SUMMARY

The ability to express different genes in different cell types is crucial to development of a multicellular organism. This cell-type specific gene expression relies on memory of activation and repression of genes through successive rounds of cell division as the organism develops from an embryo to an adult. An important component of the memory of cellular identity is the cell’s chromatin state. To maintain a transcriptional program, the cell has to maintain accessibility at active promoters and enhancers and occlusion at repressed promoters and enhancers. However, the passage of replication forks during every cell cycle obliterates cell-type specific chromatin landscapes. Our overarching goal is to determine how chromatin landscapes are sustained in spite of nucleosome dynamics throughout the cell cycle, thus maintaining cellular memory. Here, we will use genomic methods we develop(ed) to identify cellular mechanisms that maintain chromatin landscapes despite the erasing effects of replication. We will pursue two lines of investigations: First, we will identify how transcription factors find their binding sites after being stripped from the DNA during process of replication. We have shown that transcription factors are replaced by nucleosomes genome-wide post-replication. By tracking transcription factor binding to DNA and DNA accessibility as a function of time post-replication, we will uncover the determinants of transcription factor site selectivity. We will also study the effect of chromatin remodeler function in creating transcription factor binding sites post-replication. Second, we will elucidate cellular mechanisms that maintain repressed chromatin landscapes through replication at epigenetically silenced domains that are characterized by trimethylated histone H3 (H3K27me3), which we call “Polycomb domains”. Polycomb group proteins maintain repressed states at these domains. Mechanisms that carry memory of repression through replication have to act within a single cell cycle, every cell cycle. We will combine tracking chromatin states genome-wide post-replication with carefully chosen perturbations of Polycomb group proteins to uncover mechanisms that maintain this repressed state through replication. Taken together, these studies will not only resolve long-standing questions in transcription factor-site selectivity and Polycomb repression, but will also serve as a framework to understand how chromatin dynamics can shape genome function in many biological contexts.

PROJECT NARRATIVE

A cell's identity is tied to how its genome is packaged. This cell type specific genome packaging is essential for normal development of the organism and is frequently disrupted in disease. This proposal will identify how a cell remembers the specific packaging of its genome every time it replicates its genome so as to maintain its identity during normal growth and how perturbation of this memory can lead to aberrant cell states.

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Ramachandran, Srinivas

eRA COMMONS USER NAME (credential, e.g., agency login): sramacha1

POSITION TITLE: Assistant Professor, Biochemistry and Molecular Genetics

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Anna University, Chennai, Tamil Nadu	BS	05/2006	Industrial Biotechnology
University of North Carolina, Chapel Hill, NC	PHD	05/2011	Biochemistry and Biophysics
University of North Carolina at Chapel Hill, Chapel Hill, NC	Postdoctoral Fellow	07/2012	Biochemistry and Biophysics
Fred Hutchinson Cancer Research Center, Division of Basic Sciences, Seattle, WA	Postdoctoral Fellow	12/2017	Chromatin Biology

A. Personal Statement

The long-term goal of my laboratory is to develop cutting edge computational and experimental tools to understand *in vivo* dynamics of nucleosomes and transcription factors. My graduate research has trained me to view biological problems through the lens of biomolecular structure and quantitative analyses. In my postdoctoral research, I have developed several methods to map chromatin structure at high-resolution in cells, including a method termed Mapping *In vivo* Nascent Chromatin with EdU and sequencing (MINCE-seq) that can create a high-resolution, temporal map of chromatin assembly post-replication. In January 2018, I started as an Assistant Professor in the Department of Biochemistry and Molecular Genetics in the University of Colorado Anschutz Medical Campus. I have already successfully recruited three postdoctoral research associates and a technician. Two of the postdoctoral fellows will be joining the lab in November 2018. My wide-ranging experience in computational biology, biochemistry, and chromatin biology gives me the means to mentor the members of my lab to execute our research program in nucleosome dynamics.

In this proposal, we will map locus-specific chromatin landscape post-replication to uncover the mechanisms of transcription factor binding in cells and the mechanisms underlying perpetuation of repressed chromatin states through cell division by Polycomb group proteins. We are able to perform these studies with unprecedented temporal and spatial resolution because of the cutting edge experimental and computational techniques, some developed by me during my postdoctoral training, and some under development currently in our lab to study chromatin dynamics. These projects aim to answer fundamental questions in biology and are aligned to the goals of MIRA program and the mission of NIGMS.

Teaching, Training, and Mentorship: I have trained scientists at many levels including:

- Undergraduates: 1 during my graduate career
- Technicians: 1 currently in my lab, who is developing cell lines and reporters to study Polycomb function.
- Graduate students: 1 during my graduate career, and 1 during my postdoctoral career, with whom I co-authored a paper (Weber, Ramachandran, and Henikoff, Molecular Cell 2013)
- Postdoctoral fellows: 1 during my postdoctoral career, and 1 currently in my lab, who is developing methods to track locus-specific chromatin landscapes post-replication

During my postdoctoral training in the lab of Steve Henikoff, I have mentored many lab members in the analysis of complex epigenomic datasets, which I am continuing to do in my own lab. At University of Colorado Graduate School, I have taught two courses till date: i) Advanced Topics in Molecular Biology, a course where students learn to critically evaluate scientific literature and then develop research proposals that follow up the papers they discussed in class. ii) Lectures on genome function and maintenance as part of the first year graduate school

curriculum. I have also been asked (and have accepted) to be a part of thesis committees of three graduate students in the Structural Biology and Biochemistry graduate program on campus.

B. Positions and Honors

Positions and Employment

2005 - 2006	Research Associate, National Center For Biological Sciences, Bangalore
2006 - 2011	Graduate Research Assistant, Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC
2011 - 2012	Postdoctoral Research Associate, Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Advisor: Nikolay V. Dokholyan, Chapel Hill, NC
2012 - 2017	Postdoctoral Research Associate, Fred Hutchinson Cancer Research Center, Advisor: Steven Henikoff, Seattle, WA
2018 -	Assistant Professor, Biochemistry and Molecular Genetics, University of Colorado, School of Medicine, Aurora, CO

Other Experience and Professional Memberships

2007 -	Ad-Hoc Reviewer, Molecular Cell, Proceedings of the National Academy of Sciences, Nucleic Acids Research, PLoS Computational Biology, Proteins, Journal of Theoretical Biology, Central European Journal of Biology, Open Life Sciences, and Scientific Reports
2010 - 2017	Associate Faculty Member, Faculty of 1000

Honors

2009 - 2011	Predoctoral Fellowship, American Heart Association
2009	Travel Award, Graduate and Professional Student Federation
2011	Graduate Travel Award, American Society for Biochemistry and Molecular Biology
2011	Travel Award, Biophysical Society
2012	Associate Faculty Member Travel Grant, Faculty of 1000

C. Contribution to Science

Note: A "" indicates co-authorship*

1. **Newly Replicated Chromatin Landscape.** Replication represents a major challenge to chromatin as every protein-DNA contact is disrupted by the passage of the replication fork. The speed of the replication fork (2kb/min) and the assumption that transcription factors and nucleosomes rebind at the same locations post-replication meant that the newly replicated chromatin landscape was never mapped. I developed a metabolic labeling method called Mapping In vivo Nascent Chromatin using EdU and sequencing (MINCE-seq) that can map nucleosome and transcription factor footprints genome-wide within minutes after the replication fork has passed. This technical advance led to the unexpected finding that replication completely resets the chromatin landscape, with nucleosomes replacing transcription factors genome-wide. The overall high coverage of nucleosomes behind the replication fork suggests that replication creates a refractory period that also provides a basis for discrimination between functional transcription factor binding sites and cryptic and/or random, moderate affinity sites. Because nucleosomal packaging behind the replication fork effectively reduces the affinities of all transcription factor binding sites, selective binding will occur only at the best sites, providing an elegant hypothesis for how only a very small subset of possible transcription factor binding sites in the genome are bound in a given cell type.

    a. **Ramachandran S**, Ahmad K, Henikoff S. Capitalizing on disaster: Establishing chromatin specificity behind the replication fork. Bioessays. 2017 Apr;39(4)PubMed PMID: 28133760; PubMed Central PMCID: PMC5513704.
    b. **Ramachandran S**, Henikoff S. Transcriptional Regulators Compete with Nucleosomes Post-replication. Cell. 2016 Apr 21;165(3):580-92. PubMed PMID: 27062929; PubMed Central PMCID: PMC4855302.
    c. **Ramachandran S**, Henikoff S. Replicating Nucleosomes. Sci Adv. 2015 Aug 7;1(7)PubMed PMID: 26269799; PubMed Central PMCID: PMC4530793.

