

Unraveling what it means to be alive

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Biosystems Dynamics Research



Dive into BDR's intriguing research

Technique for monitoring deeper layers

Yakushiji (HY) ▶ I've noticed that the images of the area around the root of the hair are 3D movies. How are those movies made?

Morita (RM) ▶ We take multiple 2D images at different depths and stack those images to create 3D images. When we take those images at regular time intervals, you get something like a flip book, and this is what we call "time-lapse." This technique where we monitor live organisms or cells in time-lapse is called live imaging.

HY ▶ Some of the images show a deeper layer of the skin. How do you shine the light that deep?

RM ▶ We use fluorescent proteins to label the cells and structures that we are interested in. Then we use a two-photon excitation microscope to detect their fluorescence. As you can infer from the name, it applies two photons worth of energy to the fluorescent proteins to excite and make them fluoresce. Generally, long-wavelength light penetrates the animal body better. If we can use two photons to excite the fluorescent proteins, we can halve the energy of each photon, which in turn allows us to use a longer light wave. The longer the wavelength, the deeper the light can penetrate into an object.

HY ▶ So this is a similar concept to using near infrared radiation for non-destructive inspections of fruits.

RM ▶ Another benefit of using two photons is that it can reduce the damage caused by the light on a sample. This allows us to monitor a sample for a longer time period.

HY ▶ Longer exposure to light can deteriorate the samples?

RM ▶ Yes. We monitor them for a relatively long time.

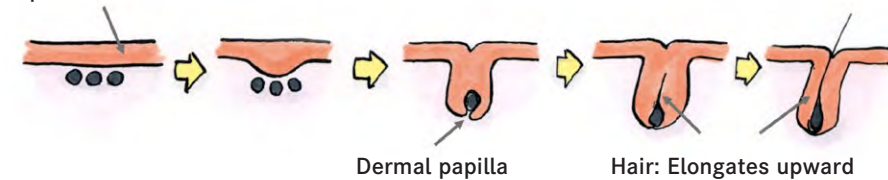
HY ▶ Do you have any moving images?

RM ▶ Sure.

Process of hair growth

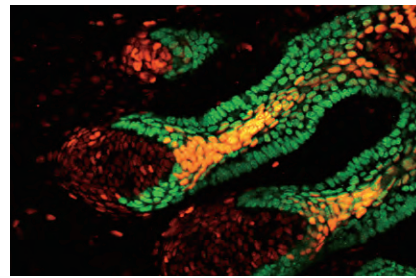
RM ▶ This is the live imaging of the early developmental stages of whisker hair follicles dissected out from a mouse embryo.

Epithelial tissue



Everything is done manually

The interview took place in January 2021, but since Ritsuko was in the middle of submitting her paper to the journal *Nature*. We held off on releasing this interview until the paper was published. Now that it's been published, we can share the interview and reveal the behind-the-scenes work of her paper.



See live-imaging movie of developing whisker hair follicles ▶



HY ▶ Wow. You can see the pores are being formed!

RM ▶ That's exactly what is being captured. The epithelial tissue invaginates downwards and wraps around the dermal papilla (mesenchyme). Then, this interaction with the dermal papilla causes the epithelial cells to proliferate and differentiate, and in turn, migrate upward and appear as hair on the surface of the skin. The slit from which hair appears is a pore.

HY ▶ Where do those cells come from?

RM ▶ Cells from the base to the middle region of a pore are undergoing differentiation. Cells can differentiate and transform into other cell types or proliferate to create copies of themselves. Within the hair follicles, there are areas where cells are actively differentiating and other areas where many cells are proliferating. In the proliferative areas, new cells are produced rapidly through cell division, but when those newly produced cells leave these areas, they start to slowly differentiate and eventually become hair.

HY ▶ Until it finally detaches and fall out.

RM ▶ That's right. The life cycle of hair is to grow, eventually fall out, and then grow again. During this hair cycle, the lower part of the hair follicle which produces hair regenerates over and over again. This repeating cycle is possible because the hair follicle has a population of stem cells capable of generating the constituent cells of hair follicles. This is a notable characteristic of the hair follicle as an organ.

HY ▶ So, something that didn't exist in the beginning, suddenly appears, grows and transforms... What's really happening there?

RM ▶ That's the very theme of our research. In order for organs like hair follicles to function correctly, the



Ritsuko Morita

Research Scientist
Laboratory for Tissue Microenvironment

After earning her doctorate degree from the Tokyo University of Science, she moved to RIKEN to start her research on the developmental process of hair follicles. Her hobby is virtual moving. She has always been fascinated by buildings and architecture, and enjoys looking at the pictures and floorplans on real estate listing sites and "moving in" to those places using her imagination. Her favorite site is R Real Estate.



