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PEOPLE & IDEAS

Sachihiro Matsunaga: FISHing the nuclear architecture of plant cells

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Sachihiro Matsunaga studies the nuclear structure and chromatin dynamics of plants.

Sachihiro (Sachi) Matsunaga often lost track of time when he was a kid, as he could spend hours observing nature. He learned how to grow plants from his mother and how to fish from his father, but he himself discovered the art of catching insects. Equipped with crayons and ink, Sachi started by drawing all kind of flowers and living beings and ended up by meticulously annotating their life cycle stages in his observation diary. It comes as no surprise that he joined the biology club in high school, where he did his first molecular biology project on bacteriophages. He quite enjoyed presenting his work in the school science fair, and that was when the idea of becoming a researcher hit him.

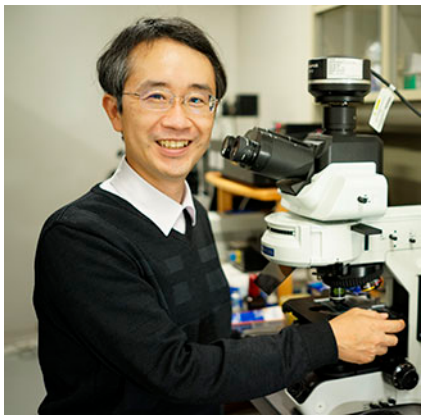
During his undergraduate at the University of Tokyo, Sachi did research on tobacco BY-2 cells in the lab of Prof. Toshiyuki Nagata, who pioneered the engineering of plant protoplasts, which are wall-less plant cells with high regenerative capacity. He later became fascinated with cell biology and joined the lab of Prof. Tsuneyoshi Kuroiwa for his PhD at the same university, where Sachi studied plant sex chromosomes and identified sex-specific genes by means of FISH, which became his favorite technique at that time. He then did a postdoc on plant molecular cell biology and genetics with Prof. Shigeyuki Kawano at the University of Tokyo and with Prof. Sarah Grant and Prof. Jeff Dangl at the University of North Carolina at Chapel Hill. Sachi became principal investigator (PI) at the Tokyo University of Science in 2011. His lab focuses on plant nuclear structure and chromatin dynamics and the regulation of histone modifications. We chatted with Sachi to learn more about his scientific journey and future projects.

What interested you about plant nuclear architecture and chromatin dynamics?

Plants can easily regenerate organs and form an entire new organism from just a small piece of tissue, and some plant species can live up to thousands of years. We all know that plants cannot move quickly, so they must live under different environmental stresses. How plants acquire their regenerative capacity and adapt to tough conditions are questions still unresolved, and I think that epigenetic and chromatin dynamic regulation play a big role. In the past, we have shown that LDL3 (lysine-specific demethylase 1-like 3) erases epigenetic H3K4me2 marks in *Arabidopsis* during the formation of the callus—a mass of pluripotent cells—to prime for the expression of shoot genes and to repress root genes (1). We continue to study epigenetic priming in regeneration and speculate that environmental stresses can trigger epigenetic priming.

What are you currently working on?

We keep working on the nuclear structure and the regulation of chromatin dynamics in organ regeneration and in response to environmental challenges. We have recently discovered the mechanism that arranges centromeres within the nuclei of *A. thaliana* (2). During cell division in yeast, wheat, and barley, centromeres are pulled to opposite poles and cluster at one side of the nucleus in the daughter cells, retaining their position throughout interphase—this is what we call the Rabl configuration. However, nematode, human, and *Arabidopsis* cells can also display a non-Rabl configuration where centromeres are evenly dispersed within the nucleus. We found that, during late anaphase, condensin II functions in coordination with the LINC (linker of nucleoskeleton and cytoskeleton) complex to scatter centromeres around the nuclear periphery in *A. thaliana*. This centromere configuration is then stabilized in interphase by the nuclear lamina protein CRWN (CROWDED NUCLEI), which we recently characterized as a regulator of chromatin distribution in response to environmental stresses (3). One of the obvious questions we asked is whether



Sachihiro Matsunaga with his favorite fluorescence microscope. Photo courtesy of Prof. Yoshikatsu Matsubayashi.

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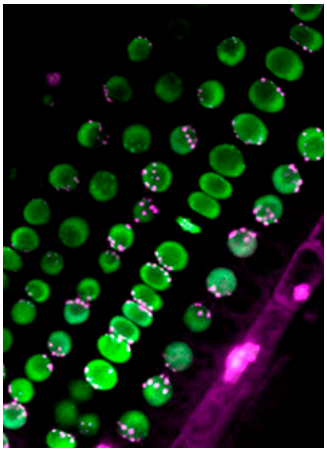

The root of a condensin II mutant of *A. thaliana* with nuclei (green) showing an either scattered, non-Rabl configuration or clustered, Rabl configuration of centromeres (magenta). Image courtesy of the Matsunaga lab.

non-Rabl configuration is essential for gene expression. It seems that the spatial distribution of centromeres doesn't matter for gene expression under normal conditions. But mutants with a Rabl conformation seem to be more sensitive to DNA damage. We're now trying to better understand how a scattered centromere conformation protects genome integrity as this could help us engineer non-genetically modified stress-resistant plants.

Is there a particular project you're pushing forward?