2. **Nucleosome Structure *in vivo*.** *In vitro* and *in silico* structural studies of the nucleosome have uncovered fundamental properties of histone-histone and histone-DNA interactions; however, these studies target only a restricted set of sequences in a simplified biochemical milieu. The complexity of *in vivo* nucleosomes is much more: they can form with millions of possible sequences, and in the presence of myriad machineries that modulate histone-DNA interactions. I have combined paired-end sequencing with nuclease assays to study nucleosome structure *in vivo*. To understand how nucleosome dynamics might facilitate transcription *in vivo*, I developed the computational framework to take full advantage of the base-pair resolution map of RNAPII and nucleosomes to determine the positions where RNAPII stalls and backtracks. We found that nucleosomes present significant, context-specific barriers to RNAPII *in vivo* that can be tuned by H2A.Z. Similar methodologies applied to H4-S47C-anchored chemical cleavage mapping allowed me to identify a nucleosomal intermediate formed during chromatin remodeling by RSC complex *in vivo*. Finally, I designed a new mutant (Histone H3-Q85C) that enabled conversion of histone H3 into a site-specific nuclease by coupling the cysteine to phenanthroline *ex vivo*, allowing us to map nucleosome positions at single base-pair and single molecule resolution.

   a. **Ramachandran S**, Zentner GE, Henikoff S. Asymmetric nucleosomes flank promoters in the budding yeast genome. Genome Res. 2015 Mar;25(3):381-90. PubMed PMID: 25491770; PubMed Central PMCID: PMC4352886.
   b. Weber CM, **Ramachandran S**, Henikoff S. Nucleosomes are context-specific, H2A.Z-modulated barriers to RNA polymerase. Mol Cell. 2014 Mar 6;53(5):819-30. PubMed PMID: 24606920.
   c. Warfield L*, **Ramachandran S***, Baptista T, Devys D, Tora L, Hahn S. Transcription of Nearly All Yeast RNA Polymerase II-Transcribed Genes Is Dependent on Transcription Factor TFIID. Mol Cell. 2017 Oct 5;68(1):118-129.e5. PubMed PMID: 28918900; PubMed Central PMCID: PMC5679267.
   d. Chereji RV*, **Ramachandran S***, Bryson TD, Henikoff S. Precise genome-wide mapping of single nucleosomes and linkers in vivo. Genome Biol. 2018 Feb 9;19(1):19. PubMed PMID: 29426353; PubMed Central PMCID: PMC5807854.

3. **Structural epigenomics of cfDNA.** I adapted Micrococcal Nuclease mapping using sequencing (MNase-seq) as a novel tool for structural epigenomics *in vivo*. I identified nucleosomal intermediates formed during transcription *in vivo* and showed that transcriptional elongation drives hexasome formation. Having established the features of nucleosomal intermediates in a cell line, I went on to show nucleosome unwrapping in promoter-proximal regions in cell-free DNA (cfDNA) from human blood plasma DNA that was naturally digested during cell turnover. I showed that subnucleosomal particles over promoter-proximal nucleosome positions in cfDNA are the relics of transcription programs of tissues where cfDNA originated. Hence, I could use subnucleosome enrichment near promoters to predict tissue-of-origin of cfDNA and to distinguish disease states from healthy states. Current paths for developing cancer diagnosis methods using cfDNA ("liquid biopsy") rely on identifying a few copies of specific onco-mutations in cfDNA. My method can predict the entire transcriptional state of tissues contributing to cfDNA, providing a robust alternative to hunting for onco-mutations. Importantly, this subnucleosome enrichment analysis requires sequence from only the first 300 bp of a transcription unit, just 0.15% of the genome which when coupled to targeted DNA sequencing can greatly reduce the cost of cfDNA tissue-of-origin testing for cancer diagnosis.

   a. **Ramachandran S**, Ahmad K, Henikoff S. Transcription and Remodeling Produce Asymmetrically Unwrapped Nucleosomal Intermediates. Mol Cell. 2017 Dec 21;68(6):1038-1053.e4. PubMed PMID: 29225036.

4. **Molecular Recognition of Platinum-DNA Adducts.** I investigated the molecular basis of the recognition of drug-DNA complexes by DNA-binding proteins. Specifically, I asked how the platinum-based anticancer drugs, cisplatin (CP) and oxaliplatin (OX), differ in their efficacy against various tumors despite forming identical adducts on DNA structure that form the basis for their cytotoxicity. Using molecular dynamics simulations of DNA adducts formed by CP and OX, I discovered that differential interactions of CP- and OX-DNA with proteins occur due to differences in flexibility of the platinum-DNA adducts rather than their steady state structure. These results establish sequence- and drug-induced DNA flexibility as the determinant of the binding affinity of damage-recognition proteins to DNA and suggest that differential conformational dynamics of CP- and OX-DNA could contribute to the differences in their efficacy.

a. **Ramachandran S**, Temple B, Alexandrova AN, Chaney SG, Dokholyan NV. Recognition of platinum-DNA adducts by HMGB1a. Biochemistry. 2012 Sep 25;51(38):7608-17. PubMed PMID: 22950413.

b. King CL, **Ramachandran S**, Chaney SG, Collins L, Swenberg JA, DeKrafft KE, Lin W, Cicurel L, Barbier M. Debio 0507 primarily forms diaminocyclohexane-Pt-d(GpG) and -d(ApG) DNA adducts in HCT116 cells. Cancer Chemother Pharmacol. 2012 Mar;69(3):665-77. PubMed PMID: 21968950; PubMed Central PMCID: PMC3777240.

c. Bhattacharyya D, **Ramachandran S***, Sharma S, Pathmasiri W, King CL, Baskerville-Abraham I, Boysen G, Swenberg JA, Campbell SL, Dokholyan NV, Chaney SG. Flanking bases influence the nature of DNA distortion by platinum 1,2-intrastrand (GG) cross-links. PLoS One. 2011;6(8):e23582. PubMed PMID: 21853154; PubMed Central PMCID: PMC3154474.

d. **Ramachandran S**, Temple BR, Chaney SG, Dokholyan NV. Structural basis for the sequence-dependent effects of platinum-DNA adducts. Nucleic Acids Res. 2009 May;37(8):2434-48. PubMed PMID: 19255091; PubMed Central PMCID: PMC2677858.

