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PEOPLE & IDEAS

Kazuhiro Maeshima: Excitement under the microscope

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Maeshima investigates the higher order structures and dynamics of chromatin.

Kazuhiro Maeshima was first introduced to microscopy in his second year of elementary school when a school friend received a toy microscope. Intrigued, Maeshima asked his father, an industrial chemist, to buy him his own microscope and teach him how to use it. Maeshima characterizes this as his first attempt at live-cell imaging and says, "I looked at a beautiful transparent body of a water flea swimming under the microscope. A lot of fun! Since then, I have been very much fascinated with the small cell world!" Nowadays, Maeshima is still using microscopes but has focused them on exploring the structure of chromatin and the motion of nucleosomes.

We contacted Maeshima to take a closer look at his scientific journey so far.

Where did you grow up?

I grew up in Nara, which was an ancient capital of Japan ∼1,400 yr ago. Around me, there were not only beautiful temples and shrines but also many ancient ruins and remains. I often played around the ruins and imagined how ancient people survived living there. So, I wanted to be an archeologist when I was a small child. Though I became a scientist, I love to visit ruins all over the world and still hope to dig up ancient remains after my retirement.

Where and with whom have you studied?

As an undergraduate at the University of Tsukuba, I became interested in DNA. Then I studied DNA–protein interactions using the RAD51 recombination protein during my PhD in the laboratory of Toshihiro Horii at Osaka University. But, to be honest, I was frustrated and wanted to work on something much more exciting. What would be a more interesting subject to pursue? I wondered how DNA is organized in the cell and how it works there. Then I decided to work on the higher-order structures of DNA–protein, chromatin and chromosomes, and joined Ulrich Laemmli's laboratory in Geneva, Switzerland, as a postdoc. Uli is very famous for his SDS-PAGE system and beautiful chromosome structure model (radial loop scaffolding model), and is amazingly creative and original. He loved bench work and taught me how to take beautiful images of chromosomes. Much later, I realized he really broadened my mind and perspective and showed me how he freely (and radically) thinks things over. After five fruitful years in Geneva, I came back to Japan in 2004 to join Naoko Imamoto's laboratory at RIKEN and learn about the nuclear structure, a shell for chromatin. Since 2009, I have been working at the National Institute of Genetics (NIG) in Mishima, Japan, as a professor. NIG is a small but very nice institute, with plenty of academic freedom. I am quite happy!

> "One of my colleagues told me, "Kazu, don't worry, be happy!" I think this is most important for a research career."

What initially drew you to the chromatin field?

I was interested in what chromatin and chromosomes are like in living cells. When I started to study chromatin around 2000, a famous textbook model was that the nucleosome fiber, where genomic DNA is wrapped around core histones, formed a regular "30-nm chromatin fiber" shaped like a zigzag or solenoid. It was thought these 30-nm fibers fold into a hierarchy of larger fibers, or radial loops, in the cell. Of course, as

Kazuhiro Maeshima in front of the NIG main entrance. Image courtesy of Kazuhiro Maeshima.

everybody did at that time, I also believed this model because I observed nice 30-nm fibers in mitotic chromosomes in my own EM work in Geneva!

To see how chromatin fibers are organized, I tried to trace them in the mitotic chromosome by EM tomography, but this did not work well because the fiber organization was too complicated. It is reasonable when I think about that in retrospect. But at that time, I felt at a loss and started to collaborate with Mikhail Eltsov, who is an EM expert. We hoped to see real chromosome organization by cryo-EM, in which cells can be observed in a near-living state. I also began a structural study of mitotic chromosomes by small angle x-ray scattering (SAXS) at SPring-8 to detect possible regularity or periodicity in the chromosome.

