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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Integrated analysis of chromatin conformation by Hi-C and electron tomography

研究課題名（英文）Integrated analysis of chromatin conformation by Hi-C and electron tomography

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：ゲノムが物理的にどのように機能しているかは、依然として不明な点が多い。このプロジェクトでは、電子顕微鏡を使ってゲノムの特定部分の3D画像を取得し、その仕組みを観察した。EMは解像度が非常に高く、細部のイメージングが可能であるが、重要な生体分子をEMで可視化するためには、金属による染色や標識が必要である。
私たちは、金属ビーズで制御性生体分子を標識する方法を開発し、DNAを染色した。このプロジェクトの最終段階として、これらのサンプルをさまざまな角度からEM画像を撮影し、これらの画像から遺伝子制御の3Dモデルを再構築する。3D画像の解析にはAIを使用し、画像内のオブジェクトを識別・分類する。

研究成果の学術的意義や社会的意義
The physical mechanisms by which genetic variants contribute to disease remain unclear. 3D imaging of the cell nucleus shows where important biomolecules are physically located relative to each other. Visualizing the genomic machinery helps to unravel the biophyical mechanisms underlying disease.

研究成果の概要（英文）：How the genome functions physically remains to a large extent unknown. In this project, we used electron microscopy to image the genome and observe its machinery. While electron microscopy has a very high resolution allowing imaging of small details, it requires staining or labeling with metal to make important biomolecules visible in EM.
We developed methods to label regulatory biomolecules with metal beads, and stained DNA. As the final step in this project, we will take EM images of these samples from different angles, reconstruct a 3D model of gene regulation from these images, and identify the labeled biomolecules in the images.

研究分野：genomics, biophysics

キーワード：electron microscopy　tomography　3D genomics　promoters　enhancers　gene regulation

１．研究開始当初の背景

Background at the beginning of the research: Genetic information is stored in the human genome in the form of DNA in the nucleus of each cell. Functional genomics methods have created detailed atlases of the genome and catalogs of biomolecules, and have revealed global patterns of gene regulation using statistical methods. Similarly, genome-wide association studies have identified important disease-associated locations in the DNA by statistically analyzing and comparing the genomes of different people. But what is the biophysical reality behind the statistics that governs the observed patterns? Progress in molecular biology and its application in biomedicine often requires understanding the precise molecular mechanisms governing specific genes, for example to elucidate regulatory pathways in cell differentiation, or to identify crucial oncogenes for targeting by anti-cancer drugs in personalized medicine.
To enable detailed mechanistic studies of such essential regulatory mechanisms, in this project we applied electron microscope imaging to obtain a detailed view of gene regulation in the cell nucleus. We are particularly interested in promoters and enhancers, which are key regulatory elements in the DNA that control gene activation and repression. Promoters and enhancers are typically far from each other along the genome (Figure 1, top panel) but can be close to each other in 3D space and can interact with each other through chromatin looping (Figure 1, bottom panel), forming the physical basis for gene regulation.
The 3D structure of the genome can be investigated using sequencing methods, in which chromatin regions close to each other in 3D space are ligated to each other and sequenced. However, such methods are expensive, limited in resolution, typically average over many (millions) cells, and do not identify any regulatory biomolecules that act on the chromatin and affect gene regulation. By applying electron microscope imaging, our aim is to obtain a complete 3D picture of the physical basis of gene regulation in individual nuclei (Figure 1, bottom panel).

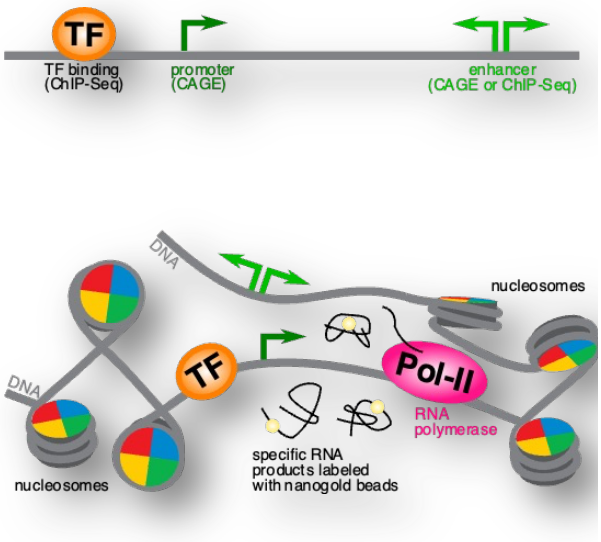

Figure 1. (top panel) Functional genomics provides annotations of functional elements on the genome represented as a straight line. (bottom panel) The 3D conformation of the genome in the cell nucleus provides the physical basis for gene regulation by placing specific regulatory elements close to each other in 3D space, allowing them to interact.

