COLUMBIA PRECISION MEDICINE INITIATIVE

ADVANCES IN PRECISION MEDICINE

CHROMATIN AND NUCLEAR ARCHITECTURE

FRIDAY, MAY 19, 2023

COLUMBIA | PRECISION MEDICINE

Welcome Letter

I am delighted to welcome you to the Seventh Annual Columbia Precision Medicine Initiative (CPMI) conference, Advances in Precision Medicine: Chromatin and Nuclear Architecture.

We are thrilled to be gathering in person once again, but we do understand that some need to attend virtually, so this year's conference is in a hybrid format.

Our topic this year is chromatin and nuclear architecture. Our objective is to provide an up-to-date perspective on the relationship between DNA sequence and genome organization in space and time during normal cellular function and in diseased states. We provide examples of the dynamic regulation of genome folding interrogated by state-of-the-art genomic and imaging technologies, and we explore the role of genome sequence variations in chromatin organization and function.

We have been fortunate to assemble an extraordinary group of pioneers in the development and application of technologies that probe and manipulate genome organization, revealing a previously unappreciated role of 3D genome architecture in the dysregulation in human disease.

I sincerely hope that you enjoy, and are informed by, the conference.

Tom Maniatis, PhD
Director, Columbia University Precision Medicine Initiative
Isidore S. Edelman Professor of Biochemistry and Molecular Biophysics

Conference Schedule

ADVANCES IN PRECISION MEDICINE: CHROMATIN AND NUCLEAR ARCHITECTURE

Columbia University, New York

May 19, 2023

9:00 a.m.	**Tom Maniatis, PhD: Welcome**
9:10 a.m.	**Marcin Imieliński, MD, PhD,** New York University
10:00 a.m.	**Jane Skok, PhD,** New York University
10:50 a.m.	**Geeta Narlikar, PhD,** University of California, San Francisco
11:40 a.m.	**Bing Ren, PhD,** University of California, San Diego
12:30 p.m.	Lunch
1:30 p.m.	**Stavros Lomvardas, PhD,** Columbia University
2:20 p.m.	**Jennifer Phillips-Cremins, PhD,** University of Pennsylvania
3:10 p.m.	**Anders Sejr Hansen, PhD,** Massachusetts Institute of Technology
4:00 p.m.	**Alistair Boettiger, PhD,** Stanford University
5:00 p.m.	Networking Reception

Marcin Imieliński, MD, PhD

Member of the Faculty, Department of Pathology, and Director of Cancer Genetics, Perlmutter Cancer Center, NYU Grossman School of Medicine; Core Member, New York Genome Center

Marcin Imieliński is a physician-scientist whose research is focused on understanding patterns of complex, noncoding, and structural genomic variation in human cancer. As a molecular genetic pathologist, he is interested in the clinical applications of whole genome sequencing (WGS). Through the development of genome graph approaches (JaBbA, gGnome) for studying cancer DNA structural variation, the Imieliński laboratory has uncovered new classes of complex genomic rearrangements (*Cell* 2020) and signatures of telomere crisis (*Nature Communications* 2021). The laboratory is also interested in how genomic rearrangements perturb genome folding, having recently developed a new, long-read sequencing assay (Pore-C) and analytic framework (Chromunity) to assess high-order 3D genome structure (*Nature Biotech* 2022). Previous work includes uncovering some of the first somatic mutational "tattoos" of cancer cell-of-origin in lung, liver, stomach, and thyroid cancer *(Cell* 2017) and leading major WGS studies in lung cancer (*Cell* 2012, *Cell Reports* 2021). Dr. Imieliński obtained his MD and PhD in genomics and computational biology at the University of Pennsylvania and a BS in computer science at Rutgers College. He completed his residency and fellowship in molecular pathology at Massachusetts General Hospital and Harvard Medical School and postdoctoral research at the Broad Institute and Dana Farber Cancer Institute. Dr. Imielinski has an h-index of 57 across over 100 publications that have been cited over 47,000 times.

