Unraveling what it means to be alive

BDR TIMES

vol. 11
2022 SUMMER

RIKEN Center for
Biosystems Dynamics Research

Dive into BDR's intriguing research

The Marvels of Shapes Revealed by Mathematics

The person who I interviewed this time is Dr. Daisuke Ohtsuka, who works in the Laboratory for Developmental Morphogeometry, one laboratory at the BDR that is headed by a team leader with a non-biology background. I thought our conversation might include a lot of references to numbers and mathematical formulas, but as Dr. Ohtsuka himself has a biology background, he enlightened me with a story of when biology and mathematics beautifully intertwine.

Coming to RIKEN made it possible to switch topics

Yakushiji (HY) ▶ What made you decide to do research at RIKEN?

Ohtsuka (DO) ▶ When I received my doctorate, I was doing research on a virus that infects silkworms that produce silk. The virus is troublesome as the silkworms die when they are infected with this virus. I was in a laboratory in the department of agriculture, so my research was more directly related to the agricultural field. But when I got my degree and began doing my own research, I found myself wanting to do something different; developmental biology was one area I was interested in.

Luckily, there were a few positions that were advertised around that time, one of which was in my current laboratory. My boss was just starting to set up the laboratory at what was then the RIKEN Center for Developmental Biology (CDB), and I was one of the first post-docs hired in the laboratory.

HY ▶ So, I guess you jumped right into a very different field. It must have been quite challenging at first.

DO ▶ Yes, it was. Not only did I switch fields, but my boss and other laboratory members had a background in mathematics, which meant we use different terminology and have different assumed knowledge, so we sometimes had difficulties communicating. I had learned calculus and linear algebra in my classes at university, but had forgotten most of it. So in the early days of joining the laboratory, my co-workers held mathematics tutorials for me, and I, in turn, taught them about biology.

At the time, though, I also did not know a lot about developmental biology. But the CDB was a research institute with a focus on developmental biology, so everyone around me was an expert in this field. I learned about developmental biology by being at the CDB. It was because I came here that I was able to switch fields smoothly.

How shapes are formed

HY ▶ When you switched fields, had you already decided what your project would be?

DO ▶ The fundamental theme of the laboratory is to understand how shapes of organisms are formed. We knew that we would start by adopting the approach taken by my boss, Dr. Yoshihiro Morishita, to analyze limb (arms and legs) formation, that is, to have experimental and theoretical researchers work together to examine the process of shape-changing quantitatively using microscopy data and mathematic formulas. We observe the developmental process of animals using microscopy to track how their shapes change and the cellular phenomena taking place during this process. Where do the cells divide, where do the cells grow, and where do they undergo rearrangement? I was to observe these phenomena and describe the process as a mathematical formula.

Among the different body regions, I have been focusing on the brain.

HY ▶ How does the brain form?

DO ▶ First, a sheet of neural cells is formed, and this then curls into a tubular structure like a straw. The brain arises from this tubular structure.

HY ▶ The shape of the tip of the tube is changing, is it not?

DO ▶ That's right. In humans, the clump of cells at one end of the tube becomes the cerebrum and the section behind the cell clump grows narrower to eventually form the spinal cord. The cell clump at the tip elongates laterally, and this is where the eyes later form.

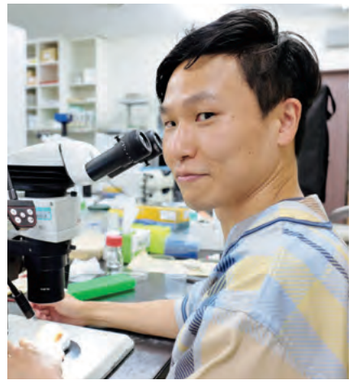
I am focusing on this area where lateral elongation takes place.

HY ▶ Why do these protrude outward on both sides?

DO ▶ In humans, there is a congenital disease called cyclopia, in which, as implied by the name, results in the formation of only one eye. In most cases, the babies are delivered stillborn, and it is known that a mutation in a particular gene is linked to the onset of this disease. We also know that the activity of this gene leads to the bidirectional protrusions. However, this gene is also known to be involved in the formation of fingers. Without this gene, bone formation would not progress normally, resulting in only one finger.

HY ▶ The eyes and bones are quite different, though.

DO ▶ Yes, that's right. The same gene is involved in generating both the eyes and fingers, but we don't know why this is the case. This was the kind of thing I wanted to know more about, and why I wanted to come here.

