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A sampling of methods to study chromosome and genome structure and function

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Abstract

As a scientist, one's perspective of the human genome is informed by the way it is studied – at the level of single nucleotides, a single gene, a specific genomic region, an entire chromosome, the complete karyotype, or the nucleus that encompasses both the genome and the nuclear components that support genome structure, function, stability, and inheritance. Experimentally investigating aspects of genome structure and chromosome number and higher order packaging requires different technical approaches that offer varying levels of resolution. This special issue of Chromosome Research provides overviews of a few current methodologies to study chromosome and genome organization and function, with a particular focus on contemporary sequencing-based approaches.

Keywords

aneuploidy; chromatin; DNA; enhancer; fluorescence in situ hybridization; histone; immunoprecipitation; replication; RNA; SNP; sequencing; structural variation; transposon

Chromosomes were termed by Wilhelm Waldeyer (Waldeyer 1888), building on Theodor Boveri's description of chromatic elements and Walther Flemming's observations of the longitudinal splitting of threadlike material in the nucleus (i.e. mitosis) [reviewed in (Opitz 2016)]. The introduction of methods that employed synthetic or natural dyes and/or fluorochromes that differentially stain DNA based on nucleotide content or affinity to the DNA major or minor grooves allowed nuclei and chromosomes to be visualized by light or fluorescence microscopy. Giemsa (G-banding) and reverse (R-banding) were ways in which chromosome-specific banding patterns allowed accomplished cytogenetics technologists to identify deleted, duplicated, or rearranged genomic material on a single cell basis (Comings 1975, 1978; Latt 1977). Because many of these techniques were performed on metaphase chromosomes, resolution was rather low and unable to reliably confirm submicroscopic chromosome aberrations. In the late 1980s and early 1990s, the development of fluorescence

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in situ hybridization with single copy, repetitive, and chromosome painting probes gave way to more precise analysis of the genome and allowed copy number or structural analysis of single loci to be evaluated even in interphase nuclei (Liehr et al. 2009; Ried 2013). However, nucleotide resolution cannot be achieved with molecular cytological methods, even using stretched DNA or chromatin fibers (Heiskanen et al. 1995; Sullivan 2010; Dechyeva and Schmidt 2016). Sequencing-based technologies have provided ways to interrogate the structure, organization, and packaging of the genome at base pair resolution.

The reviews within this special issue highlight techniques to address fundamental aspects of chromosome and genome biology. How chromosome and chromatin are spatially and temporally organized within the nucleus is important for understanding the interplay of genes, non-coding sequences, transcripts, and proteins in the function and regulation of the genome. Solmaz Khosravi, Takayoshi Ishii, Steven Dreisseg, and Andreas Houben discuss methods to visualize and track specific genomic regions or sequences in living cells (Khosravi et al. 2019). They review the basic framework of molecular tethering systems such as the *Lac* operon-*Lac* repressor, Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effectors (TALEs), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) with CRISPR-associated caspase 9 (Cas9) and their uses in monitoring the dynamics of centromeres, telomeres, and individual chromosomes in plant and mammalian cells.

Numerical and structure changes to the genome are associated with birth defects, reproductive abnormalities, and cancer. The article by Qing Hu, Elizabeth Maurais, and Peter Ly (Hu et al. 2020) describes approaches ranging from classical cytogenetics to sequencing-based technologies used to identify structural chromosome rearrangements and to understand the molecular mechanisms that lead to genome rearrangements. The authors present a historical timeline of microscopy-based approaches, from early light microscopy of chromosome banding using synthetic dyes to higher resolution fluorescence microscopy approaches that use designer oligonucleotide Oligopaint probes to identify regions of chromosomes or illuminate entire chromosomes. They present genome engineering strategies to induce DNA breaks and create chromosome rearrangements or deletions that can be characterized structurally using DNA sequencing.