Hideki Yakushiji

Business developer based in Kobe. He has a broad background in areas such as analytical chemistry, optics, biotechnology and IT. He is involved in a wide range of activities to assist in commercializing technologies and ideas born from academia, including setting up opportunities for idea sharing, finding investors, and strategic planning.

stem cells and differentiated cells need to be positioned correctly to work together. We analyzed how hair follicle stem cells and differentiated cells are produced during the fetal stages, by tracking cells in time-lapse images of hair follicle development. When we rewind those time-lapse images, we can find out where those differentiated cells were initially located. Based on that information, we aimed to find the developmental origin of the hair follicle stem cells.

HY ▶ Do the stem cells not stay in the same location?

RM ▶ In the early stages of hair follicle development, the epithelium starts off as a thin sheet, and only parts of this epithelium forms primordial hair follicles (the detailed mechanism has been omitted), which then invaginate, and only some of those epithelial cells become stem cells.

HY ▶ I thought that the stem cells were always present...

RM ▶ I can understand why you'd think that way. Actually, there hasn't been much analyses done on where the stem cells arise from.

With our live-imaging technique, we can capture the development of hair follicles from the beginning to the end where it produces hair at the single-cell level. If we can track the cells in the time-lapse movies and then rewind the images, we may be able to find where the prospective stem cells originally came from. This was the initial idea of our research.

It had to be done manually

HY ▶ As a person with no training in this field, I'd think that you would be able to automate the tracking of the cells in the images. Was this the case?

RM ▶ While it's true that automated tracking technology has been advancing, unfortunately it is still difficult to apply it to track the cells in the developing hair follicles that we handle; hence, it's all done manually.

HY ▶ Whoa! Why manually?

RM ▶ It's because of the sheer number of cells. The cells are also very densely positioned with virtually no space between the nuclei.

When imaging, the nuclei are labeled with a fluorescent protein so that we don't lose track of them when they divide. However, during early development, cell division is extremely fast and sometimes happens without producing much cytoplasm, leading to cells with the nucleus occupying most of the cell

volume. That's why the images look like the nuclei are tightly packed together.

The nuclei appear circular, but when there are two of them close together, it's hard to determine if there is one or two.

HY ▶ Unlike human faces, each nucleus doesn't have a unique feature so it must be hard to distinguish one from another.

RM ▶ Furthermore, sometimes even human eyes can't tell the difference. If human eyes can't distinguish them, then it's difficult for machines to distinguish them as well. Although artificial intelligence and other technologies are actively being developed and used, the technology that can distinguish cells with the quality we need is still not within our reach. That's why at present it's basically been done manually.

But if you move the videos back and forth, you can kind of see where and how each cell moved. We use that kind of information to aid our tracking work. It's the type of work that requires patience and perseverance.

HY ▶ Perseverance for sure.

The truth about the research

HY ▶ How many cells do you track?

RM ▶ The time and duration of tracking are different by images so it's hard to say, but the total number of cell lineages is probably over 1,200. They divide over the 24 to 72 hours we track them, so by the end of the analyses, it's probably around 2,500 cells.

HY ▶ Such time-consuming work.

RM ▶ Yes. It was a daunting task to do it all myself, so I got help from the technical staff in the lab as well as a college student who came to work part-time for a short-term to the lab. She left saying, "Research really is a repetition of long tedious work." I guess I was able to convey one hidden truth about research (laughing).

HY ▶ It's not always flashy and eye-catching.

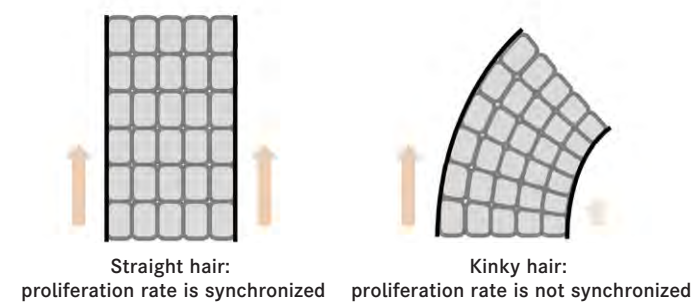
RM ▶ That's right (laughing).

HY ▶ All of that hard work gets summarized into a single diagram...

RM ▶ I think I nearly gave up about five times. I was so anxious about when I would be able to summarize my work into a paper that at one point I announced my resignation, saying, "I quit."

HY ▶ How do you overcome that defeated mindset?

RM ▶ The simple desire to publish the research that I



Postscript

Every time I speak to researchers, I am always blown away by the amount of painstaking and time-consuming work that they carry out—from generating thousands of patterns of cells and developing a system to measure them, to manually counting those cells... Congratulations on getting published in *Nature*! Hurray for tedious work!

started. So many people have helped me up to this point, and I knew that I would regret it if I didn't publish it.

