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):** 01/01/2010 – 06/30/2011

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

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

5. **Grant SAP Number: #** 4100050916

6. **Project Number and Title of Research Project:** *3: Elucidation of the Integrator Composition and Function*

7. **Start and End Date of Research Project:** 01/01/2010 – 06/30/2011

8. **Name of Principal Investigator for the Research Project:** Ramin Shiekhattar, Ph.D.

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:

   $907,003.13

   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
Shiekhattar	Principal Investigator	60%	157,695.12
Baillat	Associate Staff Scientist	100%	40,248.18
Beringer	Postdoctoral Fellow	100%	4,697.52
Carre	Postdoctoral Fellow	100%	50,393.04
Gardini	Postdoctoral Fellow	15%	7,512.78
Saviolaki	Predoctoral Fellow	100%	40,314.48
Acevedo	Research Assistant I	100%	24,148.88

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
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
None		

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 __x_____ No_____

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

NIH direct cost $230,493

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_____ No ___x_____

If yes, please describe your plans:

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

We are planning to gain an understanding of the full scope and detailed mechanism of Integrator function in mammalian transcription.

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_____ No _x_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

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.

Small nuclear ribonucleic acids (snRNAs) are components of the spliceosome, a complex assembly of ribonucleoparticles involved in pre-micro RNA (mRNA) processing. The majority of snRNAs (i.e., U1 to U5) are transcribed by RNA polymerase II (RNAPII) to yield short non-polyadenylated 3'-extended precursors. These precursors are then exported to the cytoplasm for further 3' trimming and incorporation into the small nuclear ribonucleoparticles (snRNPs). The

formation of proper snRNA precursors depends on a *cis*-acting sequence, named the 3' box, located 9-19 nucleotides downstream of the 3' end of the mature snRNA and on the presence of an snRNA compatible promoter at the 5' end of the gene. Furthermore, the C-terminal domain (CTD) of the largest subunit of RNAPII has been shown to play a crucial role in snRNA 3'-end processing. The identity of the factor(s) that associate with the CTD and result in the processing of the snRNA genes remained elusive for over two decades. We isolated a multi-protein complex termed the Integrator that associates with the CTD of RNAPII and mediates the 3'-end processing of snRNAs. This project was focused on characterization of the Integrator polypeptide composition and functional elucidation of its mechanism of action.

Specific Aims:

1. *Analysis of subunits of the Integrator and reconstitution of the complex using recombinant components.* Several of the proteins in the Integrator complex do not correspond to genes whose function or expression patterns have been described. Biochemical and immunological analyses will be performed to determine the role of these proteins in regulation of small nuclear RNA transcription and processing.

2. *Identification of the subunit(s) of the Integrator that mediate association with the CTD.* We will examine the role of the CTD phosphorylation in modulating the association of the Integrator complex and RNAPII.

To directly assess the role of Integrator complex and its individual subunits in snRNA processing, we utilized a reporter construct which allows the detection of the processed and unprocessed transcript using RNAase protection. As shown in Figure 1, depletion of INTs 1, 2, 3, 4, 9 and 11 results in a defect of snRNA processing. We have generated knock-down vectors for the rest of the Integrator subunits and are in the process of systematic analysis of all subunits. It will also be important to know the subunit composition of the complex following the knock-down of individual subunits. We have generated a number of epitope-tagged cell lines using different Integrator subunits where Integrator could be affinity-purified. Such an analysis will not only begin to reveal the subunit architecture of the complex, but also will provide valuable information regarding individual subunits whose presence is required to maintain the integrity of a large multi-protein complex. Such information will also aid in precise explanation of the functional defects following the knock-down of the individual subunits. For example, we will be able to discriminate between functional defects that result from the loss of the catalytic subunit INTS11, which may reveal a functional requirement for an individual subunit of the complex. Taken together, these studies combined with *in vitro* reconstitution should provide us with great insight into the structure and function of the Integrator complex.

Integrator displays a role in transcription beyond that of RNA processing

To further dissect the Integrator role in snRNA transcription as well as RNA processing, we designed a reporter plasmid comprising the entire U2 snRNA gene (promoter, U2 snRNA sequence and 3´ box) fused at its 3´end with the renilla luciferase (pU2G-RL in Figure 4a) and another comprising the U2 snRNA promoter directly fused to the renilla luciferase coding sequence (pU2P-RL, in Figure 2a). The pU2G-RL should express luciferase only when the

processing is impaired, while pU2P-RL should indicate the transcription rate of the U2 promoter in the different conditions used. The shRNA mediated knock-down of INTS1 and INTS11, known to impair U2 snRNA 3´end processing, resulted in an important increase of luciferase activity (Figure 2b, left panel). Unexpectedly, knock-down of INTS1 and INTS11 also resulted in the increase of transcription from the U2P construct (Figure 2a, right panel: white columns). Ratio of luciferase activity from U2G over U2P is still in favour of a processing defect over just a transcriptional activation (Figure 2a, right panel). Nevertheless, the increase in luciferase from pU2P-RL reporter seen following Integrator knock-down is suggestive of a role for Integrator in transcription of the U2 promoter distinct from its role in 3´-box-mediated snRNA processing. Importantly, we find a similar effect following the knock-down of most Integrator subunits (Figure 3). It is formally possible that Integrator has a greater role in RNA processing. However, any additional role for Integrator in RNA processing would lead to decreased luciferase activity, rather than an enhanced luciferase expression.

