BIOGRAPHICAL SKETCH

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

NAME: Marc R. Gartenberg

eRA COMMONS USER NAME (credential, e.g., agency login): gartenberg

POSITION TITLE: Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of California at Berkeley	B.Sc.	05/1985	Chemistry
Yale University, New Haven, CT	Ph.D.	05/1990	Chemistry
Harvard University, Cambridge, MA	Post-doc	05/1993	Mol. Biol.

A. Personal Statement

The long-term interests of my laboratory have been to understand how chromosome structure is related to chromosome function. I aim to define basic architectural features of chromosomes and to determine how structural anomalies in chromosomes relate to human diseases, including cancer. I use budding yeast as a model system because it offers powerful genetic, genomic and biochemical tools, and because the central features of chromosomes are conserved across all eukaryotes. Historically, my lab investigated establishment, maintenance and sub-nuclear localization of yeast heterochromatin. More recently, our work has focused on 1) how transcriptional activation and repression influence sister chromatid cohesion, and 2) how tRNA genes shape the genome. My lab has been funded continuously from the NIH for over twenty years and during that time we have published our discoveries at a steady rate in highly respected journals. We have the experience and expertise to see this next set of proposed experiments to fruitful and beneficial conclusions. In addition to my contributions as a research scientist (see section **C**), I have recently contributed as a science educator as an instructor of the historic Cold Spring Harbor Laboratory Yeast Genetics and Genomics Course (2013-16). This led to the publication of the most recent edition of the popular course manual [1]. I also recently co-published a comprehensive review of yeast heterochromatin in the YeastBook resource [2] I take pride in my hands-on training and mentorship of graduate students and post-docs in my laboratory. I have trained eight post-docs, 14 graduate students and 17 undergraduates, including four students who were under-represented minorities. Nearly all these students are involved in biological research or medicine today. Eight have become faculty members at universities or medical schools.

Ongoing and recently completed projects that I would like to highlight include:

Agency: National Institutes of Health
Type: R01 (R01 GM51402 starting in 1996)
Recent duration: 05/01/19-2/28/23 (in no cost extension)
Role: PI
Title: Binding, Sliding and Function of Cohesin on Sister Chromatids
Description: This proposal aims to determine how the dynamic distribution of cohesin on chromosomal DNA is determined by binding, sliding and function of the complex. The experiments will determine how the dimensions of the complex limit which obstacles the complex can slide past and whether single complexes embrace two sister chromatids at once. Additional experiments will determine how transcription and other ATP-dependent processes regulate cohesin movement, and define the biological consequences when movement is blocked. Lastly, the experiments will yield the first comprehensive maps of cohesin on chromosomes based on the functionality of the complex.

Citations:

1) Dunham, M., Gartenberg, M. R. and Brown, G. W. (2015). Methods in Yeast Genetics and Genomics: A Cold Spring Harbor Laboratory Manual. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY.

2) Gartenberg, M. R. and Smith, J. S. (2016). The nuts and bolts of transcriptionally silent chromatin in *Saccharomyces cerevisiae.* ***Genetics*** **203**, 1563-99. PMCID: PMC4981263

B. Positions and Honors

Academic Positions - (Note: Robert Wood Johnson Medical School was part of the University of Medicine and Dentistry of New Jersey before joining Rutgers University on July 1, 2013)

1993 to 2000 – Asst. Prof., Department of Pharmacology, Robert Wood Johnson Medical School
2000 to 2005 – Assoc. Prof., Department of Pharmacology, Robert Wood Johnson Medical School
2005 to 2013 – Prof., Department of Pharmacology, Robert Wood Johnson Medical School
2013 onward – Prof., Department of Biochemistry and Mol. Biology, Robert Wood Johnson Medical School
2002 – Visiting Prof., University of Geneva, Dept. of Mol. Biol., Geneva, Switzerland
2002 onward – Director, Graduate Program in Cellular and Molecular Pharmacology, Robert Wood Johnson Medical School at Rutgers University
2007 to 2011 – Standing member, NIH MGC study section
2013 to 2016 – Instructor, 3-week Cold Spring Harbor Laboratory Course in Yeast Genetics and Genomics