HY ▶ I can understand that. For researchers, if they don't publish a paper, it's as if all of their work until that point didn't happen.

Mysteries of organ diversity

HY ▶ Since we are talking about hair, I'd like to ask you about my kinky hair. How come it doesn't grow straight?

RM ▶ It's probably easier to see in the movie, but hair is made up of multiple layers of different cells. When the cells, which differentiate to eventually become hair, move towards the skin surface, they gradually die and become keratinized. They are tightly packed together to become hair.

HY ▶ You mean like cuticles?

RM ▶ That's right. The layers of cells are lined up in a row and move up synchronously. There are about eight layers of cells that move up orderly as they are produced. And the pace at which they move up is in sync. Isn't that amazing?

If they move up synchronously, the hair grows straight. But if there is even a slight disruption to this pace, distortion occurs which causes kinky hair.

HY ▶ So my hair doesn't like collective behavior just like me...

RM ▶ Well, it does require tight teamwork...

HY ▶ Anyway, what are your plans for future research?

RM ▶ In this study, we discovered the developmental origin of epithelial cells and hair follicle stem cells. We also proposed a new model for hair follicle development (not covered in this interview) that explains how the morphogenesis of hair follicles and the correct arrangement of constituent cells within the tissue can occur simultaneously. I'm interested in studying how organ diversity is created through cell-to-cell communications in my next research.

HY ▶ Do you mean that there must be a certain spatial pattern in order for the cells to communicate with each other?

RM ▶ By analyzing the interactions between cells and between tissues, I think that we will be able to clarify the prototype of the patterns that generate morphological diversity, for example instructing to "make a long extension here" or "make a branching here" in organ morphogenesis.



In fact, we are tracking individual cells, one by one.
How is organ diversity created?

Read other interviews ▶

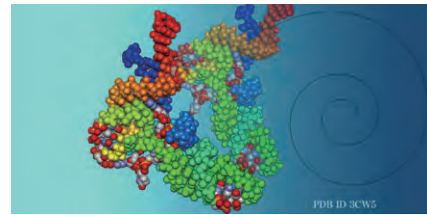


01

Creating an artificial genetic code

Over 50 years ago, biologists discovered the code for deciphering the genetic information of living organisms, and nowadays, they are capable of developing technologies to change the code itself. Yoshihiro Shimizu (Lab for Cell-Free Protein Synthesis) and his collaborators have developed an in vitro protein synthesis system that uses only in vitro transcribed tRNAs. By redesigning the anticodon sequence of some tRNAs, they were able to alter the relationship between codons and amino acids, yet generate functional proteins in vitro.

Hibi K, Amikura K, Sugiura N, et al. *Commun Biol* 3, 350 (2020)



02

Microbes are perhaps more manageable than we thought

The emergence of multidrug-resistant bacteria—pathogens that are resistant to various antibiotics—has become a worldwide concern. Chikara Furusawa and his team (Lab for Multiscale Biosystem Dynamics) developed an automated robotic cell culturing system and conducted a large-scale experiment to examine the evolutionary dynamics of *E. coli* bacteria in acquiring drug resistance. Their findings indicate that the likely adaptive trajectories for viruses and bacteria might be fewer than previously thought, and has implications for tackling the superbugs crisis by allowing researchers to predict in advance how drug resistance will develop and aid in developing countermeasures.

Maeda T, Iwasawa J, Kotani H, et al. *Nat Commun* 11, 5970 (2020)

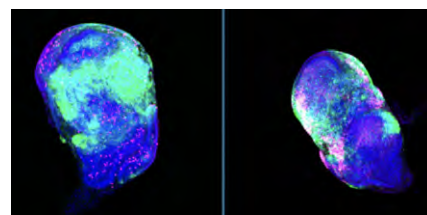


03

Dietary amino acid determines the fate of cancer cells

Oncogenes are genes that induce overproliferation of cells causing cancer and are often also known to cause cell death that prevent cancer. In a new study by Hiroshi Nishida and Sa Kan Yoo (Lab for Homeodynamics) and their collaborators, they focused on the oncogene *Src* and investigated how cell proliferation (or oncogenesis) and cell death are regulated in the fruit fly and showed that *Src* regulates both processes independently and simultaneously via parallel pathways. Once the team identified the molecular cascade leading to cell proliferation and realized that this pathway can be controlled by nutrients in the diet, they demonstrated that reducing consumption of a specific protein building block in the diet could prevent oncogenesis, but not affect cell death. Since the process of oncogenesis is known to be the same in both fruit flies and mammals, these findings can help explain how human cancers develop.