One exciting hypothesis concerns the possible role for Integrator in suppressing aberrant transcripts generated due to read-through transcripts. Normally, Integrator suppresses such transcripts and its depletion leads to accumulation of such aberrant transcripts. In the following section we will present further data that is consistent with a role for Integrator in the regulation of a large number of RNAPII-mediated genes.

Purification of Integrator from the chromatin fraction and global analysis of its genome occupancy

We initially purified the Integrator complex using a nuclear extract of a stably-expressing Flag-INTS10 293T-derived cell line. In order to gain further insight into the composition of the Integrator complex that is associated with the chromatin fraction, we purified Integrator using a new protocol that was described for purification of chromatin bound factors. Interestingly, we found that not only was Integrator quite enriched in the chromatin fraction as expected from its stable association with RNAPII, but also we were able to obtain a cleaner purification of the complex using chromatin enriched fractions (also called nuclear pellet). Interestingly, the purification enabled us to avoid the excess of over-expressed flag-tagged protein. Flag-INTS10 is now eluted at nearly stoichiometric ratio with the other subunits (Figure 4a). Mass spectrometric sequencing of this chromatin bound Integrator complex confirmed the subunit composition we had previously established for the Integrator (Figure 4b). The enriched association of the Integrator with the chromatin persuaded us to analyze its global genome occupancy using chromatin immunopreciptation followed by high throughput Solexa sequencing. We anticipate a greater role for this complex on RNAPII-mediated transcription beyond that of snRNA transcription and RNA processing.

The close association of Integrator and chromatin described above and the recent identification of Integrator in large scale purifications of the spliceosome or the mRNA 3´end processing machinery suggested a broader function for the Integrator complex in RNAPII transcription. To investigate this possibility, we designed a ChIP-sequencing experiment. A stable 293T cell line stably expressing INTS11 fused to two Hemagglutinin (HA) epitopes was established and tested for enrichment of U1 snRNA gene 3´end as a control (Figure 5a).

The ChIP material was then used for high throughput sequencing using Solexa technology. Moreover, we used an HA antibody ChIP from an untagged 293T cell line as control and subjected those to Solexa sequencing as well. The analysis of high throughput data is performed in collaboration with the Bioinformatic facility at the Wistar institute headed by Dr. Ramana Davuluri. We obtained 4,379,099 unambiguous reads from HA-Int11 cell line and 4,815,758 unambiguous reads from parental untagged control cell line. Following clustering analysis and subtraction of signals (peaks) that did not reveal a statistically significant difference between HA-Int11 cell line and the parental control cell line, we were left with 2,321 specific HA-Int11 peaks. Eighty-five percent of these peaks (1,973) displayed a close association with the transcriptional start sites of RNAPII-dependent genes (Figure 5b and c). Gene ontology analysis indicated that a large number of these genes (greater than 50) are involved in establishment or maintenance of chromatin architecture (Figure 5c).

Analysis of Genome-wide occupancy of Integrator using antibodies against the endogenous protein

We used antibodies that we developed against the INTS11 protein to perform additional chromatin immunoprecipitation followed by high throughput sequencing of the associated DNA. These results revealed the presence of Integrator at a large number of U snRNA as well as a set of protein-coding genes (Figure 6A). The profile of Integrator at U2 (Figure 6B) and average profile of Integrator at U snRNA genes revealed a localization at the 3'-end of the genes. In contrast, Integrator displayed a chromatin residence at the 5'-end of the protein-coding genes consistent with a role in regulation of transcription (Figure 6C). Detailed analysis indicated that at most protein-coding genes Integrator occupies the transcription start site while smaller quantities could be observed throughout the body of the genes.

Integrator is critical for transcriptional activation

The pattern of Integrator occupancy suggested a role for this complex in transcriptional regulation of protein-coding genes. Therefore, we tested Integrator's role in transcriptional activation of a set of genes responsive to epidermal growth factor (EGF). HeLa cells were treated with EGF, and responsiveness of a set of candidate genes including JUN, FOS, EGR1 and NR4A1 (Figure 7A) was assessed before and following depletion of Ints 11 and Ints 1 subunits of the Integrator. Interestingly, while depletion of Integrator subunits de-repressed basal levels of transcription, the responsiveness to EGF was substantially blunted (Figure 7B). This effect was not due to indirect role of Integrator in RNA processing, as similar decreased responsiveness to EGF was also observed when one analyzed the primary transcripts (prior to RNA processing) of the responsive genes (Figure 7C). Since Integrator displayed a critical role in EGF responsiveness, we asked whether Integrator may have a role in transcriptional activation by classical transcriptional activator such as VP16. Importantly, depletion of Ints11 subunit of integrator completely abrogates the responsiveness of a reporter construct to transcriptional induction by GAL4-VP16 (Figure 7D and E). These results demonstrate a role for Integrator in transcriptional activation.