Long Molecule Footprints of Backup Repair Pathways in Homologous Recombination–Deficient Cancers

ABSTRACT

Homologous recombination (HR) deficiency is associated with DNA rearrangements and cytogenetic aberrations. Paradoxically, the types of DNA rearrangements specifically associated with HR-deficient cancers only minimally impact chromosomal structure. To address this paradox, we combined a genome graph analysis of short-read whole genome sequencing (WGS) profiles across thousands of tumors with deep linked-read (LR) WGS of 46 BRCA1 or BRCA2 mutant breast cancers to discover a distinct class of HR deficiency-enriched rearrangements called reciprocal pairs. LR WGS showed that reciprocal pairs with identical rearrangement orientations gave rise to one of two distinct chromosomal outcomes, distinguishable only with long molecule data. While one (cis) outcome corresponded to the copy and pasting of a small segment to a distant site, a second (trans) outcome was a quasi-balanced translocation or multi-megabase inversion with substantial (10kb) duplications at each junction. We propose an HR-independent replication restart repair mechanism to explain the full spectrum of reciprocal pair outcomes. LR WGS additionally identified single-strand annealing (SSA) as a BRCA2-deficiency specific repair pathway in human cancers. Integrating these features in a classifier improved discrimination between BRCA1- vs. BRCA2-deficient genomes. In conclusion, our data reveal classes of BRCA1- and BRCA2-deficiency specific rearrangements as a source of cytogenetic aberrations in HR-deficient cells.

Jane Skok, PhD

Director, Cancer Genome Dynamics Program, Perlmutter Cancer Center, NYU School of Medicine; Sandra and Edward Meyer Chair of Radiation Oncology, NYU School of Medicine; Affiliate Member, New York Genome Center; Associate Director of Basic Sciences, Perlmutter Cancer Center, NYU School of Medicine

Jane Skok's laboratory applies a combination of sophisticated imaging techniques, molecular biology (including chromosome conformation capture), and genetics to investigate the contribution of nuclear organization and long-range interactions in coordinating transcriptional programs during development and redirecting these in cancer cells. Since starting her lab, Dr. Skok has continued to pioneer new applications of 3-D FISH and has set up a highly innovative CRISPR/Cas9 live imaging system. She independently established chromosome conformation capture at NYU. In collaboration with Richard Bonneau's computational biology group at NYU her team developed a method for 4C-seq analysis, 4C-ker. In addition, they developed 4Tran to identify transposable element (TE) interaction profiles for individual endogenous retrovirus (ERV) families and integration events specific to particular genomes *(Genome Biology*). Her team can now show that TEs participate in both long- and short-range contacts and could potentially be in involved in the regulation of multiple target loci. They also developed a method to simultaneously detect methylation and ATAC-seq or ChIP signal on the same DNA molecule (*Genome Biology*). Thus, Dr. Skok's lab is one of a handful of labs that has expertise in both the experimental and analytical aspects of chromosome folding. The lab shares its knowledge widely with the scientific community both inside and outside NYU, as demonstrated by its numerous collaborative publications.

The Impact of Cancer-Associated CTCF Mutations on Chromatin Structure and Gene Regulation

ABSTRACT

Though it is known that 3D genome organization plays a crucial role in gene regulation and cancer, the underlying mechanisms connecting these are poorly understood. CTCF is central to these, as it governs genome organization and is implicated in cancer. In fact, mutations in CTCF are detected in numerous cancers; however, the extent to which they perturb 3D chromosomal architecture and contribute to the malignant phenotype is unknown. *We hypothesized that each CTCF mutation will alter cellular function in a different manner depending on whether it is associated with (i) total loss of CTCF binding, (ii) a change in binding affinity, (iii) an alteration in binding motif preference, or (iv) orientation of binding. Furthermore, we propose that mutations, which occur frequently in cancers that have no apparent effect on binding or binding affinity, will have important functions in disrupting dimerization of CTCF molecules or binding of important cofactors such as cohesin*. To test these models, we have used two innovative approaches that examine the impact of mutations on (i) binding affinity and target sequence specificity and

(ii) chromosome structure and gene regulation. Determining the impact of CTCF cancer-associated mutations at these different levels provides insight into their contribution to cancer phenotypes as well as a mechanistic understanding of their involvement in TAD structure and gene regulation.