Academic Appointments

Graduate Program in Cellular and Molecular Pharmacology at RWJMS of Rutgers University
Graduate Programs in Microbiology and Biochemistry at RWJMS of Rutgers University
Graduate Program in Quantitative Biomedicine at Rutgers University
Cancer Institute of New Jersey

C. Contributions to Science

C.1) Establishment and maintenance of yeast heterochromatin

Heterochromatin assembly is operationally divided into two steps: establishment and maintenance. DNA elements termed silencers establish yeast heterochromatic silencing. When we began in the field, it was not known whether silencers also maintained the silent state. To address this question, we "uncoupled" a heterochromatin domain from silencers *in vivo* using inducible site-specific recombination (SSR, see Research Plan). The resulting DNA circles started transcribing again, even in cells that did not progress through the cell cycle. We concluded that silencers act continuously, not periodically, to maintain silencing. Our work provided the first indication that heterochromatin structures are dynamic with equilibrating components [1].

It was a long-held view that heterochromatin was established during S phase via DNA replication. To examine this possibility, we created non-replicating DNA circles by SSR, and then activated a conditional silencer within the circle. We discovered that silencing can be established without DNA replication, thereby indicating that other cell cycle events govern heterochromatin assembly [2]. This work had a major impact as it overturned dogma that had stood for 17 years (for commentary, see Smith and Boeke, *Science*, 2001)

A long-term goal of the chromatin field has been to isolate a piece of native chromatin for biochemical analysis. To this end, we developed a sedimentation strategy that enriches recombinant chromatin circles over chromosomal contaminants by 5000-fold [3]. The partially purified material retains benchmarks of intact yeast heterochromatin, indicating that studies of native chromatin *in vitro* are feasible. The technology played a key role in the chromatin purifications and subsequent studies of Roger Kornberg and other chromatin biochemists.

Sir2 is a NAD-dependent histone deacetylase that acts during yeast heterochromatin assembly. The protein is evolutionarily conserved and plays key roles in human health, disease and aging. A byproduct of the Sir2 reaction, *O*-acetyl-ADP-ribose (*O*AADPr), was thought to aid spreading of the Sir2/3/4 complex during yeast heterochromatin assembly. We tested the *O*AADPr hypothesis by creating heterochromatin devoid of Sir2. To this end, we fused a core heterochromatin factor, Sir3, to Hos3, a heterologous deacetylase that does not consume NAD nor produce *O*AADPr. This non-traditional approach conferred robust silencing that bore typical

hallmarks of yeast heterochromatin yet without Sir2 present [4]. We concluded that the only essential function for Sir2 in silencing is to generate deacetylated histone tails, not to produce *O*AADPr. (* = corresponding author)

1) Cheng, T.-H. and Gartenberg, M. R.* (2000). Maintenance of yeast heterochromatin is a dynamic process that requires silencers continuously. ***Genes Dev.*** **14**, 452-63. PMCID: PMC316382

2) Li, Y.-C., Cheng, T.-H., and Gartenberg, M. R.* (2001). Establishment of transcriptional silencing in the absence of DNA replication. ***Science*** **291**, 650-3. PMID: 11158677

3) Ansari, A. and Gartenberg, M. R.* (1999). Persistence of silent chromatin structure *in vitro.* ***Proc. Nat'l. Acad. Sci. USA*** **96**, 343-8. PMCID: PMC15138

4) Chou, C.-C, Li, Y.-C. and Gartenberg, M. R.* (2008). Bypassing Sir2 and *O*-acetyl-ADP-ribose in transcriptional silencing. ***Mol. Cell*** **31**, 650-59. PMCID: PMC2696193

