of Dr. David L. Spector
Summer 2006 Wistar Institute Undergraduate Research Program
2007-2010 Wistar Institute Biotechnician Training (BTT) Program

Professional Honors:

1996 American Society for Cell Biology Pre-doctoral Travel Award
2006 Beckman Young Investigator Award
2007 V Foundation Scholar
2007 March of Dimes Basil O'Connor Starter Scholar Research Award

C. Publications (in chronological order):.

1. Hsu C, **Janicki SM**, and Monteiro MJ. (1995) The first intron of the mouse neurofilament light gene NF-L. increases gene expression. *Molecular Brain Research* 32, 241-251.
2. **Janicki SM** and Monteiro MJ. (1997) Increased apoptosis arising from increased expression of the Alzheimer's disease-associated presenilin-2 mutation N141I. *Journal of Cell Biology* 139, 485-495.
3. **Janicki SM** and Monteiro MJ. (1999) Presenilin overexpression arrests cells in the G1 phase of the cell cycle: arrest potentiated by the Alzheimer's disease PS2N141I mutant. *American Journal of Pathology* 155,

135-144.
4. Stabler SM, Ostrowski LL, **Janicki SM**, Monteiro M.J. (1999) A myristoylated calcium binding protein that preferentially interacts with the Alzheimer's disease presenilin-2 protein. *Journal of Cell Biology* 145, 1277-1292.
5. **Janicki SM**, Stabler SM, Monteiro MJ. (2000) Familial Alzheimer's disease presenilin-1 mutants potentiate cell cycle arrest. *Neurobiology of Aging* 21, 829-836.
6. Tsukamoto T, Hashiguchi N, **Janicki SM**, Tumbar T, Belmont AS, Spector DL. (2000) Visualization of gene activity in living cells. *Nature Cell Biology* 2, 871-878.
7. Muratani M, Gerlich D, **Janicki SM**, Gebhard M, Eils R, Spector DL. (2002) Metabolic-energy-dependent movement of PML bodies within the mammalian cell nucleus. *Nature Cell Biology* 4, 106-110.
8. **Janicki SM** and Spector DL. (2003) Nuclear choreography: interpretations from living cells. *Current Opinion in Cell Biology* 15, 149-157.
9. **Janicki SM**, Tsukamoto T, Salghetti SE, Tansey WP, Sachidanandam R, Prasanth KV, Ried T, Shav-Tal Y, Bertrand E, Singer RH, Spector DL. (2004) From Silencing to Gene Expression: Real-Time Analysis in Single Cells. *Cell* 116, 683-698.
10. Shav-Tal Y, Darzacq X, Shenoy SM, Fusco D, **Janicki SM**, Spector DL, Singer RH. (2004) Dynamics of Single mRNPs in the Nuclei of Living Cells. *Science* 18, 1797-1800.
11. **Janicki SM** and Spector DL. Imaging Gene Expression in Living Cells. (2004) In: *Live Cell Imaging: A Laboratory Manual,* Goldman RD and Spector DL, editors. Cold Spring Harbor Laboratory Press, New York.
12. Rafalska-Metcalf IU and **Janicki SM**. (2007) Show and Tell: Visualizing Gene Expression in Living Cells. *Journal of Cell Science*, 120, 2301-7.
13. Shanbhag NM, Rafalska-Metcalf IU, Balane-Bolivar C, **Janicki SM**, and Greenberg RA. (2010) ATM-dependent chromatin changes silence transcription in cis to DNA double-strand breaks. *Cell* 141(6):970-81.
14. Rafalska-Metcalf IU, Joo LM, Powers SL, LeRoy G and **Janicki SM**. (2010) Single cell analysis of transcriptional activation dynamics. *PLoS ONE* 5(4):e10272.
15. Lan L, Ui A, Nakajima S, Hatakeyama K, Hoshi M, Watanabe R, **Janicki SM**, Ogiwara H, Kohno T, Kanno S, Yasui A. (2010) The ACF1 complex is required for DNA double-strand break repair in human cells. *Molecular Cell* 40(6):876-87.
16. White, DE, Rafalska-Metcalf IU, Ivanov AV, Corsinotti A, Peng, H, Lee SC, Trono D, **Janicki SM**, and Rauscher 3rd FJ. (2012) HP1 association regulates ATM-dependent phosphorylation of KAP1 during DNA repair in Heterochromatin. *Molecular Cancer Research* 10(3), 401-14.
17. Newhart A, Rafalska-Metcalf IU, Yang T, Negorev DG, and **Janicki SM**. Single cell analysis of Daxx and ATRX-dependent transcriptional repression. (2012) *Journal of Cell Science.* Nov 15;125(Pt 22):5489-501. Epub 2012 Sep 12.
18. Rafalska-Metcalf IU and **Janicki SM**. (2013) Preparation of Cell Lines for Single-Cell Analysis of Transcriptional Activation Dynamics. In: *Gene Regulation: Methods and Protocols.* Minou Bina, editor. Humana Press, New York.
19. Newhart A, Negorev DG, Rafalska-Metcalf IU, Yang T, Maul GG and **Janicki SM**. Sp100A promotes chromatin decondensation at a CMV-promoter regulated transcription site. (2013) *Molecular Biology of the Cell.* May;24(9):1454-68. Epub 2013 March 13.
20. Newhart A, Rafalska-Metcalf IU, Yang T, Joo LM, Powers SL, Kossenkov AV, Lopez-Jones M, Singer RH, Showe LC, Skordalakes E, and **Janicki SM**. Single-cell analysis of RNA-mediated histone H3.3 recruitment to a cytomegalovirus promoter-regulated transcription site. (2013) *Journal of Biological Chemistry.* July 5;288(27):19882-99. Epub 2013 May 20.
21. Newhart A and **Janicki SM**. Seeing is Believing: Visualizing Transcriptional Dynamics in Single Cells. (2013) *Journal of Cellular Physiology*. (Accepted).
22. Britton LMP, Newhart A, Bhanu NV, Sridharan R, Michelle Gonzales-Cope M, Plath K, **Janicki SM** and Garcia BA. Expanding the Language of the Histone Code: Serine, Threonine and Tyrosine O-Acetylation. (2013) *Epigenetics.* (Accepted).