Sarah Bacher,[1] Guimei Jiang,[1] Theodore Sakellaropoulos,[1] Priscillia Lhoumaud,[1] Fara Faye Regis,[1] Aristotelis Tsirigos,[1] Marcus Noyes,[1] Catherine Do,[1] Jane Skok.[1]*

[1] New York University School of Medicine, 550 1st Ave, New York, NY 10016, USA.

* These authors contribute equally to this work.

Geeta Narlikar, PhD

Professor and Vice-Chair, Biochemistry and Biophysics
Lewis and Ruth Cozen Chair I
University of California, San Francisco

Geeta Narlikar obtained her PhD in chemistry at Stanford University under the mentorship of Dr. Daniel Herschlag and carried out postdoctoral research at Harvard Medical School under the mentorship of Dr. Robert Kingston. She has been a faculty member in the Department of Biochemistry and Biophysics at UCSF since 2003, and in 2017, was appointed to the Lewis and Ruth Cozen Chair I. Her laboratory asks questions about the mechanisms underlying epigenetic regulation and genome organization. Through the application of sophisticated biophysical approaches, the team is learning (i) how nanoscale molecular motors use chemical energy to cause mechanical disruptions in the packaged genome; (ii) that the smallest unit of genome folding, a nucleosome, acts akin to a dynamic receptor rather than a static packaging unit; and (iii) that liquid-liquid phase separation processes can help organize and sequester large regions of the genome. These types of discoveries from the Narlikar laboratory are changing textbook descriptions of genome packaging and suggesting new avenues to tackle diseases caused by defects in genome organization.

Dr. Narlikar's scientific work has been recognized by various awards including the Beckman Young Investigator Award (2006); the Outstanding Faculty Mentorship Award by the UCSF Graduate Students Association (2011); the Glenn Award for Research in Biological Mechanisms of Aging (2018); and the Distinguished Alumnus Award from the Indian Institute of Technology, Mumbai (2018). She was elected to the National Academy of Sciences in 2021.

Mechanisms of Chromatin Remodeling Machines: Different or the Same?

ABSTRACT

Unlike other chromatin remodelers, the INO80 complex preferentially mobilizes hexasomes, which are formed during transcription. Why INO80 prefers hexasomes over nucleosomes remains unclear. Cryo-electron microscopy-based structures of S. cerevisiae INO80 with a hexasome show an unexpectedly large, ~180-degree rotation of INO80 compared to the structure with a nucleosome. This rearrangement places its ATPase subunit, Ino80, at superhelical location (SHL) -2 instead of SHL-6/-7 as seen on a nucleosome. We also observe substantial unwrapping of flanking DNA, binding of the Arp5 module to the exposed H3-H4 surface, and binding of the Arp8 module to unwrapped DNA. Our results indicate that INO80 action on hexasomes resembles action by other remodelers on nucleosomes, where the ATPase is maximally active at SHL-2. In contrast, INO80 action on nucleosomes appears heavily regulated by auto-inhibition and steric clashes that prevent Ino80 from readily accessing SHL-2. These novel mechanistic adaptations for preferential hexasome sliding imply that sub-nucleosomal particles play significant regulatory roles *in vivo*.

Bing Ren, PhD

Professor, Cellular and Molecular Medicine; Director, Center for Epigenomics
University of California, San Diego

Bing Ren is director of the Center for Epigenomics and professor of cellular and molecular medicine at the University of California, San Diego (UCSD). He is also a member of the Ludwig Institute for Cancer Research (LICR). Dr. Ren obtained his PhD in biochemistry from Harvard University in 1998, where he studied with Tom Maniatis. He joined the faculty at LICR and UCSD in 2001, after completing postdoctoral training with Richard Young at the Whitehead Institute.