C.2) Localization of yeast heterochromatin

Heterochromatin domains congregate at the nuclear periphery. To investigate the basis of yeast heterochromatin positioning, we uncoupled a heterochromatin domain by SSR and monitored the movement of the resulting DNA circles by fluorescence microscopy. In collaborations with the Gasser, Sternglanz and Fox labs, we made three significant advances [1-4]. First, we showed yeast heterochromatin anchors to the inner nuclear membrane via interactions with two proteins, Ku and Esc1. Second, we found an additional telomere anchoring mechanism based on palmitoylation of Rif1, a telomere-binding protein. This was the first demonstration of palmitoylation in the nucleus. Third, we showed that heterochromatin factors disperse throughout the nucleus when the anchoring sites are mutated. We concluded that anchoring sites normally sequester silenced loci and the associated silencing factors away from euchromatin. We hypothesize that the cell compartmentalizes heterochromatin to reduce the risk of inadvertently repressing the wrong genes.

1) Gartenberg, M. R.,* Laroche, T., Blaszczyk, M and Gasser, S. M. (2004). Sir-mediated repression can occur independently of chromosomal and subnuclear contexts. ***Cell*** **119**, 955-67. PMID: 15620354

2) Ansari, A. and Gartenberg, M. R.* (1997). The yeast silent information regulator Sir4p anchors and partitions plasmids. ***Mol. Cell. Biol.*** **17**, 7061-8. PMCID: PMC232562

3) Andrulis, E. A., Zappulla, D. C., Ansari, A. I., Perrod, S., Laiosa, C. V., Gartenberg, M. R.,* and Sternglanz, R.* (2002). Esc1, a nuclear periphery protein required for Sir4-based plasmid anchoring and partitioning. ***Mol. Cell. Biol.*** **22**, 8292-301. PMCID: PMC134074

4) Park, S., Patterson, E. E., Cobb, J., Audhya, A., Gartenberg, M. R.*, and Fox, C. A.* (2011). Palmitoylation controls the dynamics of budding yeast heterochromatin via the telomere-binding protein Rif1. ***Proc. Nat'l. Acad. Sci. USA*** **108**,14572-7. PMCID: PMC3167557

C.3) Heterochromatin and the cohesion of sister chromatids

Cohesin, the protein complex that mediates sister chromatid cohesion, had been genetically-linked to heterochromatin assembly. Whether cohesin acted directly on heterochromatin was not known. We showed that heterochromatic domains recruit cohesin [1]. Furthermore, by combining fluorescence microscopy with SSR we showed that cohesin mediates cohesion of heterochromatic sisters. Parallel findings in fission yeast, albeit with entirely different heterochromatin proteins, led us to conclude that heterochromatin-specific cohesion is a conserved feature of eukaryotes.

At the time cohesin was thought to hold sister chromatids together by placing both chromatids in a single channel within the ring-shaped cohesin complex (the double embrace model). While our experiments with DNA circles supported the notion that cohesin binds DNA topologically, the data were not consistent with a double embrace [1]. Our challenge to the prevailing view, at least at heterochromatin domains, had a substantial impact on the field (for commentary, see Huang and Moazed, *Genes Dev*, 2006).

We sought to identify the features of heterochromatin that dictate cohesin binding. To this end, we tethered

individual heterochromatin proteins to DNA and found that tethered Sir2 reconstituted both cohesin recruitment and cohesion [2,3]. We concluded that Sir2 was bifunctional, serving as both a histone deacetylase and a cohesin binding scaffold. Our prediction that Sir2 binding sites throughout the genome would serve as cohesin accumulation centers was validated by Jeff Smith's lab. Further studies on cohesin loading at the heterochromatic *HMR* locus uncovered a requirement for an adjacent tRNA gene [4]. We hypothesized that tRNA genes throughout the genome load cohesin complexes that then migrate to function at adjacent sites. Indeed, the Uhlmann lab showed later that tRNA genes indeed load cohesin onto chromosomes genome-wide.