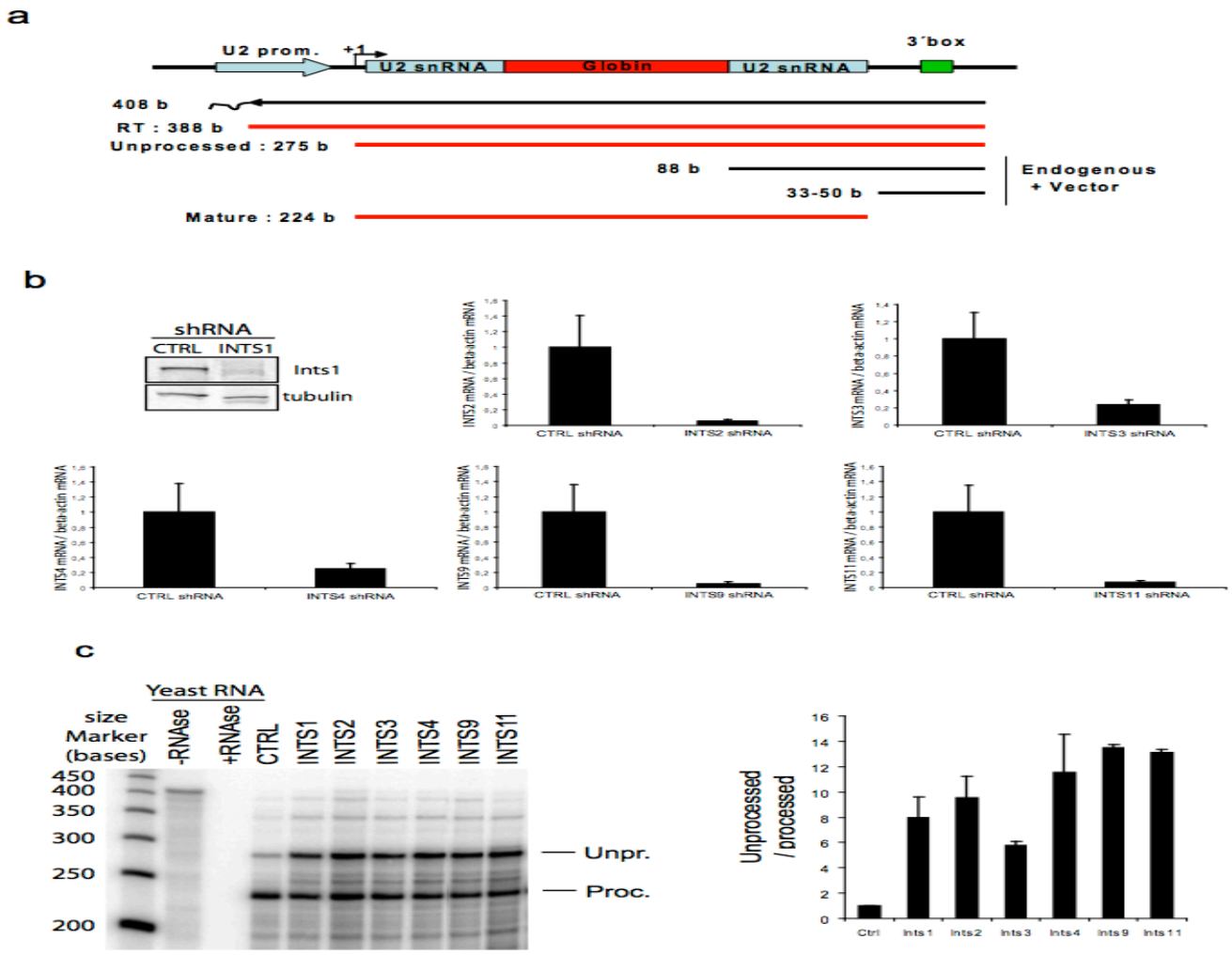


Figure 1. Integrator is essential for the processing of snRNA genes.