Dr. Ren is a pioneer of epigenetic technology development and a leader in the study of enhancers and insulators in the human genome. His pioneering research in gene regulation and epigenomics has contributed seminally to the knowledge of transcriptional regulatory elements in the human genome, the 3D chromatin architecture, and the role of noncoding DNA variants in human diseases. Over the last two decades, Dr. Ren has invented numerous tools that have revolutionized the research of epigenetics and gene regulation. Notably, the ChIP-chip technology that he developed ushered in a new era in epigenetic studies in the early 2000s. Recently, he further developed a suite of single cell epigenomic assays—including single nucleus ATAC-seq, single cell Methyl-HiC, Paired-Seq, and Paired-Tag—that have enabled the analysis of chromatin accessibility, DNA methylome, chromosomal conformation, and histone modification, either individually or in combination with RNA-seq, in single cells and at unprecedented scale, thereby setting the stage for comprehensive epigenome mapping in development and disease process. Leveraging the state-of-the-art epigenome mapping technologies that he pioneered, Dr. Ren has made seminal contributions to the annotation of cis-regulatory elements, especially enhancers and insulators, that account for more than 15 percent of the human genome. Notably, his lab produced atlases of candidate gene regulatory elements for each of over 200 fetal and adult human cell types and 160 mouse brain cell types. Dr. Ren was the first to discover that enhancers in metazoan cells carry a unique histone modification H3K4me1 that can be used to predict the location and cell-type specific activities of these cis-regulatory elements. He further identified and characterized protein complexes acting as enhancers and elucidated the functional roles of the H3K4me1 in facilitating nucleosome remodeling and long-range promoter-enhancer contacts. Dr. Ren is among the first to discover the topologically associating domains (TADs), a key 3D chromatin organization feature in the genome, establishing a mechanistic link between TADs and transcriptional insulation.

Dr. Ren is a recipient of the Chen Award for Distinguished Academic Achievement in Human Genetic and Genomic Research and is an elected fellow of the American Association for the Advancement of Science.

Single-Cell Epigenome Profiling Maps, Cell Types, and Gene Circuits Involved in Neurological Disorders

ABSTRACT

Neurological disorders and mental illnesses are the leading cause of disease burdens in the United States. Tens of thousands of sequence variants in the human genome have been linked to the etiology of neuropsychiatric disorders. However, interpreting the mode of action of the identified risk variants remains a daunting challenge since the vast majority of them are non-protein-coding. Mounting evidence suggests that a large fraction of the non-coding risk variants contribute to disease etiology by perturbing transcriptional regulatory elements and target gene expression in the disease-relevant cell types. However, a lack of the maps and tools to explore gene activities and their transcriptional regulatory sequences at high cellular and anatomical resolution in the brain prevents a clearer mechanistic understanding of the broad spectrum of neuropsychiatric disorders. To address this knowledge gap, we carried out single nucleus ATAC-seq analysis to probe the open chromatin landscape from over 1.1 million cells in 42 brain regions of three neurotypical adult donors. Integrative analysis of the resulting data identified 107 distinct cell types and revealed the cell-type-specific usage of 544,735 candidate cis-regulatory DNA elements (cCREs) in the human genome. We uncovered strong associations between specific brain cell types and neuropsychiatric disorders. We further developed deep learning models to predict regulatory function of non-coding disease risk variants.

Stavros Lomvardas, PhD

Roy and Diana Vagelos Professor and Chair of Biochemistry and Molecular Biophysics, Vagelos College of Physicians and Surgeons, Columbia University; Herbert and Florence Irving Professor of Biochemistry and Neuroscience and Principal Investigator, Zuckerman Mind Brain Behavior Institute

By studying olfaction, Stavros Lomvardas aims to understand the molecular mechanisms that give rise to the wide diversity of cell types in the mammalian nervous system. Under the mentorship of Dr. Richard Axel, co-director of the Zuckerman Institute and Nobel Prize winner, Dr. Lomvardas developed an interest in how gene regulation operates in the olfactory system, wherein each olfactory sensory neuron expresses just one from ~1,000 olfactory receptor genes. His lab continues to study not only the epigenetic mechanisms of olfactory receptor gene choice but also the molecular mechanisms that control the wiring of the olfactory circuit. By deciphering how olfactory receptor genes are turned on and off and how the axons of olfactory sensory neurons find their targets in the brain, his research sheds light on the brain's underlying biomolecular principles—and what happens when those mechanisms go awry in a variety of human conditions from COVID-19 to Alzheimer's disease.