In the cryo-EM images, we could not see any regular structures with ∼30 nm size (2). The chromosomes looked quite

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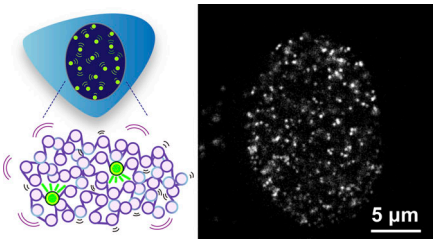

Nucleosomes are sparsely labeled with fluorescent dye (left) and each dot represents a single nucleosome (right). See Videos 1–4 in Nagashima et al. (1).

homogenous, consistent with earlier cryo-EM work by Jacques Dubochet. However, we observed an ∼30-nm peak in mitotic chromosomes by SAXS (3). I was puzzled to see our two studies produce these inconsistent results, though both reproduced older studies by the Dubochet, Langmore, and Paulson groups. After spending a year solving this puzzle, we finally found that the 30-nm peak by SAXS came from ribosome particles stuck to mitotic chromosomes with a spacing of ∼30 nm. Once the contaminating ribosomes were removed, the 30-nm peak by SAXS disappeared and so did the evidence for the 30-nm fiber chromatin model (2, 3).

We were very happy to see that our cryo-EM and SAXS studies finally converged (2, 3). I was thus convinced that chromatin is rather irregular and that the 30-nm fiber is not the basic structure. Many laboratories are providing new data that support this. So why have we seen pictures of 30-nm chromatin fibers in textbooks for so long? Because the chromatin was in low salt, which could make it a regular 30-nm fiber (4). Chromatin is a negatively charged polymer and without salt, nucleosomes are repulsed by one another and the nucleosome fiber is extended. With a little salt, repulsion decreases and one nucleosome can bind to its neighbor and form the 30-nm fiber like zigzag or solenoid. If you add even more salt, repulsion goes away, and one nucleosome can bind to any nucleosome. The fibers become interdigitated with one another and form a droplet-like structure without 30-nm fibers (4, 5). We think this is the chromatin structure in the cell (4, 5).

We wondered if irregularly folded nucleosomes behave as a fluid, because they are less physically constrained. To address this, we wanted to visualize local nucleosome motion by single nucleosome imaging. But it was still not so easy to do that at that time because there are more than 30 million nucleosomes in a single nucleus. After a long struggle, using sparse nucleosome labeling and a special microscope called an oblique illumination microscope, we finally saw the nucleosomes swaying like fluid in the living cell (5, 6): ∼50 nm of movement in only 50 ms! Quite amazing! This nucleosome swaying might make it easier for the proteins moving around to reach their targets on the genome. We have been enthusiastic about this fluid-like nucleosome motion in living cells (5).

"We can statistically analyze individual nucleosome motion and extract more and more information from our motion data. Something like digging for treasure!"

What questions is your laboratory currently pursuing?

We are looking at how local nucleosome motion occurs across the whole genome in living human cells (5). The motion looks to be driven partly by thermal fluctuation but can be constrained by different proteins, such as cohesin. Cohesin captures chromatin fibers to make a loop, which constrains chromatin as loss of cohesin increases chromatin motion. Recently, we found another constraining factor is the transcription machinery including RNA polymerase II (1). We were quite excited about this finding—when a gene is activated, chromatin in the transcribed region is thought to be more open and dynamic. However, we found that this is not necessarily the case—inhibition of transcription globally increases chromatin motion. This suggests that the genome chromatin forms loose networks organized by the transcription machinery. I think this finding is also compatible with classical models of transcription factories or more recent models of liquid droplet formation by transcription-related factors.

Local chromatin motion should reflect how chromatin is organized in living cells, so we can provide new insight into how chromatin is structured by using our data. In collaboration with Masaki Sasai at Nagoya University, we can statistically analyze individual nucleosome motion and extract more and more information from our motion data. Something like digging for treasure! We are now focusing on how nucleosome motion changes with epigenetic modifications, chromatin protein knockdown, the cell cycle, and cell differentiation, etc. We very much welcome those who are interested in this line of work to join our laboratory.

What has been the biggest challenge in your career so far?

Challenging the textbook model of chromatin organization. Although it took us more than 10 yr, this model has been updated in some molecular cell biology textbooks and hopefully others will follow in the near future.

What is the best advice you have been given?

My postdoc supervisor Uli Laemmli advised me to practice free and radical thinking: look at things from a different angle. Doubt what people are doing. It is still possible a current trend is wrong!

Any tips for a successful research career?

When I was in Geneva, one of my colleagues told me, "Kazu, don't worry, be happy!" I think this is most important for a research career. Of course, other factors such as persistence are also important.

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Maeshima laboratory members at the rooftop of NIG with a view of the Izu mountains. Image courtesy of Kazuhiro Maeshima.