Nishida H, Okada M, Yang L, et al. *Elife* 10, (2021)

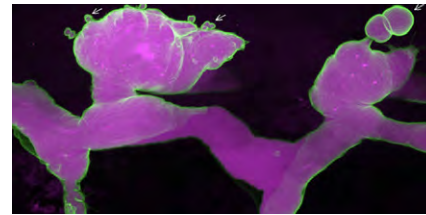


04

Marcks11 keeps blood vessels in shape

The failure to form or maintain a well-ordered network of tubular blood vessels is detrimental to tissue physiology and consequently, health. Endothelial cells form the innermost lining of blood vessels and are subjected to the mechanical stresses of pressurized blood flow. A new study by Li-Kun Phng (Lab for Vascular Morphogenesis) and her team using zebrafish revealed a protective mechanism by which endothelial cells resist deforming forces of blood flow to regulate cell and vessel morphology during vessel development.

Kondrychyn I, Kelly DJ, Carretero NT, et al. *Nat Commun* 11, 5476 (2020)



05

Proteins enable tendons and muscles of fruit flies to develop in sync

While much is known about the internal mechanisms of cells and how they are assembled into tissues, there is comparatively little known about how cells secrete materials and assemble them into external structures such as extracellular matrix (ECM). In a new study, Wei-Chen Chu and Shigeo Hayashi (Lab for Morphogenetic Signaling) revealed a mechano-chemical mechanism of a filament-like transformation of the ECM protein Dumpy that anchors tendon cells to the cuticle of the *Drosophila* pupa during muscle development. In addition, they found that another protein called Quasimodo, an unconventional ECM protein that diffuses through the whole body and modifies the activity of other ECM proteins, is needed to enhance the strength of the ECM.

Chu WC, Hayashi S. *Curr Biol* 31, 1366-1378.e7 (2021)



06

Age-related stem cell dysfunction linked to eye-color gene

The *white* gene, which was discovered more than a century ago, has been assumed to mainly control eye color in fruit flies and is used as an identifiable marker in genetic experiments using fruit flies since defects arising from mutations in the gene are assumed to be mild enough not to greatly impact other aspects of biology under investigation. The team led by Sa Kan Yoo (Lab for Homeodynamics) has now discovered a new role of the *white* gene in these flies—that it also drives aging-related defects in intestinal stem cells through changes in the metabolism of nutrients. As an analogous gene is expressed in the human gut, it could be playing a role in human colorectal cancer.

Sasaki A, Nishimura T, Takano T, et al. *Nat Metab* 3, 546-557 (2021)



Peek-a-LABoo

Structural biology is a discipline for studying of the molecular structure of biological molecules, such as proteins, to reveal how they are formed, how they function and how they interact with other molecules. In April 2020, the Laboratory for Dynamic Structure of Biomolecules, led by Team Leader Ichio Shimada, that is undertaking structural biology research using nuclear magnetic resonance (NMR) was launched at the Yokohama Campus. Here, we introduce this new laboratory.

Laboratory for Dynamic Structure of Biomolecules



What are the main research themes of the laboratory?

In addition to conventional NMR analysis methods, we develop new NMR technology to analyze the structure of biomolecules under physiological conditions. We have been focusing on analyzing and dissecting the function-related dynamics of membrane proteins (e.g., G protein-coupled receptors) and related proteins that play crucial roles in many biological phenomena and are also important targets for drug discovery. RNAs have emerged as a novel target for drug development, however, detailed three-dimensional (3D) RNA structural data that can serve as the basis for RNA-targeted drug discovery is limited. To accelerate research for RNA-targeted drug discovery, it is necessary to develop basic strategies to experimentally obtain atomic-resolution 3D structures of target RNAs and their complexes under physiological conditions. We recently started a new RNA project to address this, which is part of a larger collaboration with other institutions and industry partners and has been in the works since 2019.



How many people are there in the laboratory?

There is a total of eight people working in the laboratory—the team leader, one senior scientist, one research scientist, one technical staff and four graduate students (student trainees).



Is there anything that the laboratory is particular about with respect to research?

Solution NMR is the best approach for obtaining dynamic structural information about membrane proteins under physiological conditions. We are dedicated to carrying out highly original research using NMR, including our newly developed NMR methodology to investigate the function-related dynamics of biomolecules.

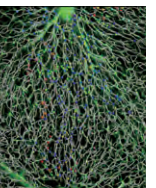


How has the COVID-19 pandemic affected the research activities of the laboratory?

We were just beginning to set up our lab when the pandemic began to gain speed last year. Our assistant did a good job of ordering and arranging for the equipment and supplies needed for our lab, so when the first state-of-emergency was declared, we were just waiting for the orders to arrive. Fortunately for us, our research was not overly affected by the events of the past year.



On the cover!



Tangled roots of a tree?

This is a capillary network in the liver called sinusoids. The liver has a high regenerative capacity. It has been shown that when the liver is damaged, there is an increase in blood flow rate and this is detected as a mechanical stimulus by cells in the sinusoid, which is important for initiating regeneration.

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Lab for Organ Regeneration

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