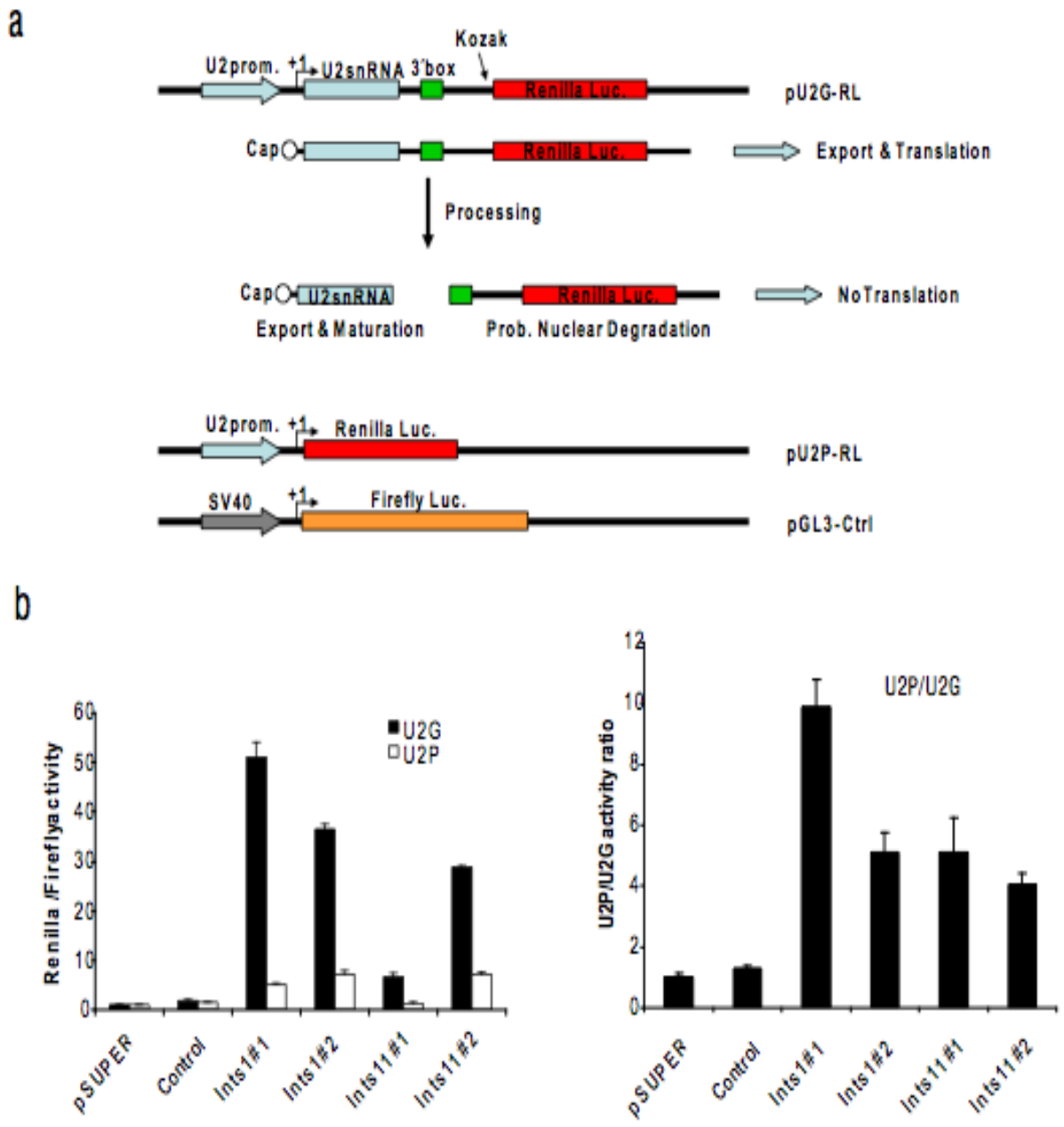


Figure 2. Integrator displays a function beyond that of 3'-end processing.

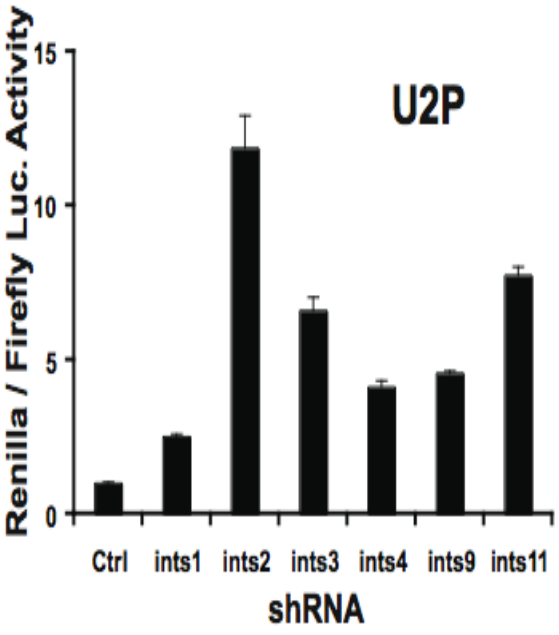
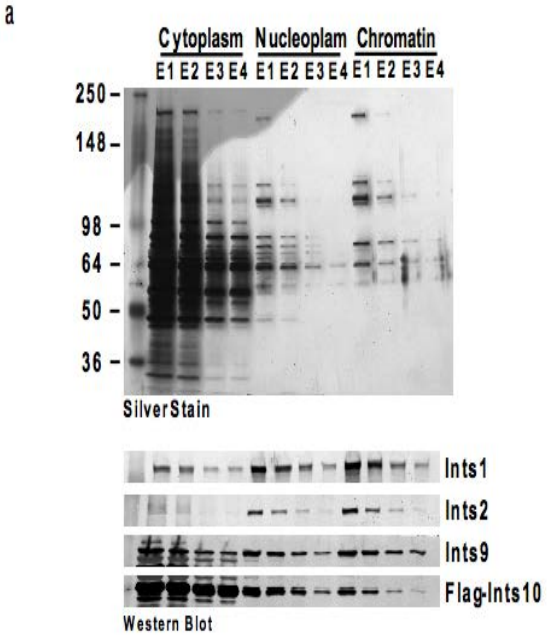