5. **Ryanodine Receptor Structure and Function.** Ryanodine Receptor type 1 (RyR1), found in the sarcoplasmic reticulum (SR) membrane of skeletal muscle, responds to effector binding at its cytoplasmic side and releases high levels of calcium into the cytoplasm from the SR through channel opening to trigger skeletal muscle contraction. The molecular mechanism that links effector binding to channel gating has long been unknown due to lack of structural data. I constructed an all-atom structural model of the membrane-spanning region of the channel, which uncovered an intramolecular interface that linked effector binding to channel opening. This structural model was independently validated by comparison with cryoelectron microscopy in collaboration with Dr. Montserrat Samso. Using this structural model, I designed RyR mutants that completely uncoupled channel opening from effector binding. These mutants were functionally validated using electrophysiology in collaboration with Dr. Gerhard Meissner. This integrated computational and experimental approach has significantly advanced our understanding of the structure and function of RyR.

a. Shirvanyants D, **Ramachandran S**, Mei Y, Xu L, Meissner G, Dokholyan NV. Pore dynamics and conductance of RyR1 transmembrane domain. Biophys J. 2014 Jun 3;106(11):2375-84. PubMed PMID: 24896116; PubMed Central PMCID: PMC4052289.

b. **Ramachandran S***, Chakraborty A*, Xu L*, Mei Y, Samsó M, Dokholyan NV, Meissner G. Structural determinants of skeletal muscle ryanodine receptor gating. J Biol Chem. 2013 Mar 1;288(9):6154-65. PubMed PMID: 23319589; PubMed Central PMCID: PMC3585052.

c. **Ramachandran S**, Serohijos AW, Xu L, Meissner G, Dokholyan NV. A structural model of the pore-forming region of the skeletal muscle ryanodine receptor (RyR1). PLoS Comput Biol. 2009 Apr;5(4):e1000367. PubMed PMID: 19390614; PubMed Central PMCID: PMC2668181.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40284203/?sort=date&direction=descending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing

None

Completed Research Support

None

BACKGROUND

Time-resolved Epigenomics. Distinct and dynamic chromatin landscapes underly growth, development, differentiation, and disease states including tumor initiation. **Our long-term goal is to understand how distinctive chromatin landscapes that reflect cellular identity are established and maintained.** We want to uncover mechanisms that sculpt chromatin landscapes by developing novel computational and experimental genomic methods that map chromatin structure at high temporal and spatial resolution. To maintain identity and its transcriptional program, the cell has to keep active regions of the genome accessible and repressed regions of the genome occluded. However, DNA replication obliterates these distinct chromatin landscapes. Most cells divide continuously, yet they maintain their identity. **It is unknown how chromatin landscapes are sustained in spite of the disruptive events of replication, because most current approaches generate steady-state snapshots of epigenomes in which chromatin dynamics are completely invisible.** To understand how epigenomic landscapes are established and maintained, I developed a metabolic labeling method called Mapping In vivo Nascent Chromatin using EdU and sequencing (MINCE-seq) that generates a high-resolution, genome-wide map of chromatin within minutes after the replication fork has passed[3]. Here, we will use MINCE-seq and other novel methods we develop(ed) to identify cellular mechanisms that maintain chromatin landscapes despite the erasing effects of replication.

Theme 1. Active chromatin. The DNA replication fork strips every protein-DNA interaction genome-wide. Nucleosomes are deposited on newly replicated DNA by assembly factors associated with the fork. I have shown that immediately post replication, transcription factors (TFs) and nucleosomes compete for binding to the newly synthesized DNA[3,4]. Furthermore, I have shown that TF binding sites are occluded by nucleosomes post-replication in *Drosophila* cells, and in human cells (see RECENT PROGRESS), and the same has also been observed in *Drosophila* early embryo[5]. My data suggest that nucleosomes persist for hours at TF binding sites before being displaced. We hypothesize that this refractory period for TF rebinding post-replication may enforce discrimination between functional binding sites and spurious sites: occlusion by nucleosomes post-replication effectively reduces the affinities for all binding sites, and this increased stringency post-replication may allow binding to occur only at the best sites (**Fig. 1**). TFs are at the apex of regulatory pathways that specify cell identity and they spearhead developmental transitions essential for the spectrum of cell differentiation necessary for an organism to reach adulthood. However, our understanding of what determines TF binding site selectivity in metazoan genomes is limited and until now, no experimental approaches were available to address this question in cells. **We will analyze protein binding on DNA post-replication as a unique means to study how genome-wide TF binding site selectivity is achieved**.

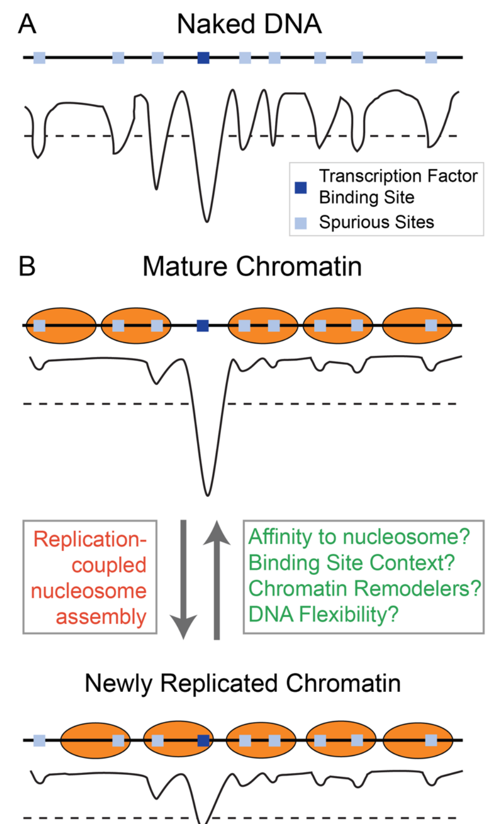

Figure 1. Nucleosome occlusion post-replication restricts TF binding. The TF binding free energy landscape on naked DNA (**A**), where dashed line indicates effective binding energy cut-off for stable binding. Spurious sites (light blue) outnumber functional high-affinity sites (dark blue). (**B**) At steady state, spurious sites are occluded by nucleosomes but true functional sites are accessible. Immediately post-replication, all binding sites are occluded by nucleosomes, and only true sites have sufficient affinity for TFs to displace nucleosomes.

Theme 2. Repressed chromatin. Histone post-translational modifications associated with cellular identity are diluted 2-fold every round of replication[6], but mechanisms that maintain these key, locus-specific modifications are poorly understood. One class of well-studied and key repressive structures are Polycomb (Pc) domains[7]. We still do not know how memory of repression is carried through replication and significantly, if the modified histones themselves are epigenetic. Repressed Pc domains are fundamental to a cell's identity and essential for normal development, and lack of understanding of how Pc repression is maintained through replication is a significant gap in our knowledge. Mechanisms that carry memory of repression through replication have to act within a single cell cycle, every cell cycle. We will elucidate mechanisms that establish and maintain chromatin landscapes essential for cellular memory by developing means to perturb Pc pathway function in the time-scale of minutes to hours coupled with tracking Pc domains temporally post-replication. **Our studies will**

identify causal mechanisms that maintain cellular memory through repression and determine the contribution of the histone modification itself to cellular memory.

RECENT PROGRESS

Mapping nucleosome structure in cells. During my postdoctoral training, I developed the approach of inferring structure of nucleosomal intermediates from sequencing data, which we term as "structural epigenomics"[8-11]. This is a general strategy applicable to a wide variety of datasets as detailed here. I used molecular modeling to interpret data from a chemical mapping technique that was originally used to identify nucleosome positions[12], to deconvolve individual contacts of histone H4 with DNA in cells[9,11]. This enabled me to identify a chromatin remodeling intermediate *in vivo*[9,11] and to characterize the structure of centromeric nucleosomes in budding yeast[9]. Furthermore, I exploited the structural information of the nucleosome to design cysteine mutants that converted the histone H3 into a site-specific nuclease by coupling the cysteine to phenanthroline *ex vivo* to enable direct mapping of single nucleosome positions at base-pair resolution in budding yeast[8].

