Impact Objectives

- Establish a model to demonstrate physical and mathematical properties of chromatin fibres in fission yeast cells
- Validate our chromatin structure dynamics model
- Apply our model-based analyses to predict the defects in gene regulation

Inside the nucleus

***Professor Shin-ichi Tate**, from the **University of Hiroshima**, seeks to uncover exactly how chromatin transforms inside a cell's nucleus*

Would you please give us a brief overview of your project?

We currently exploring the physical properties of chromatin (a DNA-protein fibre complex used to make DNA, packed inside a tiny nucleus) structural dynamics in cells that remain in the interphase stage of cell cycle using the data collected by time-resolved confocal fluorescence microscopy. One of my favourite parts of the research is relating chromatin's 'fractal structure' to a mathematical meaning. Given this mathematical property, we can calculate the spatial size of the movable area of a specific gene position inside the nucleus. In another interesting example, we also characterised the dynamic properties of chromatin in cell nuclei through the trajectories of fluorescent-labelled gene loci. From the hidden Markov analyses of the trajectories, we confirmed that chromatin fibre adopts only limited numbers of conformations, typically four to six states. In cell nuclei, chromatin fibre transfers between the discrete conformational states. We have collected over 100 gene loci in fission yeast, all of which consistently show that chromatin has discrete conformations. Based on the results, we are now building the structural models to show the forms that chromatin can take within the cell nucleus of fission yeast.

What are the key objectives of this study and what is the timeframe you are working to?

Establishment of the model to reproduce the experimental data and physical and mathematical properties of chromatin fibres in fission yeast cells is what we hope to accomplish within a couple of years. The genomics, epi-genomics and proteomics data are already available publicly. In combination with those data, we can validate our chromatin structure dynamics model to see if the model explains the gene regulatory events. Once we have qualified the model, we will apply the model-based analyses to predict various defects in gene regulation that cannot be readily explained without considering the higher-order architecture of chromatin. We hope to achieve the application of this model within the next three to four years.

How have you approached the challenge of achieving your project goals?

The challenge we had was in collecting the trajectories of each fluorescence-labelled gene locus. The data tracking was quite important in collecting the data, but the current computer software cannot precisely track the fluorescence point in the microscopic images. We tried to improve the tracking programme but failed to get much improvement. Eventually, one of our postdocs very patiently collected the tracking data for over 100 gene loci. Without his efforts, we could not have proceeded to the next stage of the work.

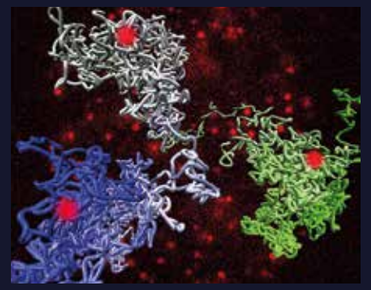
How do the results you've achieved so far match up with your expectations? Have there been any surprises?

What we did in this project gave us several surprising findings that have never been previously reported in conventional biological research. These include the fractal structure of chromatin and the fact that it has a limited number of discrete conformational states, as noted above. Besides those mentioned, we have published various theoretical papers: one of which describes the theory to elucidate the energy dissipation associated with molecular diffusion inside the cell, as applied to chromatin dynamics. The chromatin modelling based on the genome-wide data on chromatin in fission yeast is now underway, but the data will give what we anticipated. As an extension of the project, we have started the research with electron microscopy to directly observe the chromatin structure inside the nucleus on a nano-scale. This new project is now running well and will be used to validate the chromatin models built from the genome-wide chromatin dynamics data collected by fluorescence microscopy.

Unveiling the architecture of chromatin

By understanding how chromatin remodels itself, ***Professor Shin-Ichi Tate*** *and his team from* ***Hiroshima University*** *will be able answer the age-old question of how chromatin and protein transcription factors work together to regulate genes*

The field of molecular biology has provided great insights into the structure and function of key molecules. Thanks to this area of research, we can now grasp the biological details of DNA and have characterised an enormous number of molecules in massive databases. These 'biological periodic tables' have allowed scientists to connect molecules to particular cellular events, furthering scientific understanding of biological processes. However, molecular biology has yet to answer questions regarding 'higher-order' molecular architecture such as that of chromatin. Chromatin is the molecular material that serves as the building blocks for chromosomes, the structures that carry an organism's genetic information inside of the cell's nucleus. Understanding the physical properties of chromatin is crucial to developing a more thorough picture of how chromatin's structure relates to its key cellular functions. Moreover, by establishing a physical model of chromatin, scientists will be able to open the doors into the true inner workings of the cell nucleus.



Dynamic nucleosome movement and topological chromatin domain found in living human cells

Professor Shin-ichi Tate and his team of researchers at Hiroshima University's Research Center for the Mathematics on Chromatin Live Dynamics (RcMcD) are attempting to do just that. Through a five-year grant funded by the Platform for Dynamic Approaches to Living Systems from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Tate is aiming to gain a clearer understanding of the structure and dynamics of chromatin. His team includes theoretical physicists, mathematicians and experimental researchers, all of whom offer diverse backgrounds, scientific viewpoints and project ideas. With this interdisciplinary team, Tate can expand existing research past traditional biological knowledge and, in doing so, facilitate scientific understanding of the biological phenomena that is chromatin.

