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Exploring the cell nucleus: From chromosome structure to single-cell omics

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The cell nucleus is a fascinating organelle. The myriad of fundamental processes that ensure that the genetic information is read correctly and faithfully transmitted provides a rich subject of research across time scales and model systems. Topics ranging from chromosome 'metascale' organization and division, enabled by molecular machineries like the kinetochore, to the organization of the genome that ensues cell division during interphase, which enables fine tuning of gene regulation, are subjects that we cover in the 'Cell Nucleus' issue of *Current Opinion in Cell Biology*.

Chromosome organization

During the M-phase of cell division, cell nuclei are condensed and mitotic chromosome structure is formed. Answering how chromosomes are organized and create mitotic structures is an important issue. Structural maintenance of chromosomes (SMC) protein complexes such as Cohesin and Condensin have key roles for chromosome organization. These SMC complexes have a loop extrusion activity for chromatin or DNA *in vitro*, and this activity of SMC complexes is considered important for chromosome construction in this field. However, this alone does not explain chromosome construction. Hirano and Kinoshita introduce a mechanism of chromosome construction that cannot be explained by the loop extrusion activity of SMC complexes. Cohesin, an SMC protein, is thought to attach the two sister chromatids, but its molecular mechanism remains unclear. *In vitro* reconstitution of the Cohesion complex is essential to tackle the problem, and Murayama describes the recent advances of Cohesin complex reconstitution and its biochemical properties. Various kinases may also be important in the regulation of chromosome construction and cell cycle progression; Aurora B kinase, a member of the chromosome passenger complex (CPC), plays an important role in M-phase progression. Matsui et al. introduce how CPCs assemble at centromeres in M-phase.

Centromere/kinetochore organization

Various functional domains exist in mitotic chromosomes, and the centromere is the functional domain that plays an important role in chromosome segregation during M-phase. On centromeres, a protein complex called kinetochore is formed, which binds to spindle microtubules. Chromosomes bound to spindle microtubules via kinetochores are

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pulled and divided into daughter cells. Much progress of studies on centromere/kinetochore assembly has been made using cultured cells and model organisms in recent years [1]. Unexpectedly, centromere and kinetochore composition is surprisingly diverse between species. For example, centromeres/kinetochores are formed at a single locus on a chromosome in many organisms; however, centromeres in some insects and plants are spread over the entire chromosome, which is called a holocentromere. Marques and Drinnenberg describe the holocentromere architectures and discuss how such organisms acquired holocentromeres and how holocentromeres have had evolutionary consequences. The centromere/kinetochore also plays an important role in meiosis as well as mitosis. Meiosis separates homologous chromosomes by two successive divisions, meiosis I and meiosis II. Koch and Marston introduce the meiosis-specific organization of meiosis-specific kinetochores and explain how gametes are produced by meiosis.

Mitotic progression and aneuploidy

Mitotic progression must happen in a timely fashion. In normal cells, mitosis is completed in about 30 min, but mitosis can sometimes take longer for various reasons, such as spindle abnormalities and cell stress. Recent studies have shown that cells have a stopwatch (mitotic stopwatch) that monitors the time of mitosis, and Sparr and Meitinger discuss how the mitotic stopwatch monitors the length of mitosis and what cellular stresses and/or damages prolong mitosis. Prolonged mitosis and incorrect chromosome segregation often cause aneuploidy. Aneuploidy is a hallmark of cancer. Cao et al. discuss the profiles and consequences of aneuploidy in human cancer. They also describe aneuploidy-targeted cancer therapy. Centrosomes, the major microtubule-organizing centers, are also critical for proper cell division. Centrosome dysfunction also causes aneuploidy, so understanding their structure and function is important. However, centrosome biology remained limited until the last century due to technical limitations. In recent years, technical advances in proteomics, genetics, and microscopic imaging such as cryo-electron microscopy and expansion microscopy have allowed us to gain studies in this field. Camila Fernandes Mariano et al. summarize recent advances in centrosome biology.

Making chromosomes ready for cell division

Before the cell divides, the genome has to be duplicated. This process is highly regulated and relies on our genome to be faithfully replicated once and only once per cell cycle. The process of replication must occur both, within the highly regulated three-dimensional (3D) organization of the nucleus and in coordination with processes such as transcription. How DNA replication and transcription co-occur, in particular in the context of chromatin assembly, is reviewed by Segura et al. They posit that the organization of the genome in the 3D nuclear space is key to enable coordination of such processes. Indeed, the lack of coordination of transcription and replication can lead to collisions of their respective machineries, referred to as transcription and replication conflicts, which can alter the chromatin template and lead to genome stability [2,3]. Coordination of the replication programme is also linked to chromatin and 3D organization features. For example, during S-phase, genomic regions that replicate early tend to be located in A-compartments and locate in the nuclear interior [4]. Likewise, regions that replicate late tend to belong to heterochromatic B-compartments and show proximity with specific nuclear landmarks such as the nuclear lamina. Approaches to study DNA replication are discussed by van den Berg et al.

Nuclear landmarks and genome organization during interphase

Following cell division, the genome adopts an interphase configuration, whereby nuclear organelles and structural components act as references for genome organization and function. One such reference is the nuclear pore, which, in addition to ensuring nucleocytoplasmic transport, has been associated to genome regulation through specific interactions with active chromatin regions. The nuclear pore reforms at each cell division, and the structural components and dynamic changes of this process are reviewed by Dultz et al. The 3D folding of the genome, including specific cell-type regulatory long-range interactions, also re-emerges at each cell cycle upon the exit of mitosis. Lières et al. discuss recent insights into the enhancer mode of action, which have been illuminated by innovative single-molecule perspectives. Technological advancements in recent years have taught us that the physical properties of the chromatin and stochasticity in transcription factor binding are key factors to consider when studying enhancer function. Three-dimensional interactions between enhancers and promoters are also governed by insulators, but the looping model alone cannot account for experimental measures of their proximity, where nonlinear models bring additional parameters to better understand interphase nuclear organization in the context of gene regulation and its underlying enhancer-promoter function. Enhancers, insulators, and promoters are all cis-regulatory regions (CREs) that provide platforms of transcription factor binding sites, and they rely on their binding for their function. How the corresponding transcription factors recognize their CRE target and bind, even when wrapped around the nucleosomal organization of chromatin is discussed by Gómora-García et al. A specific type of factors recognized for their ability to recruit proteins that will open-up and remodel the chromatin template are pioneer transcription factors. Interestingly, binding of such pioneer factors can have genome-wide effects on chromatin features such as chromatin accessibility. How transcription factors like CTCF impact genome organization is also discussed by Gómora-García et al., in particular within topological associating domains (TADs). The (statistical) stability of TADs is also discussed by Liéres et al. and both reviews thus together provide a combined perspective of approaches and molecular players to understand gene regulation within the 3D interphase genome.

The emerging single-cell view of the nucleus

All nuclear processes occur within individual cells, and work over the recent decades has indicated an enormous heterogeneity between cells within a population, tissue, or organ with regards to transcriptional output and, to a certain degree, genome organization. Van der Berg et al. present an exhaustive review of current methodologies to study the nucleus using single-cell genomics. They discuss their adaptability and limitations and point toward future directions and the need of applying approaches that enable the study of different nuclear features simultaneously in single cells. Ranging from the state of DNA methylation to DNA damage and DNA secondary structure, van der Berg et al. discuss the value of multiomic measurements to contextualize DNA and chromatin features across cells and tissues.

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Appendix A. Supplementary data

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