Micrococcal nuclease (MNase) preferentially degrades linker DNA and is inhibited when it encounters a protein-DNA contact. MNase has traditionally been used to map positions of whole nucleosomes in intact nuclei by purifying ~147 bp DNA after MNase digestion and subjecting this DNA to massively parallel short read sequencing[13]. With sequencing library protocols that capture all fragment lengths combined with paired-end sequencing, I was able to uncover the full spectrum of fragments generated by MNase treatment of nuclei[10]. I showed that these intermediate length protections (between 50 and 147 bp) represent discrete loss of contacts asymmetrically from either side of the nucleosome as it unwraps during transcription and remodeling. By correlating a base-pair resolution map of RNA polymerase II that we developed[14] with the high-resolution distribution of nucleosomal intermediates at the first nucleosome downstream of the promoter (the "+1 nucleosome"), I found that nucleosome unwrapping occurs in a step-wise manner – first the contacts to the H2A-H2B dimer proximal to the promoter are lost; then as RNAPII elongates through the nucleosome, contacts to the H2A-H2B dimer distal to the promoter are lost. Inhibiting topoisomerases or depleting histone chaperones increased unwrapping, whereas inhibiting the release of paused RNAPII or reducing RNAPII elongation decreased unwrapping. Our results indicated that positive torsion generated by elongating RNAPII causes transient loss of histone-DNA contacts[10]. Cryo-electron microscopy of *in vitro* reconstituted unwrapped nucleosomes yielded structures that matched the structures we inferred from paired-end sequencing, thus validating our general approach[15].

Mapping In vivo Nascent Chromatin using EdU and sequencing (MINCE-seq). In MINCE-seq (**Fig. 2**), nucleotide analogue ethynyl deoxyuridine (EdU) is added to asynchronous cells to label newly replicating DNA[16]. EdU can be linked with biotin *ex vivo* to facilitate affinity isolation and when combined with micrococcal nuclease footprinting and high-depth, short-read sequencing, the biotin linkage allows specific characterization of the newly replicated chromatin landscape. To track chromatin maturation as a function of time, we can chase the pulse of EdU with thymidine for different lengths of time. Thus, we can use MINCE-seq to generate a temporal map of chromatin landscape post-replication. Recent studies in yeast indicate that TFs are replaced by nucleosomes in *Drosophila* but not in yeast[17,18], raising the question whether nucleosome occlusion of TF binding sites post-replication is a *Drosophila*-specific phenomenon. To extend MINCE-seq beyond *Drosophila* cells we have recently optimized the protocol for mammalian cell lines. To ask if TFs are replaced by nucleosomes in mammals, we analyzed nucleosome landscape centered at CTCF binding sites genome-wide in K562 cells, at steady state, immediately post-replication, and 1-hour post-replication (**Fig. 3**, unpublished data). We observed depletion of nucleosomes at CTCF binding sites and ordered nucleosome arrays on either side of the binding sites at steady state, as has been observed before. Strikingly, there is complete loss of nucleosome depletion immediately post-replication (85% loss of nucleosome depletion on average), indicating that nucleosomes replace CTCF behind the replication fork. 1-hour post-replication, there is still significant nucleosome occupancy over the CTCF binding sites, with only 65% recovery of binding compared to steady state, indicating that CTCF rebinding post-replication occurs in time-scale of hours. In

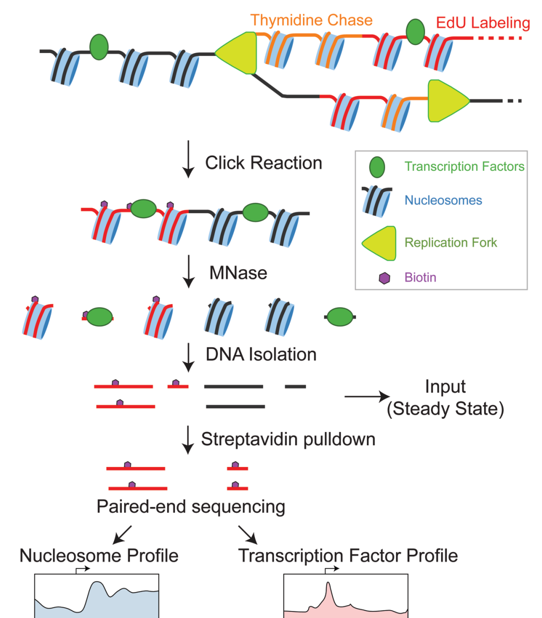

Figure 2. MINCE-seq schematic

summary, we have demonstrated that MINCE-seq can be performed in mammalian cells and have also shown that nucleosomes replace TFs behind replication fork in human cells.

Nucleation sites in mESCs. Polycomb Response Elements (PREs) are DNA segments that are genetically sufficient to initiate and maintain silencing through development. PREs are thought to nucleate the histone methyltransferase complex, Polycomb Repressive Complex 2 (PRC2) for trimethylation of histone H3 lysine 27 (H3K27me3). Although PREs have been mapped in *Drosophila*, it has been difficult to genetically map mammalian PREs. We hypothesized that kinetics of establishment of H2K27me3 can be used to identify PREs as these DNA segments should display the fastest kinetics of establishment of H3K27me3 in a Polycomb (Pc) domain in cells. This provides an alternative to genetic reporters for identifying PREs, and is much more tractable. A recent study[19] provided the best dataset to test our hypothesis. Mouse Embryonic Stem Cells (mESCs) were treated with an inhibitor of PRC2 catalytic activity until all H3K27me3 was diluted out. The inhibitor was then washed out and H3K27me3 ChIP was performed at 0, 4, 8, 16, 24, 48, and 96 hours after drug washout to monitor reappearance of H3K27me3[19]. We developed the computational framework to infer locus-specific kinetics of reappearance of H3K27me3 from the timed ChIP-seq data post drug washout. We hypothesized that PRC2 nucleation sites would have the fastest kinetics of H3K27me3 accumulation within a domain even if they do not have the highest enrichment of H3K27me3 at steady state. Based on this hypothesis, we were able to identify 1084 PRC2 nucleation sites genome-wide (**Fig. 4**, unpublished analysis). As proof-of-concept, the one PRE identified in human ES cells (D11.12)[2] corresponds to a nucleation site in our analysis in the syntenic location of the mouse genome. These computationally identified nucleation sites are the first step in our effort to define PREs in mammals in Question 2b.

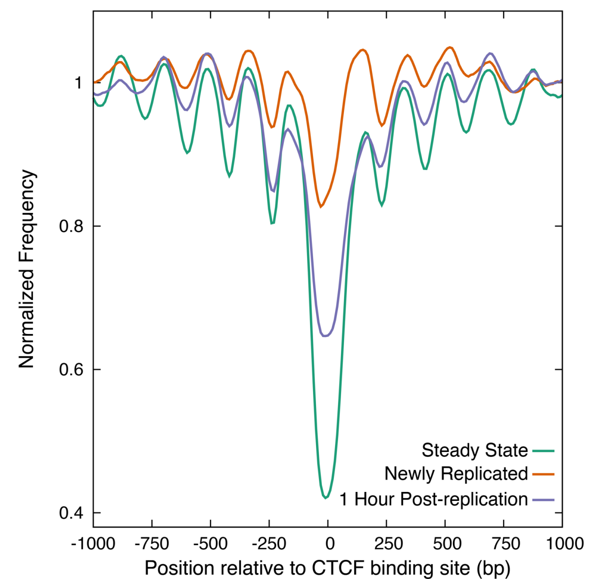

Figure 3. MINCE-seq in mammalian cells. Average nucleosome profile centered at CTCF binding sites genome-wide from ref.[1].