Dr. Lomvardas joined Columbia and the Zuckerman Institute in 2014, when he was recruited from the University of California, San Francisco. Prior to this, he completed his postdoctoral training at Columbia University with Dr. Axel, where he started dissecting the molecular underpinnings of olfactory receptor gene choice in the mouse olfactory system. He received his PhD in biochemistry and molecular biophysics in 2002, and his MA in genetics and development in 1999, both as a graduate student at Columbia University. A native of Greece, Dr. Lomvardas received his BS in molecular biology from the University of Crete in 1998.

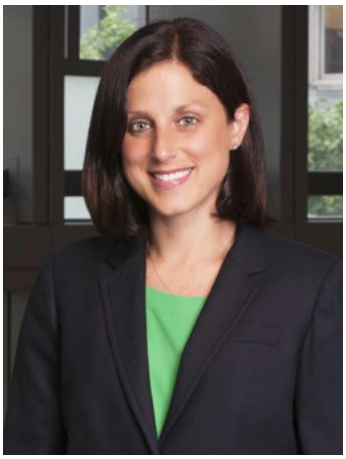
Dr. Lomvardas has received a variety of honors throughout his career, including the Harold Weintraub Graduate Student Award, the Vilceck Prize for Creative Promise, the Faculty Scholar Award from the Howard Hughes Medical Institute, the Young Investigator Award for Research in Olfaction, the McKnight Scholar Award, the EUREKA Award from the National Institutes of Health, and the NIH Director's New Innovator Award.

Achieving Singularity and Diversity in Olfactory Receptor Gene Expression

ABSTRACT

Olfactory receptor (OR) choice provides an extreme example of allelic competition for transcriptional dominance, as every olfactory neuron stably transcribes one out of more than ~2,000 OR alleles. OR gene choice is mediated by a multi-chromosomal enhancer hub that activates transcription at a single OR, followed by OR-translation dependent feedback that stabilizes this choice. However, with more than one OR enhancer hub in each nucleus, the mechanism by which only one OR allele is chosen for transcription remains enigmatic. Here, using single cell genomics, we show that only one of the OR enhancer hubs formed in each

neuron retains euchromatic features and sustains transcriptional competence. Further, we provide evidence that OR transcription recruits enhancers and reinforces enhancer hub activity locally, while OR RNA inhibits transcription in competing hubs over distance, promoting OR protein-independent transition to singularity. While OR RNA synthesis is sufficient to break the symmetry between equipotent enhancer hubs, OR translation stabilizes transcription at the prevailing hub, revealing dual non-coding and coding mechanisms implemented by OR RNAs for transcriptional prevalence. We propose that coding mRNAs can function as "selfish" non-coding RNAs that influence nuclear architecture, enhance their own transcription, and inhibit their competitors, with generalizable implications in probabilistic cell fate decisions.



Jennifer Phillips-Cremins, PhD

Associate Professor and Dean's Faculty Fellow in Engineering and Medicine
University of Pennsylvania

Jennifer Phillips-Cremins is an associate professor and Dean's Faculty Fellow in Engineering and Medicine at the University of Pennsylvania (UPenn) with primary appointments in the Departments of Genetics and Bioengineering. Dr. Cremins obtained her PhD in biomedical engineering from the Georgia Institute of Technology, in the laboratory of Andres Garcia. She conducted a multidisciplinary postdoc in the laboratories of Job Dekker and Victor Corces. Dr. Cremins now runs the Laboratory of Chromatin and Spatial Neurobiology at UPenn. Her primary research interests lie in understanding the long-range chromatin architecture mechanisms that govern neural specification and synaptic plasticity in healthy neurons and how chromatin is dysregulated in neurodevelopmental and neurodegenerative diseases. She was selected as a 2014 New York Stem Cell Foundation Robertson Investigator, a 2015 Albert P. Sloan Foundation Fellow, a 2016 and 2018 Kavli Frontiers of Science Fellow, a 2015 NIH Director's New Innovator Awardee, a 2020 NSF CAREER Awardee, and a 2020 CZI Neurodegenerative Disease Pairs Awardee and as a recipient of the 2022 ISSCR Susan B. Lim Outstanding New Investigator Award and the 2021 NIH Pioneer Award.