1) Chang, C.-R., Wu, C.-S., Hom, Y., and Gartenberg, M. R.***** (2005). Targeting of cohesin by transcriptionally silent chromatin. ***Genes Dev. 19*****,** 3031-42. PMCID: PMC1315406

2) Wu, C.-S., Chen, Y.-F. and Gartenberg, M. R.***** (2011). Targeted sister chromatid cohesion by Sir2. ***PLoS Genetics* 7**, e1002000. PMCID: PMC3033385

3) Chen, Y.-F., Chou, C-.C and Gartenberg, M. R.* (2016). Determinants of Sir2-mediated, silent chromatin cohesion. ***Mol. Cell. Biol.* 36**, 2039-50. PMCID: PMC4946433

4) Dubey, R.D. and Gartenberg, M. R.***** (2007). A *tDNA* establishes cohesion of a neighboring silent chromatin domain. ***Genes Dev.* 21,** 2150-60. PMCID: PMC1950854

C.4) Transcription and the fate of sister chromatid cohesion

In addition to binding heterochromatin, cohesin binds throughout euchromatic domains at discrete sites within and around genes. Transcription by RNA polymerase II redistributes cohesin to the ends of genes, but whether cohesin moves by sliding or by eviction/rebinding was not certain. We used fluorescence microscopy and SSR to study the fate of mobilized cohesin complexes and found that moves cohesin processively along DNA [1]. These study demonstrated that sister chromatid cohesion and gene expression are mutually compatible chromosomal events because cohesin complexes retain their function when mobilized by RNA polymerase.
Further studies with synthetic roadblocks showed that obstacles of a discrete size block translocation of cohesive complexes and that larger obstacles are needed to block non-cohesive complexes. Moreover, we showed that stalled cohesive complexes block translocation of non-cohesive complexes [2]. These studies reveal unexplored limitations of cohesin movement on chromosomes.

1) Borrie, M., Campor, J. S. Joshi, H. and Gartenberg, M. R.* (2017). Binding, sliding and function of cohesin during transcriptional activation. ***Proc. Nat'l. Acad. Sci. USA*****. 114**, E1062-E1071. PMID: 28137853

2) Borrie, M. S. , Kraycer, P. M. and Gartenberg, M. R.* (2023). Transcription-driven translocation of cohesive and non-cohesive cohesin *in vivo*. ***Mol. Cell. Biol.***, under revision.

C.5) Gene-gating by RNA polymerase III-transcribed tRNA genes

In yeast, pol III-transcribed genes were thought to cluster at the nucleolus. While studying how tRNA genes shape chromosome architecture, we discovered that their transcription fluctuates with cell cycle progression, and that the genes dock at nuclear pore complexes when their transcription peaks in M phase [1,2]. Importantly, NPC docking was abolished by elimination of Los1, the primary mediator of tRNA nuclear export. We concluded that tRNA synthesis and nuclear export are coupled processes, as anticipated by Gunter Blobel's gene-gating hypothesis. Further study found an association between tRNA-docking at nuclear pores and upregulatd protein synthesis in human lung cancers.

1)) Chen, M. and Gartenberg, M. R.* (2014). Coordination of tRNA transcription and export at nuclear pore complexes in budding yeast. ***Genes Dev.*** **28**, 959-970. PMCID: PMC4018494

2) Ruben, G. J., Kirkland, J. G., MacDonough, T., Chen, M. Dubey, R. N., Gartenberg, M. R. and Kamakaka, R. T.* (2011). Nucleoporin mediated nuclear positioning and silencing of *HMR*. ***PLoS One* 6**, E21923. PMCID: PMC3139579

3) Chen, M.*, Long, Q., Borrie, M. S., Sun, H., Zhang, C., Yang, H., Shi, D., Gartenberg, M. R.* and Deng, W.* (2021). Nucleoporin TPR promotes tRNA nuclear export and protein synthesis in lung cancer cells. *PLoS Genetics* **17**, e1009899. PMID: 34793452

Complete list of published work in MyBiography
https://www.ncbi.nlm.nih.gov/sites/myncbi/1zsSdoDE_jxkd/bibliography/46033406/public/?sort=date&direction=descending