２．研究の目的

Purpose of research: In electron microscopes, an image is obtained by passing a beam of electrons through a sample, and using an electron detector to count the electrons that passed through or were scattered by heavy atoms in the sample. As DNA and other biomolecules consist of light atoms, they do not scatter electrons efficiently and appear to be almost transparent to the electron beam. It is therefore essential to stain DNA with heavy (metal) atoms to improve its contrast in the electron microscope, and also to label the regulatory biomolecules of interest using metal beads for easy observation in the electron microscope. The purpose of our project is to stain DNA and label promoters, enhancers, and specific RNA molecules and obtain an integrated 3D image of the biophysical basis of gene regulation.

３．研究の方法

Research method: (1) We used the ChromEMT protocol to stain the chromatin with heavy metal. This protocol activates a dye localized to the DNA double helix using light of the appropriate color, which stimulates polymerization of diaminobenzidine in the local vicinity of the DNA double helix. The heavy metal osmium is then added, which binds to the diaminobenzidine polymer and thereby created a local enrichment of heavy metal around the DNA double helix that is easily observed in the electron microscope.
(2) Next, we designed primary probes against specific RNA molecules, and employed a biotinylated secondary probe targeting a generic region of the primary probe. An antibody with an attached gold bead was used to target the biotin on the secondary probe. The gold bead had a very small size to allow it to enter the nucleus. Gold enhancement was used to increase the size of the gold bead in-situ, making it visible in the electron microscope and thus revealing the position of the targeted RNA molecule in the electron microscope images.
(3) Likewise, we use primary antibodies against specific histone modifications, and a secondary antibody with a gold bead attached to label and identify specific histone modifications in the electron microscope. We targeted the histone modifications H3K4me3, H3K27Ac, and H3K27me3 to identify active promoters, active enhancers, and silenced genomic regions, respectively, in the electron microscope images.

４．研究成果

Research results:
(1) We successfully stained chromatin by applying the ChromEMT protocol in MCF-10A cells. Stained chromatin is visible as dark areas in the image (Figure 2).
(2) We labeled the non-coding RNA XIST, which is involved in inactivation of the X chromosome in females by interacting with the inactive X chromosome. Our electron microscope images show the Barr body containing the inactive X chromosome as a dark area in its typical location in the cell nucleus adjacent to the nucleolus (Figure 3). We observe a high concentration of XIST in the Barr body, indicating that the labeling of XIST was successful. In future experiments, we plan to use the same approach to label other RNAs, including messenger RNAs to be able to identify the location of specific genes in the electron microscope images.
(3) Next, we labeled histone modifications H3K4me3, H3K27Ac, and H3K27me3. Figure 4 shows an example where we used a primary antibody against H3K27ac, and a secondary antibody with a nanogold bead attached. As expected, we find a high concentration of H3K27Ac in the cell nucleus, where active enhancers characterized by H3K27Ac are located, but a low background signal in the nucleolus and in the cytoplasm, which are devoid of enhancers.
With these three elements, we can now stain chromatin as the structural basis of gene expression, we can identify promoters, enhancers, and silenced chromatin by labeling the corresponding chromatin marks, and we can identify specific RNA molecules to locate specific genes in the electron microscope images. This month, we will proceed to tomography by taking images of the generated samples at different angles in the electron microscope, and combining these images into a 3D model of nuclear regions with the relevant regulatory biomolecules labeled. In future sample preparations, we will label both promoters and enhancers at the same time, distinguishing them using different sizes of

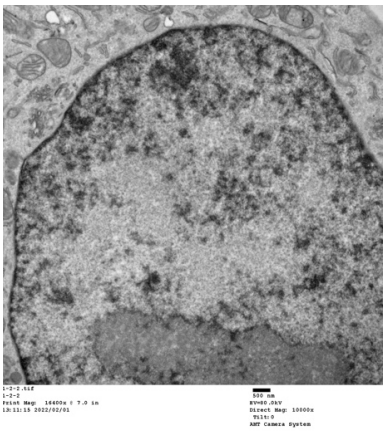

Figure 2. Transmission electron microscope image of a MCF-10A cell nucleus in which we used the ChromEMT protocol to stain chromatin by osmium, appearing as dark areas in the image.