Dissecting the 3D Genome's Structure-Function Relationship in the Mammalian Brain

ABSTRACT

The Cremins lab aims to understand how chromatin works through long-range physical folding mechanisms to influence neuronal specification and enduring changes in synaptic plasticity in normal neurophysiology and in neurological disorders. We pursue a multidisciplinary approach integrating data across biological scales in the brain, including molecular Chromosome-Conformation-Capture sequencing technologies, single-cell imaging, optogenetics, genome engineering, and induced pluripotent stem cell differentiation to neurons/organoids. At the lab's inception, it was unclear how genomes are folded in the mammalian neurodevelopment below the resolution of a Megabase, and whether and how higher-order structure could deterministically influence genome function. We have developed and applied new molecular and computational technologies to elucidate chromatin folding patterns at kilobase-resolution genome-wide, thus discovering that long-range looping interactions in cis and inter-chromosomal interactions in trans change substantially during neural lineage commitment, somatic cell reprogramming, activation of post-mitotic neural circuits, and in neurological disorders. We have demonstrated that cohesin-mediated loops are necessary for the establishment of new gene expression programs in post-mitotic neurons, including the upregulation of genes encoding axon guidance, dendritic spine morphology, and synaptic plasticity during neuron maturation in vivo as well as activity-dependent transcription during neural stimulation in vitro. We have also identified cohesin-mediated loops anchored by divergently oriented CTCF binding sites that are necessary and sufficient for the firing efficiency and localization of human replication origins during S phase reentry after mitosis. Using fragile X syndrome as a natural perturbation, we have uncovered BREACHes (Beacons of Repeat

Expansion Anchored by Contacting Heterochromatin)—rare inter-chromosomal interactions connecting heterochromatinized synaptic genes susceptible to repeat instability. Our work provides early insights into the genome's structure-function relationship during the establishment of new transcription, repeat instability, and replication patterns when mammalian cells transition states in the healthy and diseased brain.

Anders Sejr Hansen, PhD

Underwood-Prescott Career Development Professor
Department of Biological Engineering
MIT

Anders Sejr Hansen obtained his undergraduate and master's degree in chemistry at Oxford University in 2010. He received his PhD in chemistry and chemical biology from Harvard University in 2015, where he worked with Erin O'Shea and applied systems biology approaches to understand how cells can encode and transmit information in the dynamics of transcription factor activation. For his postdoc at UC Berkeley with Robert Tjian and Xavier Darzacq, Dr. Hansen developed new imaging approaches to track single proteins in living cells and applied these to understand the mechanisms of key architectural proteins involved in 3D genome organization.

Dr. Hansen began his independent lab in 2020 at MIT, where he is currently the Underwood-Prescott Career Development Professor of Biological Engineering. The Hansen lab is broadly interested in 3D genome structure and function and develops new super-resolution and single-molecule imaging methods to track chromatin looping, transcription, and protein dynamics in living cells as well as new 3D genome structure mapping methods. Current application areas of interest include the dynamics of chromatin looping and transcription, how misfolding of the genome causes disease, the basic mechanisms of 3D genome folding, and synthetic 3D genome biology.

Dynamics of 3D Genome Structure and Function

ABSTRACT

3D genome structure regulates gene expression by regulating the interactions between enhancers and promoters. CTCF and loop-extruding cohesins fold the genome into loops and domains known as Topologically Associating Domains (TADs). However, whether these domains were stable or dynamic was not clear. First, we will briefly discuss our recent work live-imaging and quantifying the dynamics of CTCF- and cohesin-mediated chromatin looping and the implications of our finding that these loops are both highly dynamic (~10–30 min median lifetime) and rare (~3%–6.5% looped fraction). Second, we will discuss more recent work focused on quantifying the interactions between enhancers and promoters (E-P). Specifically, Hi-C has poor sensitivity and depth for capturing E-P interactions. To overcome this limitation, we have developed Region-Capture Micro-C (RCMC) to generate the deepest 3D genome structure maps reported so far. With RCMC, we find extensive multi-way looping interactions between enhancers and promoters that are largely independent of loop extrusion. Instead, our results suggest that E-P interactions form through a compartmentalization mechanism, and we therefore refer to these fine-scale interactions as "microcompartments." We will discuss the implications of these findings.