The Human Genome Project, initiated in the early 1990s, set out to provide an organized and annotated base pair (bp) resolution reference assembly of a (nearly) complete human genome (Venter et al. 2001; International Human Genome Sequencing Consortium 2004). However, increasingly lower costs for whole genome sequencing has allowed many individual genomes to be compared, revealing that any two unrelated humans differ by approximately 3 million nucleotides. These heritable differences can be represented by single nucleotide polymorphisms (SNPs) or larger structural variants (SV) that involve >50bp and include deletions, insertions, duplications, or inversions. SVs can alter genome stability due to their mechanisms of formation and affect phenotypes owing to alterations in gene copy number or through position effects on nearby genes or gene clusters (Weischenfeldt et al. 2013). Parithi Balachandran and Christine Beck review structural variation in the human genome and discuss cytological, molecular, and genomic approaches to identify SVs (Balachandran and Beck 2020). They discuss whole genome and targeted

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sequencing methods to identify SVs and computational tools to align sequencing reads to accurately place SVs within the genome assembly. They elaborate on how these methods will allow us to understand mechanisms of SV formation and predict the influence of SVs on genome architecture and function.

Many SVs are caused by errors in DNA replication, a fundamental nuclear process by which the genome is copied and genome integrity is maintained (MacAlpine and Bell 2005). The dynamics of DNA replication and the identification of factors involved in precisely copying the genome has been an area of intense study for decades. In less complex organisms such as bacteria or budding yeast, the origins of replication and the process of new DNA synthesis have been precisely identified and intensely studied (Brewer and Fangman 1991; Marczynski and Shapiro 1993). However, in larger eukaryotes, the process is more complicated due the increase in genome size, non-coding DNA content, and more complex chromatin structure. DNA replication occurs over a longer time frame that partitions the genome into early and late replicating regions. The identification of replication origins in large eukaryotes has been less clear so that the replication timing of the genome is typically more broadly studied. Replication dynamics have been historically studied using biochemical, molecular and/or cytological approaches, but these methods are often laborious and are concentrated on small portions of the genome (Brewer and Fangman 1991; Boggs and Chinault 1997). Michelle Hulke, Dashiell Massey, and Amnon Koren review contemporary genomic approaches for studying replication dynamics globally (Hulke et al. 2019). They compare the advantages and challenges of multiple sequencing-based approaches, including BrdU-seq, bubble-seq, and SNS-seq, and comment on the promise of single cell replication assays that incorporate long-read sequencing technologies.

Beyond DNA replication, it is important to understand genome function more broadly. Mapping chromatin accessibility provides insight into where transcription factors bind and offers a deeper understanding of gene function and regulation. Regulation and packaging of transcriptionally active versus silent regions of the genomes is determined by how and where nucleosomes are positioned, modified histones are located, and chromatin proteins are enriched. David Klein and Sarah Hainer comprehensively review methods to characterize chromatin organization and gene function (Klein and Hainer 2019). They compare and contrast approaches such as DNase-seq and ATAC-seq, that map genome accessibility and discuss the unique elements of protein profiling techniques such as ChIP-seq, DamID, and CUT&RUN. Notably, they emphasize methods that are particularly amenable to single cell or low input analyses.

Non-coding RNAs are a class of abundant RNAs found within the cell that are classified into multiple categories and that have functions in gene regulation, development and differentiation, and genome defense. LncRNAs have roles in nuclear compartmentalization, chromatin and chromosome organization, and other aspects of nuclear architecture. Two reviews in this Special Issue explore the numerous approaches to study RNAs implicated in fundamental nuclear processes. In the first article, Saja Medha Akkipeddi, Anthony Velleca, and Dawn Carone zoom in on the nucleus to review types of long non-coding RNAs (lncRNAs) and their functions as molecular bridges with proteins, chromatin, and other RNAs (Akkipeddi et al. 2020). They discuss the range of cytological, biochemical, and

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genomic strategies to identify lncRNAs, with an emphasis on approaches that have been used to study the structure, function, and dynamics of specific lncRNAs within the nucleus and their relevance to disease. Methods to identify RNA binding proteins (RBPs) in single and multi-cellular eukaryotes are compared and the advantages and limitations of the approaches are discussed. This review illuminates the complexity of lncRNAs and introduces the reader to current questions about the roles of these non-coding elements.