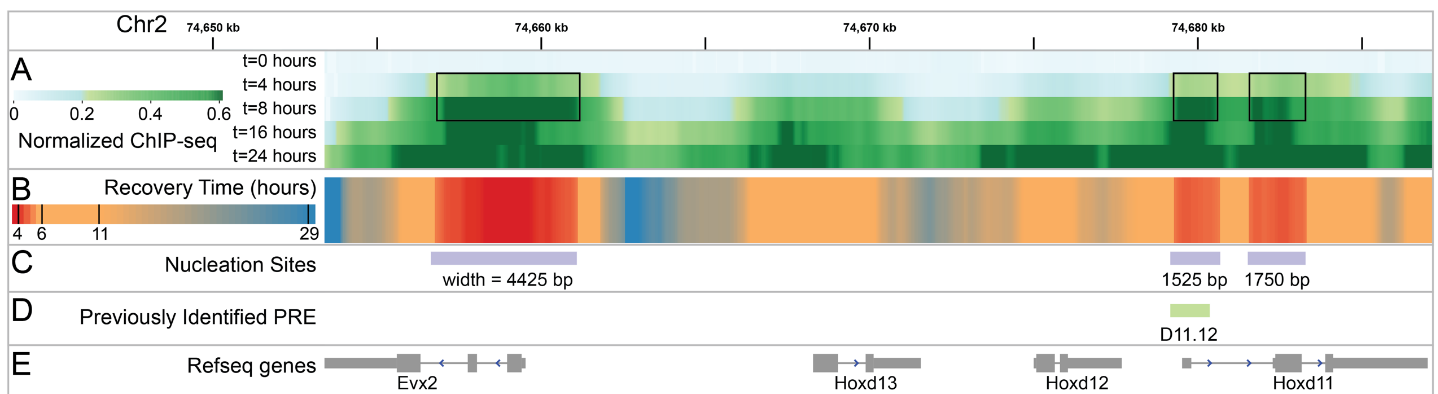

Figure 4. Identifying nucleation sites for PRC2 by tracking kinetics of recovery of H3K27me3 after drug-washout. (A) Normalized H3K27me3 ChIP-seq signal shown as a green heatmap for the first 24 hours post drug washout. At 4 and 8 hours, we can observe three regions in the locus having higher signal than the rest of the locus (indicated by black boxes). Data from ref.[19] (**B**) We calculated the time to reach 40% recovery and plotted it as a heatmap. Red indicates fastest recovery times and the three regions indicated in (B) have the fastest recovery time in the locus. (**C**) We clustered the regions based on recovery time and we predict the clusters with fastest recovery times as nucleation sites for PRC2, which is indicated in this track. (**D**) D11.12, a PRE identified in human embryonic stem cells is mapped at its syntenic location on the mouse genome[2]. We can see that it overlaps with one of the three nucleation sites identified in the HoxD locus. (**E**) Genes in this region.

PROPOSED RESEARCH PROGRAM

Theme 1. Active chromatin

We hypothesize that TF site selectivity occurs due to three factors: i) TF hierarchy as has been proposed for reprogramming[20]: The TFs most adept at disrupting nucleosomes (e.g. "pioneer factors") will be at the top of the hierarchy, creating accessibility for other TFs that require naked DNA to bind. ii) Clustered binding sites to maintain a high local concentration of the TF(s)[21,22] iii) Action of ATP-dependent chromatin remodelers on newly

replicated chromatin could expose TF binding sites as the remodelers slide and/or evict nucleosomes[21,22] (schematized in **Fig. 5**). By tracking specific transcription factor binding behind the replication fork in the time-scale of minutes to hours, we will test to what extent each of the above factors determine TF site selectivity in cells.

Question 1a. What are the kinetics of TF binding to specific chromatin loci in the cell?

We will track transcription factor binding by three different means – first, we will use high-resolution MINCE-seq to identify all transcription factor footprints genome-wide post-replication in the time-scale of minutes to hours: MINCE-seq captures both nucleosome and TF footprints in a single assay and can distinguish individual TF binding events for even closely placed TF binding sites due to the high-resolution afforded by MNase footprinting[10,23]. Second, we will map TF binding directly by coupling MINCE-seq to ChIP, where we will perform ChIP of transcription factors from cells labeled with EdU, purify the DNA and then pull-down biotinylated DNA (**Fig. 6**). This way, we will directly capture TF rebinding post-replication. Third, we will track accessibility post-replication by coupling a transposase assay (Assay for Transposase-Accessible Nascent Chromatin using sequencing, ATAC-seq[24]) to EdU labeling. For nucleosome disrupting TFs, we expect to capture TF binding to nucleosomes post-replication followed by direct binding to DNA upon nucleosome disruption (**Fig. 5**), which will be distinguished by the size of the protected DNA fragment in MINCE-seq and delayed appearance of accessibility as measured by EdU-ATAC-seq. For a TF which does not disrupt nucleosomes, we expect appearance of accessibility post-replication to coincide or precede TF binding post-replication. Through combined analysis of MINCE-seq, MINCE-ChIP-seq, and EdU-ATAC-seq, we will construct a map of TF hierarchy to uncover the basis of genome-wide binding site selection by different TFs.

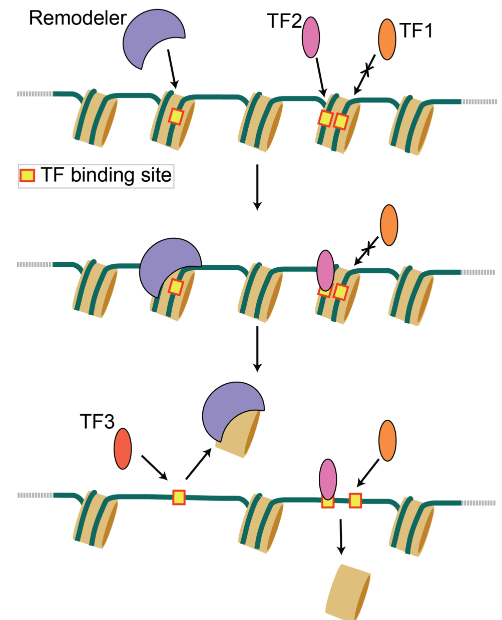

Figure 5. Model for establishment of TF site selectivity. After replication, all TF binding sites are occluded by nucleosomes. TF2 can bind its site even in the presence of nucleosome, but TF1 can only bind to naked DNA. TF2 binding displaces the nucleosome, making the site for TF1 available to bind. A remodeler mobilizes a nucleosome to expose the binding site of TF3.

Question 1b. How do remodelers facilitate transcription factor binding?

Maintenance of accessibility at active promoters and enhancers genome-wide after the disruptive events of replication also requires the action of ATP-dependent chromatin remodelers. However, it is unknown how remodelers determine their genomic targets and how they may cooperate with TFs to set up accessible chromatin landscapes. This is because most genome-wide studies of remodelers have been at steady state. By mapping chromatin landscapes as a function of time after specific and fast perturbation of remodeler function, we will uncover remodeler specificity in maintaining accessible chromatin landscapes.

In *Drosophila*, I showed that the patterns of nucleosome gain at promoters post-replication correlates with distribution of the Brahma (BRM) remodeler at steady state, indicating that BRM action during maturation could lead to the steady state landscape[3]. To test the hypothesis that ATP-dependent chromatin remodelers direct TF site-selectivity post-replication, we will map TF rebinding post-replication after disrupting two important remodelers in *Drosophila*, BRM[25] and ISWI[26]. BRM and ISWI both have an ATPase domain and they form the core of several remodeling complexes with diverse functions. To rapidly perturb remodeler function, we will use auxin inducible degron (AID) system[27], which can deplete a protein of interest in a time-scale of hours upon addition of the plant hormone auxin. We will perform MINCE-seq after rapid depletion of BRM and ISWI and compare the kinetics of TF rebinding post-replication in the absence of