THE VITAL CHROMATIN

In all eukaryotes or organisms whose genetic information is stored in the form of DNA, DNA is tightly wound into a complex called chromatin. Chromatin has a number of primary functions within the cell, including packaging DNA into a more compact shape inside the nucleus, reinforcing DNA during mitosis (the process of cell division), preventing DNA damage and controlling gene expression and DNA replication. Of particular interest to Tate's team is chromatin's role in gene regulation. During gene regulation, specific genes can only be expressed if they are accessed by proteins called transcription factors. In chromatin's default state, the DNA is carefully coiled and protected with limited accessibility to these proteins. Therefore, when gene regulation occurs, the chromatin must 'open' in a process known as chromatin remodelling, thereby allowing the transcription factors to reach their DNA destination.

So far, molecular biologists have been able to use microscopes and other genome-based approaches to view the chromatin's three-dimensional structure and its dynamic structural rearrangement. However, Tate explains, 'We still lack the consolidated views regarding chromatin structure and dynamics on which protein-DNA interaction happens.' In other words, scientists still do not understand exactly how the higher-order architecture of chromatin changes forms, nor how it works with the transcription factors to transcribe specific genes at the appropriate time. Uncovering the methodology behind this biological process requires a new and innovative approach. Previous attempts to understand chromatin remodelling have used a 'bottom-up' design, where information is processed by piecing smaller systems together as a means to understand a larger, more complex system. Instead, Tate aims to directly explore chromatin using data from fluorescence microscopy, in which images are viewed using light that has been emitted from the object itself, along with physical modelling using the mathematical properties of chromatin.

To do this, the lab is using *Schizosaccharomyces pombe*, otherwise known as fission yeast. Used in traditional brewing, fission yeast is also well-known by molecular biologists as a model organism for studying basic cellular principles. This organism contains one of the smallest number of genes of any eukaryote and has only three chromatin fibres. Additionally, fission yeast's cylindrically-shaped cells make for easy viewing under a microscope. To quantitatively analyse chromatin remodelling, Tate and his team labelled eight gene loci within the fissure yeast chromatin. A gene locus is the fixed position of that gene on a chromatin structure and it is very useful in mapping the location and movement of a gene. They then tracked the movement of the genes as the chromatin underwent remodelling in an attempt to further understand the movements and dynamic structure of chromatin.

> *'Chromatin fibres within the cell nucleus are allowed to take a limited number of discrete conformations. Each specific chromatin conformation may have a specific role in gene regulation'*

A NEW ARCHITECTURAL MODEL

The data from this pilot study provided new information regarding the distinct conformational stages of chromatin in the nucleus. 'We have found unexpected behaviour of chromatin fibres in fission yeast cell nuclei,' Tate explains. 'Chromatin fibres within the cell nucleus are allowed to take a limited number of discrete conformations. Each specific chromatin conformation may have a specific role in gene regulation.' This pilot data served as the foundation for a more extensive study where Tate's team painstaking analysed the trajectories of over 100 labelled gene loci. With the help of four technical staff members who constructed each individual yeast cell with their properly-labelled genes, the team collected time-lapsed images of the cells. Results were frequently exchanged between the theoretical and experimental teams to confirm proper statistical analysis of the gene locus trajectory.

The next step for the team is to focus on creating a physical model of chromatin. Luckily, 'The chromatin modelling based on the genome-wide data on chromatin in fission yeast is now underway,' Tate states. To validate the chromatin model, the team is also using electron microscopy, where a beam of electrons is used to create an image of a specimen. This will allow the researchers to observe the chromatin structure inside the nucleus on a nano-scale.

REVEALING THE ANSWER

Moving forward, Tate hopes to continue to spread the word about chromatin structure and dynamics. Currently, the lab has created the 4D nucleome programme to facilitate international studies surrounding chromatin. The programme's goals are to understand the principles behind the 3D organisation of the nucleus in space and time. Developing this information at an international level will help scientists rationally explain the experimental observations that have that have been made regarding chromatin. The establishment of a chromatin model will finally clarify its higher-order architecture and unveil how this key molecule cooperates with gene regulatory proteins in the nucleus.

With this research, scientists can move one step closer to understanding the relationship between chromatin organisation and function in both normal and diseased cells. Understanding gene regulation on that level could help scientists stay one step ahead of dysfunctional chromatin alterations and subsequent disease development, thus contributing to the future of disease treatment.

Project Insights

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BIO

Professor Shin-ichi Tate was awarded his PhD from the University of Tokyo in 1989, after which he worked at many illustrious institutions including the Tokyo Metropolitan Institute of Medical Science, the Tokyo Metropolitan University and the Japan Advanced Institute of Science and Technology's (JAIST) New Material Center. He has served as Visiting Scientist at the Swiss Federal Institute of Technology (ETH) and the National Institute of Health (NIH). Tate was Research Director at the Biomolecular Engineering Research Institute (BERI) between 2002 and 2006 and has since been a professor at the Hiroshima University, Department of Mathematical and Life Sciences.

RcMcD Research Center for the Mathematics on Chromatin Live Dynamics

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