The article authored by Rachel O'Neill extends the topic of nuclear noncoding RNAs by reviewing recent technological advances to identify nascent transcripts and RNAs that interact with chromatin and DNA. Dr. O'Neill provides historical context for the study of noncoding RNAs, underscoring the nuances in defining and classifying them, and highlighting key functional roles. She outlines various approaches, including RNA-seq and sequence-based nuclear run-on based techniques (GRO-seq and PRO-seq) used to globally identify transcripts and nascent transcripts. RNA-DNA interactions that occur through complementary base pairing have emerged as regulators of genome and chromosome. The article outlines methods such as DRIP-seq and RDIP that globally profile RNA:DNA heteroduplexes and larger RNA-DNA-chromatin protein complexes in the context of gene regulation and 3D genome organization. Finally, the challenges of capturing and characterizing repetitive DNAs and transposable elements (TEs) genome-wide and studying their transcription and function are presented. A particular emphasis is given to the latest ultra-long read sequencing technologies and computational pipelines required to achieve full genome assemblies that include repetitive and mobile elements.

Admittedly, this special issue covers only a few of the many methodologies used in chromosome and genome biology. However, the articles in this issue offer a balance of historical and contemporary methodologies that are responsible for moving the field from largely microscopy-based observational experiments to high resolution genomic and functional studies. I hope that the selection of reviews presented here will offer readers new perspectives of chromosome biology and encourage them to explore the latest technologies in their own research.

Seven years ago, the late Professor Herbert MacGregor, founding editor of Chromosome Research, chromosome biologist, and expert in lampbrush chromosome structure and function (Gall 2018), and his Co-Editor-in-Chief Dr. Conly Rieder tasked me with assembling annual special issues comprised of reviews contributed by experts in chromosome and genome research. In the special issues that I have overseen, the journal has highlighted exciting topics in chromosome research, including non-coding RNAs (2013), avian genomics (2014), *Arabidopsis thaliana* cytogenetics and (epi)genomics (2014), chromosome and genome engineering (2015), repetitive DNA (2015), centrosomes (2016), genome organization (2017), transposable elements (2018), and ribosomal DNA (2019). Through the special issues, I have had the privilege to work with many outstanding scientists in their roles as guest editors and authors. This latest special issue on Methods in Chromosome and Genome Biology is no exception - I enjoyed working with all of the authors and thank them for their excellent contributions. It is also a special issue swan song for me, since as of January 1st, I have moved to the role of Co-Editor-in-Chief of the journal. Going forward, Chromosome Research will continue to publish reviews as part of themed

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special issues, however, we also encourage submission of review articles for consideration in regular issues or as part of the Waldeyer-Flemming Special Collection. We look forward to many more years of publishing new discoveries and amazing insights into the fascinating area of chromosome biology!

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Abbreviations

ATAC-seq	Assay for Transposase-Accessible Chromatin sequencing
bp	basepair
BrdU	Bromodeoxyuridine
Cas9	CRISPR-associated caspase 9
ChIP	Chromatin immunoprecipitation
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CUT&RUN	Cleavage Under Targets & Release Using Nuclease
DamID	DNA adenine methyltransferase identification
DNA	Deoxyribonucleic acid
DNase-seq	DNase I hypersensitive sites sequencing
DRIP-seq	DNA-RNA Immunoprecipitation sequencing
GRO-seq	Global nuclear run-on sequencing
FISH	Fluorescence in situ hybridization
lncRNA	long non-coding RNA
PRO-seq	Precision nuclear run-on sequencing
RBP	RNA binding protein
RDIP	RNA-DNA Immunoprecipitation
RNA	Ribonucleic acid
RNA-seq	Ribonucleic acid sequencing
seq	sequencing
SNP	Single Nucleotide Polymorphism

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SNS-seq	Small Nascent Strand sequencing
SV	Structural Variant
TALE	Transcription Activator-Like Effector
TE	Transposable Element
ZFN	Zinc Finger Nuclease

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