【Grant-in-Aid for Scientific Research (S)】

Super-resolution imaging reveals chromatin domains and their regulation of cellular functions

Principal Investigator	National Institute of Genetics, Department of Chromosome Science, Professor MAESHIMA Kazuhiro　Researcher Number : 00392118
Project Information	Project Number : 24H00061　Project Period (FY) : 2024-2028 Keywords : genome, chromatin, live-cell imaging, superinsulation microscopy

Purpose and Background of the Research

●Outline of the Research

Our body is composed of approximately 40 trillion cells, and each cell nucleus contains two meters of genomic DNA. This thin string encodes the genetic information necessary for RNA and protein synthesis. Genomic DNA is wrapped around histones to form nucleosome structures (Fig. 1, left). Nucleosomes associated with other proteins and RNA are called chromatin. How are these long strings of nucleosomes stored in the cell nucleus, and how do they behave? These questions remain a fundamental issue in biology, even 70 years after the elucidation of the DNA structure.

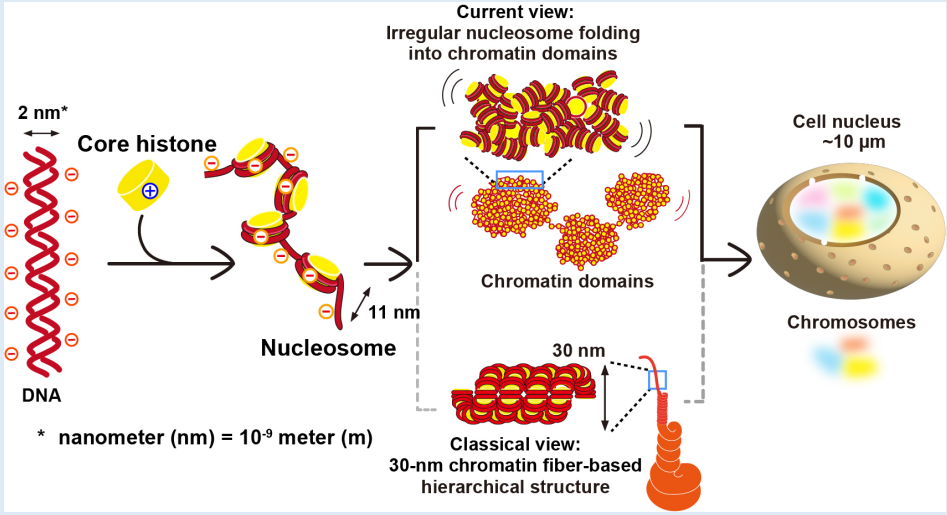

Figure 1. Schematic diagram of DNA, nucleosome, and chromatin domains. The new chromatin model is depicted in the upper, and the classical textbook model in the lower. Figure is modified from Ide et al., *BioEssays* 2023.

In the classical textbook model, nucleosomes form regularly arranged fibers with 30 nanometers in diameter, referred to as 30-nm fiber. This fiber is then folded into a helical hierarchical structure (Fig. 1, lower). However, recent findings, including those by Maeshima et al., have revealed no such regular hierarchical structure in human cells; instead, nucleosome fibers are folded irregularly (Fig. 1, upper) (Maeshima et al., *Curr Opin Cell Biol* 2019). This irregular folding suggests that nucleosomes are less physically constrained. Indeed, using single-molecule microscopy, we have revealed nucleosomes fluctuate dynamically like a liquid (Iida et al., *Science Adv* 2022; Nozaki et al., *Science Adv* 2023). Super-resolution microscopy has demonstrated that numerous fluctuating nucleosomes form clumps of approximately 200 nm in diameter, termed chromatin domains (Fig. 1, upper) (Nozaki et al., *Mol Cell* 2017; Miron et al., *Science Adv* 2020; Nozaki et al., *Science Adv* 2023).

This chromatin domain is thought to be a functional unit of genomic functions such as DNA replication and RNA transcription. It has also become clear that nucleosome fluctuations facilitate protein access to DNA within the chromatin domain (Hihara et al., *Cell Rep* 2012).

In this study, we aim to elucidate the physical properties of chromatin domains in living cells and further analyze how chromatin domains contribute to genomic functions, such as DNA replication and RNA transcription.

Expected Research Achievements

●A new super-resolution microscopy system

We will build a new super-resolution microscopy system by combining a structured illumination microscope, which can visualize the chromatin domains, and a single-molecule microscope (Fig. 2).

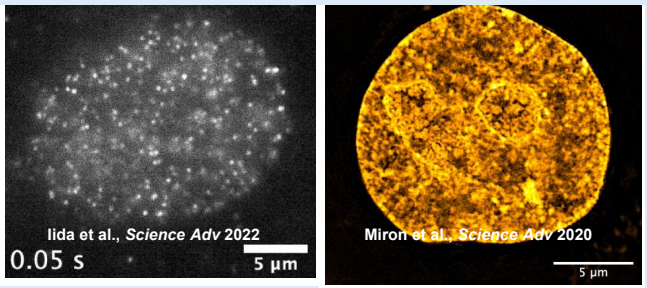

Figure 2. Left: Each dot represents a single nucleosome. By precisely determining the center of each dot, the nucleosome motion can be measured with an accuracy of less than 10 nm. Right: A structured illumination microscopy image. Chromatin forms clumps (chromatin domains).

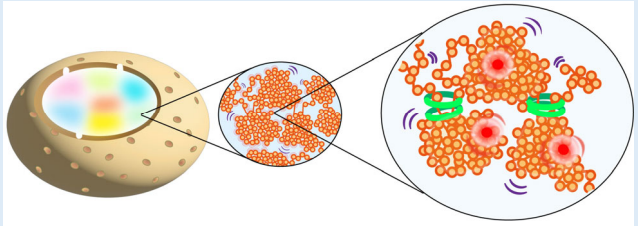

Figure 3. Structures of euchromatin and heterochromatin domains in the cell nucleus and the movement of nucleosomes within the domains.

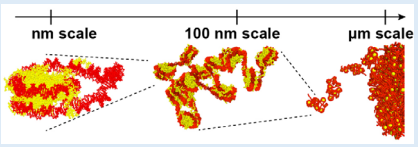

Figure 4. Multi-scale chromatin modeling: From nucleosomes to chromatin domains, the behavior of nucleosomes is reproduced computationally.

●Euchromatin- and heterochromatin-specific labeling methods

Euchromatin is an actively transcribed gene-rich region, and heterochromatin is an almost inactivated gene-poor region. We will develop a method to specifically label euchromatin and hetero-chromatin with fluorescent markers in living cells.

●Physical properties of euchromatin and hetero-chromatin domains

We will observe the structures of euchromatin and hetero-chromatin domains, as well as the movement trajectories of nucleosomes and protein molecules inside and outside these domains in living cells, using the new microscopy system (Figs. 2 and 3). By incorporating computational modeling (Fig. 4), we will then analyze the physical properties of chromatin domain and mechanisms behind their formation.

●Regulation of cellular functions by chromatin domains

We will investigate how changes in the physical properties of chromatin domains drive cellular function/dysfunction, as well as cell differentiation.

Homepage Address, etc.	HP https://maeshima-lab.sakuraweb.com Researchmap https://researchmap.jp/7000007143?lang=en Twitter X @kazu_Maeshima