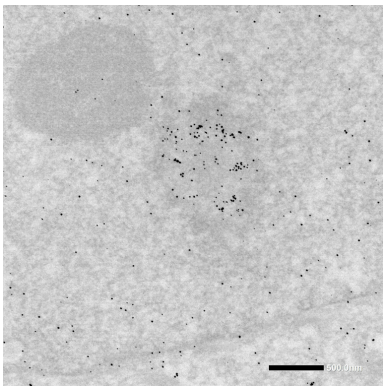

Figure 3. Transmission electron microscope image of a section of the nucleus of an MCF-10A cell. The dark region in the upper left corner is the nucleolus; the dark region near the center of the image is the Barr body containing the inactive X chromosome. XIST molecules labeled using nanogold beads are observable as black dots in the Barr body.

gold beads after gold enhancement, revealing their position in 3D space relative to each other in transcription factories. To our knowledge, this will be the first time that the location of these key regulatory regions in the genome are directly imaged as a 3D model of gene regulation.

While electron microscope tomography experiments are rare in Japan, in other countries (including the United States and Germany) such methods are currently under active development for various purposes. We have the unique goal of using electron microscope tomography to study gene regulation and its biophysical basis in the cell nucleus. For our purpose, it is essential to be able to identify the specific genomic locus being observed in the image. We plan to use a combination of light microscopy and electron microscopy, with colored in-situ hybridization probes to identify the rough localization of specific genes in the light microscope, followed by imaging the identified region at high resolution in the same sample in the electron microscope.

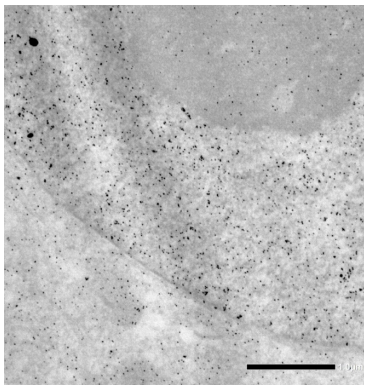

Figure 4. Section of the nucleus of an MCF-10A cell after labeling the H3K27Ac histone modification as a marker for active enhancers. Active enhancers are abundantly observed as black dots inside the nucleus, with little background in the nucleolus (upper right) and in the cytoplasm (lower left).

As the number of different objects that can be labeled with gold beads of different sizes is limited in practice, further progress will depend on our ability to identify important biomolecules directly from the image without labeling. Methods have been developed using convolutional neural networks to identify large biomolecular complexes such the ribosome from electron microscope tomography images. Similar approaches may be applied in the future to identify RNA Polymerase II and other large complexes in our images of the cell nucleus. For this purpose, we plan to take advantage of sequencing data that reveal the location of RNA Polymerase II and regulatory biomolecules on the genome, and to perform an integrated analysis of the sequencing and imaging data to generate a fully annotated 3D model of gene regulation.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計5件（うち招待講演　1件／うち国際学会　0件）

１．発表者名 Michiel de Hoon, Saumya Agrawal
２．発表標題 Functional genomics and its application in biomedicine
３．学会等名 Seminar, Kyoto University, Japan（招待講演）
４．発表年 2023年

１．発表者名 Michiel de Hoon
２．発表標題 Functional genomics and its application in biomedicine
３．学会等名 Seminar, McGill University, Canada
４．発表年 2023年

１．発表者名 Michiel de Hoon, Saumya Agrawal
２．発表標題 Functional genomics and its application in biomedicine
３．学会等名 Seminar Jackson Laboratory, Connecticut
４．発表年 2023年

１．発表者名 Michiel de Hoon
２．発表標題 Functional genomics for disease applications
３．学会等名 Seminar, University of Toronto, Canada
４．発表年 2023年

１．発表者名 Michiel de Hoon
２．発表標題 Functional genomics for disease applications
３．学会等名 Seminar, Cornell University, New York
４．発表年 2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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７．科研費を使用して開催した国際研究集会

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８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関