We're working hard on generating animal and plant hybrid cells, what I call "planimal cells," by using cell fusion and synthetic biology techniques to generate chimeric chromosomes derived from the genomes of both algae and animals (4). We chose the microalgae *Cyanidioschyzon merolae* because it has a relatively simple genome encoding for only one nucleus, one mitochondrion, and one chloroplast. However, fusing these microalgae with cultured animal cells has indeed been a challenge—and we are still in the process of characterizing these hybrid cells. We are investigating how nuclear structure, chromatin dynamics, epigenetic regulation, and gene expression change when the plant and animal genomes are intermingled in a single cell.

What is the idea behind planimal cells?

The inspiration stems from both the endosymbiotic events that occurred during plant evolution and the symbiotic relationships that exist today. Aquatic unicellular organisms that fed on cyanobacteria began to associate symbiotically with them—they retained cyanobacteria within their cells without digesting them. This is called primary endosymbiosis, and the symbiotic cyanobacteria eventually became chloroplasts, resulting in the origin of algae. Through secondary endosymbiosis, unicellular organisms engulfed algae, which were then transformed into secondary plastids, surrounded by three or four membranes. Representative examples include kelp, wakame seaweed, and euglena. There are also a few examples of endosymbiosis between algae and animal cells in nature, such as corals containing symbiotic brown algae. The idea behind the planimal cell project is to reproduce secondary endosymbiosis in the test tube. If successful, and in a yet distant future, animals with transplanted photosynthetic function could plausibly sustain life using energy produced by light irradiation—hypothetically, if they could endure a "dormancy" state with light irradiation, this could be the gateway to long-term planetary migration. But, more importantly, these planimal cells would serve us to better identify which cell biological processes are universal or specific of plants and animals.

What kind of approach do you bring to your work?

I start with a close observation of the phenomena—maybe a remnant of my childhood days when I used to spend an insane number of hours scrutinizing nature. Therefore, my approach is to observe each single phenomenon in detail by fluorescence live imaging and then use molecular biological methods to elucidate the underlying mechanisms.

It's often difficult to detect the signals of fluorescent proteins or probes in thick plant tissues; how do you do that?

This has been a challenge in the plant field for a while, mainly because auto-fluorescent pigments of plant tissues absorb the light and some cell components refract or reflect it. A few years ago, we developed a clearing method to make plant organs transparent, which we have just improved—iTOMEI (5). This makes easier to precisely determine the expression pattern of fluorescence proteins, the distribution of nucleic acids, and the location of organelles in individual cells in thick plant tissues, keeping the morphology. Although I can't become invisible, which was my childhood dream, I'm satisfied with having developed a method to make plants transparent.

And you would be one step closer to defining plant organs at the single-cell level, right?

If we can integrate imaging of cleared tissues with single-cell analysis, we would be able to understand the epigenetic and gene expression dynamics of individual cells in organs and explain phenomena that otherwise are incomprehensible by bulk analysis. I believe that the era of single cells in plant biology has indeed arrived, but we need to train researchers with analytical skills to perform single-cell analysis.

Your lab made it to the one-decade mark last year. Going back to your beginnings, what did you learn during your PhD and postdoc that helped prepare you for being a group leader?

Heartfelt communication between PI and lab member. My supervisors were always happy to discuss research with lab members, and they were also kind enough to help me with any problems I had in my personal life. I often draw strength from Dangl's words, "Accept any physical characteristic or illness as a phenotype and enjoy life," when I have to overcome difficulties in life. I recognize that the laboratory is a family and the PI sometimes takes the role of a parent.

Did your mentors give you any good advice?

Prof Kuroiwa used to say, "Discovery is everything. Rather than going into a research theme that someone else has established, study what you have discovered, no matter how small it may be. If a large pyramid stands out, each stone you pile up would not attract attention, but if you build a new pyramid, even if small, it will be noticed by future generations." He is also a fishing master and told me several times, "Whenever you are busy with your research and have no time, go fishing and listen to the voice of nature. Then you will get ideas for your research and overcome difficulties." I have followed these teachings and still do my research always thinking about my

The Matsunaga lab boat fishing at the summer lab retreat in 2015.

originality, and I always go fishing when I'm busy (laughs).

So, then I guess fishing is a hobby of yours?

Hehe, of course! Fishing is the best trigger for epigenetic priming to maintain my good health. Catching fresh fish, cooking them carefully, eating them in the company of family and friends, and enjoying the bounty of nature are my hobbies. Many biologists love fishing. I think the reason for this is that biological research and fishing have similarities. Expensive fishing tackle doesn't guarantee you catch the fish you aim for—the weather conditions may be against you! But, most likely, you would catch a fish, although it may be an unexpected one. Biological research, too, sometimes fails to produce excellent achievements even with superb facilities and abundant funds. But unexpected discoveries are made in biology, which is exciting. There is a Japanese saying for this: "Hyōtan kara koma" (often translated as "a horse coming out of a gourd").

To finish, could you give us some tips for a successful research career?

When I became PI, I set four ground rules for my lab, which I think are key for a successful research career. (1) Be serious: do your best to achieve your goal. (2) Be enthusiastic: focus on the research that you are really interested in. (3) Have your strengths: rediscover yourself and find your own strengths for the future. (4) Keep your professional spirit: spare no effort to raise your level.

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