Alistair Boettiger, PhD

Assistant Professor of Developmental Biology
Stanford University

Alistair Boettiger is an assistant professor of developmental biology at Stanford University, where his lab develops and applies quantitative super-resolution microscopy to study the role of 3D genome organization in transcriptional regulation and differentiation. Dr. Boettiger completed his undergraduate training in the Department of Physics at Princeton University, where Stansilav Shvartsman introduced him to quantitative imaging in Drosophila development. He conducted his PhD research in biophysics under the guidance of Michael Levine at UC Berkeley, whose team is known for pioneering work understanding cis-regulation in Drosophila and Ciona, where his work identified several molecular and genetic mechanisms ensuring transcriptional precision and robustness in early embryogenesis. His postdoctoral research was conducted in the laboratory of Xiaowei Zhuang at Harvard, known for pioneering work in single-molecule super-resolution. There, he co-led the development of methods for image-based spatial transcriptomics with fellow physicist Jeffrey Moffitt and graduate student Kok-Hao Chen, and adapted super-resolution microscopy approaches to uncover organizational principles of how epigenetic differences affect 3D chromatin folding. Since 2016, his team at Stanford combines the power of both Drosophila genetics and mouse embryonic stem cell genetics with single-molecule and super-resolution imaging to better understand transcriptional regulation during development. His awards include the Dale Frey Award for Breakthrough Scientists and recognition as a New Innovator from NIH, a Packard Foundation Fellow, a Beckman Young Investigator, a Kavli Fellow from the National Academy of Sciences, and a Searle Scholar.

Untangling Genetic Puzzles with Spatial Genome Imaging

ABSTRACT

Over the past decade, advances in sequencing-based methods have helped advance our understanding of genome organization and function from a 1D view into a new field of 3D genome organization, mapping structural organization genome-wide and identifying key regulators. These advances have opened an array of new questions not easily addressed by the available technologies but central to understanding the results. What physical 3D structures underlie the patterns in frequency-of-contact among genomic loci revealed by sequencing approaches such as Hi-C? That is to say, what does a “topologically associating domain” (TAD), a “CTCF loop,” or an “architectural stripe” look like under the microscope; and how do such measurements help us understand the transcriptional regulatory potential of such features? I will describe our work developing single molecule super-resolution methods to image the 3D organization of the genome and describe how these approaches are helping us understand both the mechanisms that regulate genome structure and the impact of this regulation on transcriptional control in animal development. Using an imaging approach we’ve called Optical Reconstruction of Chromatin Architecture (ORCA), I will first show how the microscopy data,

averaged across a population of cells, can map de novo these same TADs, loops, and stripes, identified by deep Hi-C, helping to validate both methods, while uncovering artifacts in some related sequencing methods. I will then describe how the single-cell nature of the 3D traces uncover the highly heterogeneous, likely dynamic, nature of chromatin folding, and exclude some earlier explanations of cis-regulatory specificity based on chromatin globules. Applying ORCA in spatially organized embryonic tissues, combined with multiplexed single molecule RNA analyses, we are able to follow how divergent 3D folding leads to divergent cell-fates during development and how disruption of this 3D structure can produce homeotic fate transformations. Combining ORCA with fast-acting degrons to rapidly deplete architectural proteins, I will show unpublished work on how we have mapped the key proteins CTCF and cohesin to organize 3D genome folding in single cells, from the kilobase scale of single genes to the whole chromosomes. These data reveal unexpected roles for CTCF in the bypass of TAD borders and unexpected roles for cohesin in chromosome-scale 3D structure, which we find has an essential noise-damping effect of global transcription regulation. I will close with a final unpublished example of how imaging 3D chromatin structure has helped us evaluate and understand the potential roles of phase-separation and droplet formation in the function of the epigenetic repressive system of Polycomb factors.