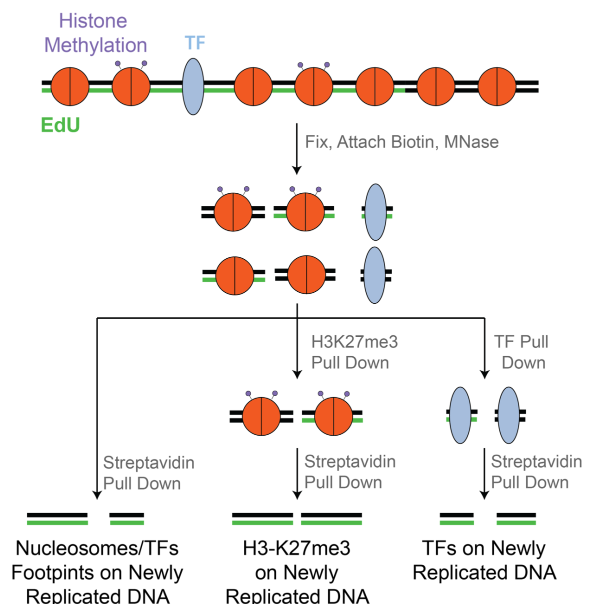

Figure 6. MINCE-ChIP-seq schematic

remodelers to that in control cells. Sites with biggest changes in TF rebinding kinetics will be the sites that require remodeler action to facilitate TF binding. Due to the time-scale of few hours between remodeler depletion and its effect on TF rebinding post-replication, we will uncover the direct kinetic effects of remodeler function on TF site selectivity genome-wide. We will validate these results by using inducible dominant-negative constructs of both ISWI[28] and Brm[29]. These mutants can form remodeling complexes and bind to their substrates, but cannot remodel chromatin due to loss of ATPase activity. We will induce expression of the dominant negative remodelers and then generate MINCE-seq and EdU-ATAC-seq maps, which will help validate sites of remodeler action determined after remodeler depletion using the AID system.

Determining the basis of TF site selectivity in metazoan genomes is a fundamental requirement for understanding regulated gene expression. The significance of studies under Question 1 are that we will uncover how a combination of TF hierarchy, binding site context, and site-specific remodeler action determine TF site selectivity.

Theme 2. Repressed chromatin

Question 2a. How are Polycomb domains maintained through replication?

Mutations in Polycomb group (PcG) genes result in loss of maintenance of cellular identity during development, resulting in altered body segment identity[7]. However, with mutations that disrupt cellular memory, the end stages do not inform us of the events that led the loss of cellular memory in the first place. For Pc domains, the preexisting histone methylation could act as a memory for the methyltransferase to modify nearby unmodified nucleosomes[30], or short DNA segments that recruit PRC2 (Polycomb Response Elements, PREs) could direct the maintenance of Pc domains[31], or compaction by PRC1 could carry the memory of repression[32] (**Fig. 7**). Genome-wide kinetics of H3K27me3 post-replication coupled with carefully chosen perturbations of PcG proteins will enable us to determine which of the three mechanisms operates in cells. We will perform these studies in *Drosophila* cell lines because they have well defined Pc domains with high enrichment of H3K27me3, similar to the developmental stage they were derived from, enabling us to map changes in H3K27me3 as a function of time at high-resolution relative to established PREs.

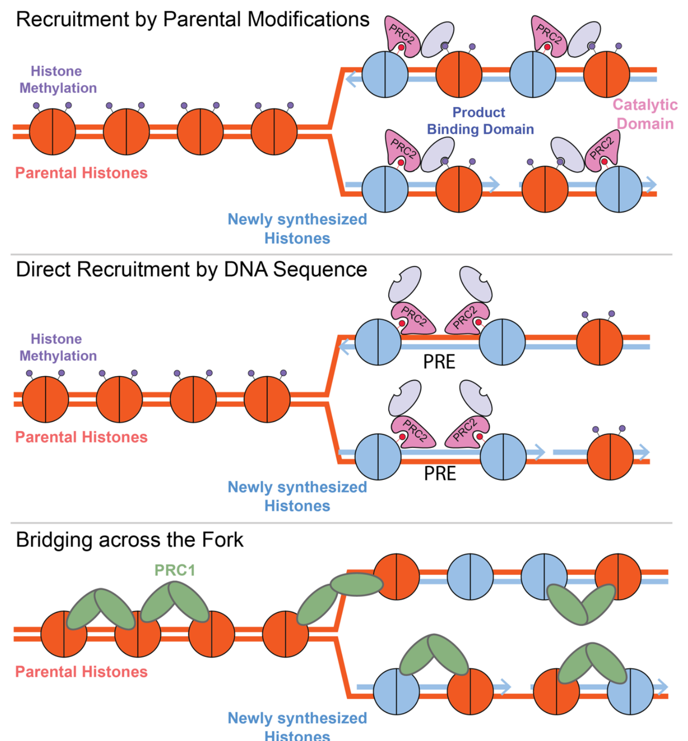

Figure 7. Models for maintenance of H3K27me3 post-replication at the replication fork. Recruitment of Polycomb Repressive Complex 2 (PRC2) methyltransferase complex by redeposited parental H3K27me3 (top), by PREs, short DNA segments that are genetically sufficient to initiate and maintain silencing through development (middle). Bridging of chromatin compacting Polycomb Repressive Complex 1 (PRC1) across the replication fork, which can subsequently recruit PRC2 (bottom).

We will perform MINCE-ChIP-seq to map the real time distribution of H3K27me3 and PRC1 genome-wide immediately post-replication. Auxin inducible degron (AID) system can deplete proteins in the time-scale of hours[27]. Combining AID and MINCE-ChIP-seq, we can temporally map the immediate effect of loss of a particular PcG protein on the Pc domain. Apart from AID, we will use two other strategies for fast perturbation: small molecules with IC_{50} in the nanomolar range[33,34], that will act in the time scale of minutes and inducible expression of dominant negatives, that will perturb the system in the time-scale of hours. Here are few examples of how these experiments will uncover the memory mechanism:

i) Pre-existing H3K27me3. Dominant negative mutants of the module of PRC2 that recognizes pre-existing H3K27me3 (EED) will prevent association of PRC2 with parental H3K27me3[35]. If Pc domains are maintained by PRC2 recruitment to pre-existing H3K27me3, we will observe weakened replenishment post-replication throughout the Pc domain.

ii) PREs. PRC2 could be recruited to PREs by DNA binding proteins like Pho[36]. By depleting Pho using AID, we will ask if PRC2 is still able to bind to PREs post-replication. Absence of nucleation after depletion of Pho would support the model that Pho recruits PRC2 to PREs post-replication to maintain proper H3K27me3 dosage.

iii) Compaction. Overexpression of just the SAM domains of PRC1 components Ph and Scm can disrupt compaction of Pc domains by competing with full length proteins that can interact with chromatin[37,38]. If compaction were essential for memory, we would see impaired replenishment of H3K27me3 post-replication upon reduction in compaction due to induced overexpression of the SAM domains.

Maintenance of cellular memory through replication is fundamental to multicellular life. The significance of studies in Question 2a is that we would have determined the memory mechanism that maintains Pc domains through replication.

Question 2b. Where are mammalian PREs located?

Although PcG proteins are conserved between *Drosophila* and mammals, and mammals have Pc domains over developmentally regulated genes, PREs, the genetic determinants of Pc silencing have been hard to identify in mammals. Only two PREs have been identified in mammals[2,39], due to the relative difficulty of genetically identifying PREs in mammals compared to *Drosophila*. We will ask if our list of mouse H3K27me3 nucleation sites (n=1084, **Fig. 4**) are PREs in mESCs via two approaches: i) high-throughput reporter assays and ii) asking if H3K27me3 reappears first at these nucleation sites post-replication in unperturbed mESCs.