Figure 3. Depletion of multiple Integrator subunits leads to enhanced transcription.



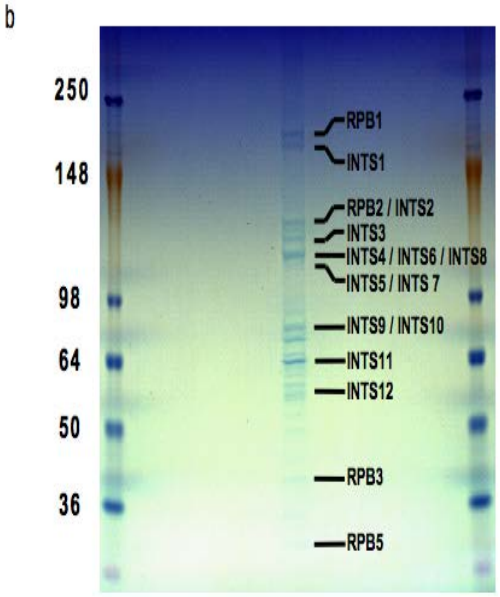


Figure 4. Purification of Integrator from chromatin fraction.

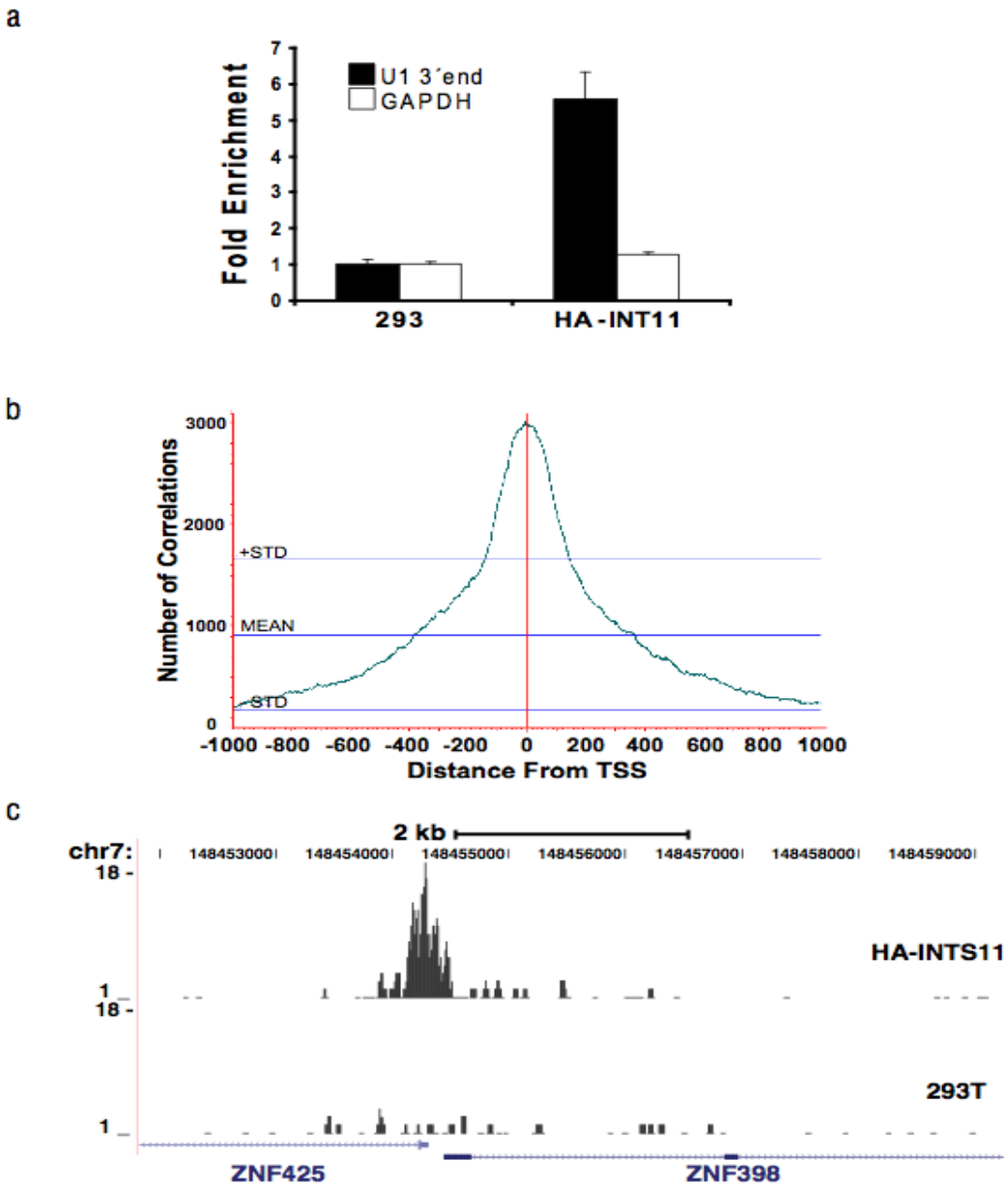


Figure 5. Global analysis of Integrator using ChIP-Sequencing