High-throughput reporter assay. Reporters in *Drosophila* embryo that go through development can identify PREs, but such assays are not possible in most cell lines because of their static cell identity and the lack of factors that establish Polycomb domains *de novo*. Cell lines only have the factors to maintain Polycomb domains already established in their progenitors. However, recent studies show that murine Embryonic Stem Cells (mESCs) are an exception. Polycomb domains are accurately established in mESCs *de novo* after their complete ablation either by deleting the methyltransferases Ezh1 and Ezh2[19], treatment with an Ezh2 inhibitor[19], or mutation of EED[35]. Thus, mESCs have the factors for both establishment and maintenance of Polycomb domains. This allows us to use reporter assays in mESCs to identify PREs.

To ask if our computationally identified nucleation sites can initiate silencing *de novo* in a PRC2-dependent manner, we will use reporters that have candidate nucleation sites upstream of a housekeeping promoter driving GFP expression. Bonafide nucleation sites will initiate a Polycomb domain that will silence the promoter upstream of GFP gene[2]. Thus, lower signal of GFP will indicate stronger PRC2 nucleation. Same experiment in the presence of PRC2 inhibitor EPZ6438[33] during and after transfection will confirm whether silencing is dependent on PRC2. We will make a library of the 1084 nucleation sites we identified using pooled PCR with specific primer pairs having unique barcodes. mESCs will be transfected with the GFP reporter library and GFP negative cells will be collected after flow sorting. Primers to the adapter sequences will be used to amplify just the nucleation sites, which will then be sequenced on the Illumina platform along with the starting library used for transfection. Enrichment of nucleation sites in GFP negative population will indicate de novo silencing of the reporters, which will be confirmed by control experiments performed in the presence of EPZ6438. Thus, we will construct an experimentally validated genome-wide map of mouse PREs using this method.

MINCE-ChIP-seq. We hypothesize that nucleation sites will recruit PRC2 faster than the rest of the Polycomb domain post-replication. We have standardized MINCE-seq for mammalian cells and will perform MINCE-ChIP-seq on mESCs to map H3K27me3 profiles post-replication. We will compare the early peaks of H3K27me3 to our computationally predicted nucleation sites to validate PREs in mESCs using a method that is orthogonal to the reporter assay.

Not knowing the identity of PREs means we do not know how Pc domains initiate in mammals. Studies under Question 2b would directly address this gap in knowledge and identify the genetic determinants of initiation of repression in mammals.

SUMMARY

Epigenomics has been mostly characterized by studies of large populations of cells in steady state[40]. However, it is difficult to identify causal mechanisms for dynamic processes using steady state techniques. We have established genomic techniques to map chromatin states as a function of time, which we believe will uncover both kinetics of TF rebinding and remodeler action post-replication and the mechanisms of maintenance of Pc domains through successive cell divisions. These studies will not only resolve long-standing questions in TF-site selectivity and Pc biology, but also will serve as a framework to understand the role of chromatin dynamics in a variety of systems and processes.

References cited

1. Skene PJ, Henikoff S. An efficient targeted nuclease strategy for high-resolution mapping of DNA binding sites. Elife. 2017;6. doi: 10.7554/eLife.21856. PubMed PMID: 28079019; PMCID: PMC5310842.
2. Woo CJ, Kharchenko PV, Daheron L, Park PJ, Kingston RE. A region of the human HOXD cluster that confers polycomb-group responsiveness. Cell. 2010;140(1):99-110. doi: 10.1016/j.cell.2009.12.022. PubMed PMID: 20085705; PMCID: PMC3324942.
3. Ramachandran S, Henikoff S. Transcriptional Regulators Compete with Nucleosomes Post-replication. Cell. 2016;165(3):580-92. Epub 2016/04/12. doi: 10.1016/j.cell.2016.02.062. PubMed PMID: 27062929; PMCID: PMC4855302.
4. Ramachandran S, Ahmad K, Henikoff S. Capitalizing on disaster: Establishing chromatin specificity behind the replication fork. Bioessays. 2017;39(4). Epub 2017/01/31. doi: 10.1002/bies.201600150. PubMed PMID: 28133760; PMCID: PMC5513704.
5. Blythe SA, Wieschaus EF. Establishment and maintenance of heritable chromatin structure during early Drosophila embryogenesis. Elife. 2016;5. Epub 2016/11/24. doi: 10.7554/eLife.20148. PubMed PMID: 27879204; PMCID: PMC5156528.
6. Alabert C, Barth TK, Reveron-Gomez N, Sidoli S, Schmidt A, Jensen ON, et al. Two distinct modes for propagation of histone PTMs across the cell cycle. Genes Dev. 2015;29(6):585-90. doi: 10.1101/gad.256354.114. PubMed PMID: 25792596; PMCID: PMC4378191.
7. Steffen PA, Ringrose L. What are memories made of? How Polycomb and Trithorax proteins mediate epigenetic memory. Nat Rev Mol Cell Biol. 2014;15(5):340-56. doi: 10.1038/nrm3789. PubMed PMID: 24755934.
8. Chereji RV, Ramachandran S, Bryson TD, Henikoff S. Precise genome-wide mapping of single nucleosomes and linkers in vivo. Genome Biol. 2018;19(1):19. Epub 2018/02/11. doi: 10.1186/s13059-018-1398-0. PubMed PMID: 29426353; PMCID: PMC5807854.
9. Henikoff S, Ramachandran S, Krassovsky K, Bryson TD, Codomo CA, Brogaard K, et al. The budding yeast Centromere DNA Element II wraps a stable Cse4 hemisome in either orientation in vivo. Elife. 2014;3:e01861. Epub 2014/04/17. doi: 10.7554/eLife.01861. PubMed PMID: 24737863; PMCID: PMC3983907.
10. Ramachandran S, Ahmad K, Henikoff S. Transcription and Remodeling Produce Asymmetrically Unwrapped Nucleosomal Intermediates. Mol Cell. 2017;68(6):1038-53 e4. doi: 10.1016/j.molcel.2017.11.015. PubMed PMID: 29225036.
11. Ramachandran S, Zentner GE, Henikoff S. Asymmetric nucleosomes flank promoters in the budding yeast genome. Genome Res. 2015;25(3):381-90. Epub 2014/12/11. doi: 10.1101/gr.182618.114. PubMed PMID: 25491770; PMCID: PMC4352886.
12. Brogaard K, Xi L, Wang JP, Widom J. A map of nucleosome positions in yeast at base-pair resolution. Nature. 2012;486(7404):496-501. Epub 2012/06/23. doi: 10.1038/nature11142. PubMed PMID: 22722846; PMCID: PMC3786739.
13. Gaffney DJ, McVicker G, Pai AA, Fondufe-Mittendorf YN, Lewellen N, Michelini K, et al. Controls of nucleosome positioning in the human genome. PLoS Genet. 2012;8(11):e1003036. Epub 2012/11/21. doi: 10.1371/journal.pgen.1003036. PubMed PMID: 23166509; PMCID: PMC3499251.
14. Weber CM, Ramachandran S, Henikoff S. Nucleosomes are context-specific, H2A.Z-modulated barriers to RNA polymerase. Mol Cell. 2014;53(5):819-30. doi: 10.1016/j.molcel.2014.02.014. PubMed PMID: 24606920.
15. Bilokapic S, Strauss M, Halic M. Histone octamer rearranges to adapt to DNA unwrapping. Nat Struct Mol Biol. 2018;25(1):101-8. Epub 2018/01/13. doi: 10.1038/s41594-017-0005-5. PubMed PMID: 29323273; PMCID: PMC5800490.
16. Ramachandran S, Henikoff S. MINCE-Seq: Mapping In Vivo Nascent Chromatin with EdU and Sequencing. Methods Mol Biol. 2018;1832:159-68. Epub 2018/08/04. doi: 10.1007/978-1-4939-8663-7_8. PubMed PMID: 30073526.
17. Fennessy RT, Owen-Hughes T. Establishment of a promoter-based chromatin architecture on recently replicated DNA can accommodate variable inter-nucleosome spacing. Nucleic Acids Res. 2016;44(15):7189-203. Epub 2016/04/24. doi: 10.1093/nar/gkw331. PubMed PMID: 27106059; PMCID: PMC5009725.