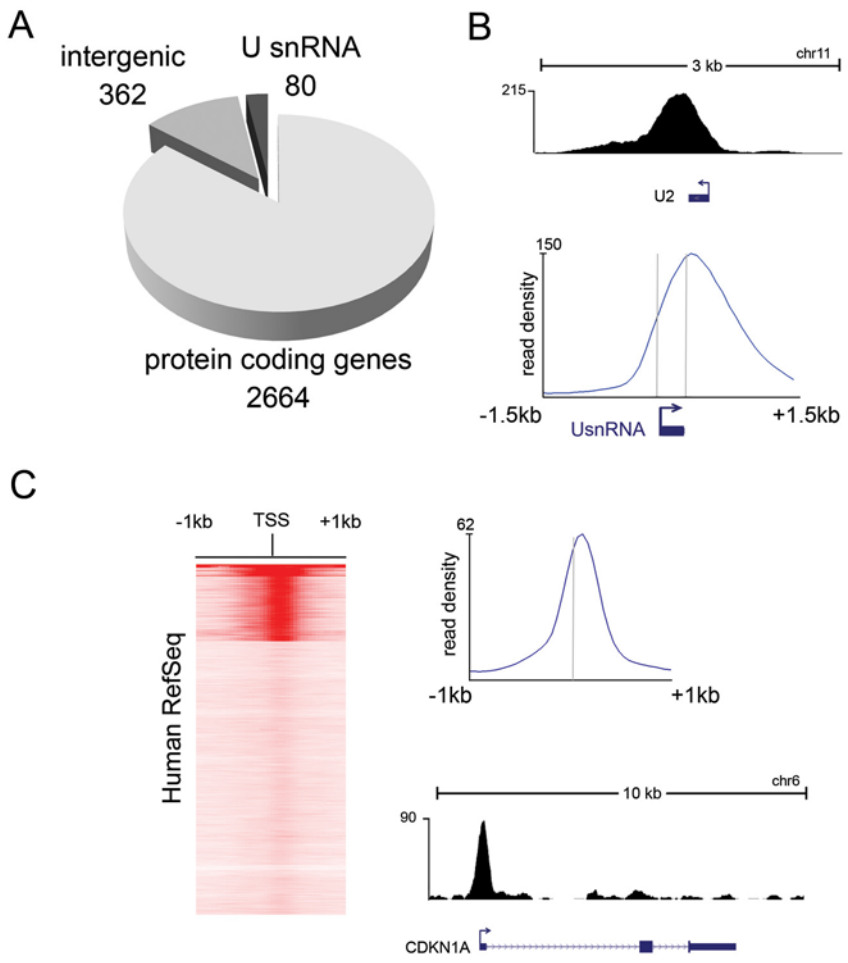


Figure 6. Analysis of genome-wide occupancy of Integrator. (A) Pie chart indicating the number of sites occupied by Integrator. (B) Profile of Integrator at U snRNAs. (C) Average profile at all protein-coding genes and individual profile of Integrator at CDKN1A gene.