18. Vasseur P, Tonazzini S, Ziane R, Camasses A, Rando OJ, Radman-Livaja M. Dynamics of Nucleosome Positioning Maturation following Genomic Replication. Cell Rep. 2016;16(10):2651-65. Epub 2016/08/30. doi: 10.1016/j.celrep.2016.07.083. PubMed PMID: 27568571; PMCID: PMC5014762.
19. Hojfeldt JW, Laugesen A, Willumsen BM, Damhofer H, Hedehus L, Tvardovskiy A, et al. Accurate H3K27 methylation can be established de novo by SUZ12-directed PRC2. Nat Struct Mol Biol. 2018;25(3):225-32. doi: 10.1038/s41594-018-0036-6. PubMed PMID: 29483650; PMCID: PMC5842896.
20. Soufi A, Garcia MF, Jaroszewicz A, Osman N, Pellegrini M, Zaret KS. Pioneer transcription factors target partial DNA motifs on nucleosomes to initiate reprogramming. Cell. 2015;161(3):555-68. Epub 2015/04/22. doi: 10.1016/j.cell.2015.03.017. PubMed PMID: 25892221; PMCID: PMC4409934.
21. Berman BP, Nibu Y, Pfeiffer BD, Tomancak P, Celniker SE, Levine M, et al. Exploiting transcription factor binding site clustering to identify cis-regulatory modules involved in pattern formation in the Drosophila genome. Proc Natl Acad Sci U S A. 2002;99(2):757-62. Epub 2002/01/24. doi: 10.1073/pnas.231608898. PubMed PMID: 11805330; PMCID: PMC117378.
22. Crocker J, Abe N, Rinaldi L, McGregor AP, Frankel N, Wang S, et al. Low affinity binding site clusters confer hox specificity and regulatory robustness. Cell. 2015;160(1-2):191-203. Epub 2015/01/06. doi: 10.1016/j.cell.2014.11.041. PubMed PMID: 25557079; PMCID: PMC4449256.
23. Henikoff JG, Belsky JA, Krassovsky K, MacAlpine DM, Henikoff S. Epigenome characterization at single base-pair resolution. Proc Natl Acad Sci U S A. 2011;108(45):18318-23. Epub 2011/10/26. doi: 10.1073/pnas.1110731108. PubMed PMID: 22025700; PMCID: PMC3215028.
24. Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nat Methods. 2013;10(12):1213-8. Epub 2013/10/08. doi: 10.1038/nmeth.2688. PubMed PMID: 24097267; PMCID: PMC3959825.
25. Kal AJ, Mahmoudi T, Zak NB, Verrijzer CP. The Drosophila brahma complex is an essential coactivator for the trithorax group protein zeste. Genes Dev. 2000;14(9):1058-71. Epub 2000/05/16. PubMed PMID: 10809665; PMCID: PMC316570.
26. Tsukiyama T, Daniel C, Tamkun J, Wu C. ISWI, a member of the SWI2/SNF2 ATPase family, encodes the 140 kDa subunit of the nucleosome remodeling factor. Cell. 1995;83(6):1021-6. Epub 1995/12/15. PubMed PMID: 8521502.
27. Nishimura K, Fukagawa T, Takisawa H, Kakimoto T, Kanemaki M. An auxin-based degron system for the rapid depletion of proteins in nonplant cells. Nat Methods. 2009;6(12):917-22. doi: 10.1038/nmeth.1401. PubMed PMID: 19915560.
28. Deuring R, Fanti L, Armstrong JA, Sarte M, Papoulas O, Prestel M, et al. The ISWI chromatin-remodeling protein is required for gene expression and the maintenance of higher order chromatin structure in vivo. Molecular cell. 2000;5(2):355-65. PubMed PMID: 10882076.
29. Elfring LK, Daniel C, Papoulas O, Deuring R, Sarte M, Moseley S, et al. Genetic analysis of brahma: the Drosophila homolog of the yeast chromatin remodeling factor SWI2/SNF2. Genetics. 1998;148(1):251-65. PubMed PMID: 9475737; PMCID: 1459776.
30. Margueron R, Justin N, Ohno K, Sharpe ML, Son J, Drury WJ, 3rd, et al. Role of the polycomb protein EED in the propagation of repressive histone marks. Nature. 2009;461(7265):762-7. doi: 10.1038/nature08398. PubMed PMID: 19767730; PMCID: PMC3772642.
31. Laprell F, Finkl K, Muller J. Propagation of Polycomb-repressed chromatin requires sequence-specific recruitment to DNA. Science. 2017;356(6333):85-8. doi: 10.1126/science.aai8266. PubMed PMID: 28302792.
32. Lo SM, Follmer NE, Lengsfeld BM, Madamba EV, Seong S, Grau DJ, et al. A bridging model for persistence of a polycomb group protein complex through DNA replication in vitro. Mol Cell. 2012;46(6):784-96. doi: 10.1016/j.molcel.2012.05.038. PubMed PMID: 22749399; PMCID: PMC3389374.
33. Knutson SK, Kawano S, Minoshima Y, Warholic NM, Huang KC, Xiao Y, et al. Selective inhibition of EZH2 by EPZ-6438 leads to potent antitumor activity in EZH2-mutant non-Hodgkin lymphoma. Mol Cancer Ther. 2014;13(4):842-54. doi: 10.1158/1535-7163.MCT-13-0773. PubMed PMID: 24563539.
34. Kruidenier L, Chung CW, Cheng Z, Liddle J, Che K, Joberty G, et al. A selective jumonji H3K27 demethylase inhibitor modulates the proinflammatory macrophage response. Nature. 2012;488(7411):404-8. doi: 10.1038/nature11262. PubMed PMID: 22842901; PMCID: PMC4691848.

35. Oksuz O, Narendra V, Lee CH, Descostes N, LeRoy G, Raviram R, et al. Capturing the Onset of PRC2-Mediated Repressive Domain Formation. Mol Cell. 2018;70(6):1149-62 e5. doi: 10.1016/j.molcel.2018.05.023. PubMed PMID: 29932905.
36. Frey F, Sheahan T, Finkl K, Stoehr G, Mann M, Benda C, et al. Molecular basis of PRC1 targeting to Polycomb response elements by PhoRC. Genes Dev. 2016;30(9):1116-27. doi: 10.1101/gad.279141.116. PubMed PMID: 27151979; PMCID: PMC4863741.
37. Kang H, McElroy KA, Jung YL, Alekseyenko AA, Zee BM, Park PJ, et al. Sex comb on midleg (Scm) is a functional link between PcG-repressive complexes in Drosophila. Genes Dev. 2015;29(11):1136-50. doi: 10.1101/gad.260562.115. PubMed PMID: 26063573; PMCID: PMC4470282.
38. Wani AH, Boettiger AN, Schorderet P, Ergun A, Munger C, Sadreyev RI, et al. Chromatin topology is coupled to Polycomb group protein subnuclear organization. Nat Commun. 2016;7:10291. Epub 2016/01/14. doi: 10.1038/ncomms10291. PubMed PMID: 26759081; PMCID: PMC4735512.
39. Schorderet P, Lonfat N, Darbellay F, Tschopp P, Gitto S, Soshnikova N, et al. A genetic approach to the recruitment of PRC2 at the HoxD locus. PLoS Genet. 2013;9(11):e1003951. Epub 2013/11/19. doi: 10.1371/journal.pgen.1003951. PubMed PMID: 24244202; PMCID: PMC3820793.
40. Roadmap Epigenomics C, Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, et al. Integrative analysis of 111 reference human epigenomes. Nature. 2015;518(7539):317-30. doi: 10.1038/nature14248. PubMed PMID: 25693563; PMCID: PMC4530010.