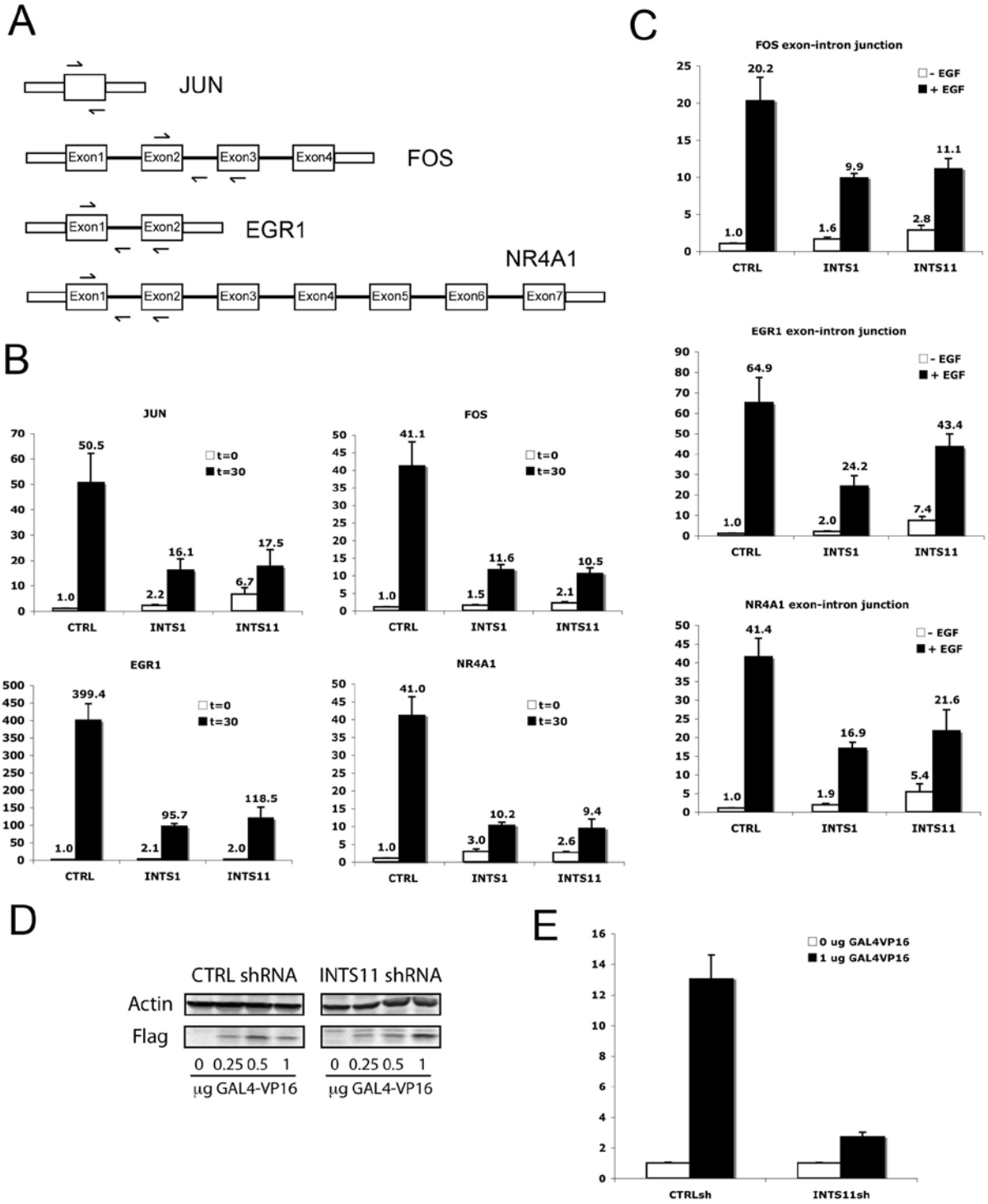


Figure 7. Integrator is critical for activation of transcription. (A) Description of primer pairs used for analysis of primary transcript shown in (C) and mature transcript shown in (B). (D) Demonstration of the increasing amounts of Flag-GAL4-VP16 used in each experiment. (E) Reporter induction after GAL4-VP16 and abrogation of activation following depletion of Integrator.

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, 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. None				☐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:

By the end of 2011, we anticipate submitting a paper that describes the findings reported on this project.

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.*

BIOGRAPHICAL SKETCH

NAME **SHIEKHATTAR, Ramin**	POSITION TITLE **Professor**
eRA Commons Username: **RAMIN_SHIEKHATTAR**	

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
University of Kansas, Lawrence, KS	B.S.	1980-1984	Chemistry
University of Kansas, Lawrence, KS	Ph.D.	1984-1989	Biochemistry

A. Personal Statement

Regulation of genomic silencing and its link to cancer

We are interested to understand the mechanisms by which protein-coding genes are silenced and how disruption of negative regulation may lead to neoplasia. Such genomic repression is achieved at both transcriptional and post-transcriptional levels. Recent evidence has implicated changes in chromatin structure as an important mechanism in gene regulation. Moreover, besides a classic role for proteins in mediating transcriptional and post-transcriptional effects, it has become clear that non-coding RNAs play an integral role in silencing through fine-tuning gene expression patterns during development and differentiation. We believe that this "signaling by RNA" is an emerging field of research that will uncover novel pathways in genome organization and regulation. In the past few years my laboratory has employed a biochemical approach to uncover a number of key factors that mediate transcriptional repression through regulation of chromatin structure, or post-transcriptional silencing via non-coding RNAs. We will continue our studies through a detailed structure/function analysis of these factors in regulation of genomic silencing and extend our experiments to elucidate the role these factors in the genesis of cancer.

B. Positions and Honors:

Research Experience:

1989-1993	Postdoctoral Fellow, Division of Behavioral Neurobiology, Department of Mental Health Sciences, Hahnemann University, Philadelphia, PA
1993-1997	Postdoctoral Fellow, Department of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ
1997-2000	Assistant Professor, The Wistar Institute, Philadelphia, PA
2001-12/31/05	Associate Professor, The Wistar Institute, Philadelphia, PA
1/1/07-5/31/09	Professor, Center for Genomic Regulation, Barcelona, Spain
1/1/06-present	Herbert Kean Professor, The Wistar Institute, Philadelphia, PA

Honors and Awards:

1993-1996	National Institute of Health National Research Service Award
1997-1998	American Cancer Society Institutional Research Grant
1999-2001	Scholar of V Foundation for Cancer Research
1998-2001	W.W. Smith Charitable Trust

C. Selected Peer-Reviewed Publications (in chronological order):

1. Dong, Y., Hakimi,, M-A,, Chen. X., Kumaraswamy. E., Cooch. N.S., Godwin. A.K., and **Shiekhattar, R.** Regulation of BRCC, a Holoenzyme Complex Containing BRCA1 and

BRCA2, by a Signalosome-like Subunit and its Role in DNA Repair. Molecular Cell 12; 1087-1099 (2003).

2. Gregory, R.I., Yan, K., Amuthan, G., Chendrimada, T., Doratotaj, B., Cooch, N., and **Shiekhattar, R.** The microprocessor complex mediates the genesis of miRNA. Nature 432; 235-240 (2004).
3. Chendrimada, T., Gregory, R.I., Kumaraswamy, E., Norman, J., Cooch, N., Nishikura, K., and **Shiekhattar, R.** TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature 436; 740-744 (2005).
4. Lee, M. G. Wynder, C., Cooch, N., **Shiekhattar, R.** An essential role for CoREST in nucleosomal histone H3 lysine 4 demethylation. Nature 437; 432-435 (2005).
5. Baillat, D., Hakimi, MA, Naar, A., Shilatifard, A., Cooch N., and **Shiekhattar, R**. Integrator, a multiprotein mediator of small nuclear RNA processing associates with the C-terminal of RNA polymerase II. Cell 123; 256-276 (2005).
6. Wynder, C. Lee, M., Hakimi, M-A., Epstein, J and **Shiekhattar, R**. Recruitment of MLL by HMG-domain protein iBRAF promotes neural differentiation. Nature Cell Biology 7; 1113-1117 (2005).
7. Gregory, R. I., Chendrimada, T., Cooch, N., and **Shiekhattar, R**. Human RISC Couples MicroRNA biogenesis and posttranscriptional gene silencing. Cell 123; 631-640 (2005).
8. Yang W, Chendrimada TP, Wang Q, Higuchi M, Seeburg PH, **Shiekhattar R** and Nishikura K. Modulation of microRNA processing and expression through RNA editing by ADAR deaminases. Nat Struct Mol Biol 13: 13-21 (2006).
9. Lee MG, Villa R, Trojer P, Norman J, Yan KP, Reinberg D, Di Croce L and **Shiekhattar R.** Demethylation of H3K27 regulates polycomb recruitment and H2A ubiquitination. Science 318: 447-450 (2007).
10. Lee MG, Norman J, Shilatifard A and **Shiekhattar R.** Physical and functional association of a trimethyl H3K4 demethylase and Ring6a/MBLR, a polycomb-like protein. Cell 128: 877-887 (2007).
11. Chendrimada TP, Finn KJ, Ji X, Baillat D, Gregory RI, Liebhaber SA, Pasquinelli AE and **Shiekhattar R.** MicroRNA silencing through RISC recruitment of eIF6. Nature 447: 823-828 (2007).
12. Savas JN, Makusky A, Ottosen S, Baillat D, Then F, Krainc D, **Shiekhattar R**, Markey SP, Tanese N. Huntington's disease protein contributes to RNA-mediated gene silencing through association with Argonaute and P bodies. Proc Natl Acad Sci U S A105(31):10820-5 (2008). PMC2504805
13. Baillat D and **Shiekhattar R**. Functional dissection of human TNRC6, (GW182-related) family of proteins. Mol Cell Biol 29: 4144-4155 (2009). PMC2715800
14. Melo SA, Ropero S, Moutinho C, Aaltonen LA, Yamamoto H, Calin GA, Rossi S, Fernandez AF, Carneiro F, Oliveira C, Ferreira B, Liu CG, Villanueva A, Capella G, Schwartz S Jr**,** **Shiekhattar R**, Esteller M. A TARBP2 mutation in human cancer impairs microRNA processing and DICER1 function. Nat Genet 41(3): 365-70 (2009).
15. Orom UA, Derrien T, Beringer M, Gumireddy K, Gardini A, Busscotti G, Lai F, Zytnicki M, Notredame C, Huang Q, Roderic G, and **Shiekhattar R**. Long non-coding RNAs with enhancer-like function in human cells. Cell 143(1):46-58 (2010).