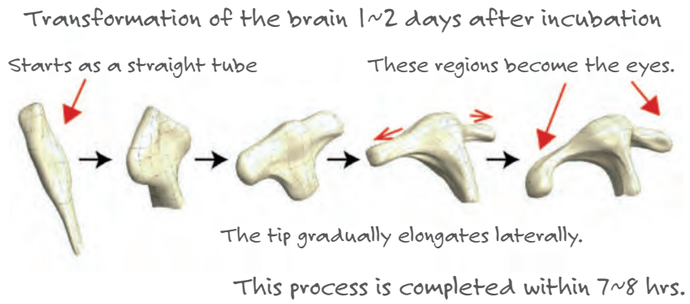


Daisuke Ohtsuka

Senior Research Scientist in the Laboratory for Developmental Morphogeometry. After obtaining his doctorate degree for research in agricultural sciences, he came to RIKEN to do developmental biology research and has now been here for almost ten years. He enjoys drinking tasty beer and collecting sneakers. His research related to the eyes have also led him to develop an interest in a goldfish species called *Chotengan* (celestial eye goldfish), which have protruding eyes with pupils that point skyward.

Hideki Yakushiji

Business developer based in Kobe, and a RIKEN alumni. 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.



How to change the shape of structures

HY ▶ How do the tissues elongate laterally? Oh, I guess the cells can just proliferate... ?

DO ▶ Actually, it is not that simple (laughing).

HY ▶ What do you mean?

DO ▶ When a structure changes by elongating laterally, there are several possible cellular behaviors to explain this phenomenon.

For example, let's assume that some of the cells are proliferating (see figure below; upper left panel). Only the green cells are dividing and proliferating, while the white cells hardly increase in number. This would result in the lateral elongation of the tissue overall, right?

HY ▶ I see that the cells in lower left panel have simply doubled in number.

DO ▶ In this case, the cells are all dividing in the same orientation as the direction of tissue elongation, which leads to the tissue elongating laterally. Or even if the cells do not proliferate and they simply expand their area in the lateral direction (upper right panel), this would also be an explanation for how the shape of the tissue elongated laterally. In the lower right panel, the cells have changed their alignment, with white cells moving vertically to integrate between the colored cells to change which cells they neighbor. In the latter two cases, the number of cells do not change, but can still result in lateral elongation.

So even though you can see that there was an overall change in shape, if you do not closely examine cell behavior and observe how the cells are actually moving, the dynamics of the cells within the tissue remains unclear.

HY ▶ I can certainly see how the tissue (in all the examples) results in lateral elongation.

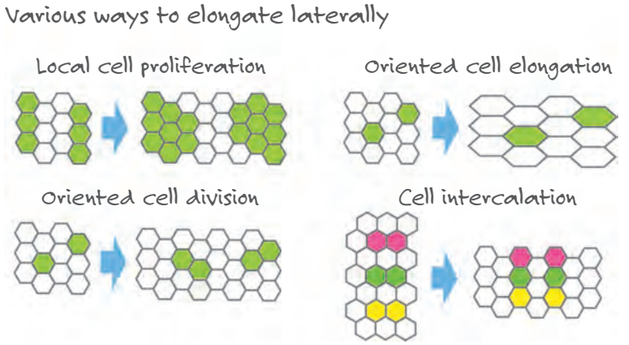
DO ▶ The gene I mentioned earlier which causes cyclopia is known to stimulate cell proliferation when added to cell culture. Therefore, it was thought that the tissue cannot elongate laterally when this gene is impaired because the cells could not proliferate.

But when we examined the behaviors of cells during the protrusion of the eyes, we discovered that cell proliferation was not involved in this process at all.

HY ▶ I guess you really do need to observe the process to know what is taking place. Are you sure the cells are not proliferating?

DO ▶ We see lateral elongation even if cell proliferation is inhibited. For starters, this elongation process is completed within about seven to eight hours, so that is only enough time for cells to divide once. Even if there was a localized proliferation of cells, it would not have much of an effect on elongation. Therefore, changes in cell alignment have a more significant effect on elongation than an increase in cell numbers.

Under normal conditions, we see cells rearranging to elongate laterally, while this does not occur under abnormal conditions. We looked at what happens when the function of the gene I mentioned earlier is disrupted, and found that direction of elongation becomes randomized, not unidirectional, with the cells displaying disorganized movements within the tissue. And as a result, the tissue does not elongate.



Updating gene annotations

HY ▶ How is the direction determined?

DO ▶ For example, white blood cells which are involved in immunity use concentration gradients to determine the direction of their movement. But in our case, we hypothesized that physical forces were the determinant for how these cells move.

HY ▶ Finally, we talk about forces!

DO ▶ I asked a co-worker in the laboratory, who is an expert in mechanical simulations, to calculate the forces exerted on the tissues, which revealed that the forces are at right angles to the direction of elongation.

HY ▶ But that was just a simulation, right? (sounding unconvinced)

DO ▶ Well, I actually carried out the experiments as well.

HY ▶ (Impressive!)

DO ▶ We attached a patch of tissue to an elastic-like sheet and stretch them in longitudinal and uniaxial directions.

HY ▶ It sounds surprisingly like a simple experiment.

DO ▶ Yes, it is. It was a very simple experiment.

If we stretch the patch of tissue in the direction of the force, we see the patch elongating in a direction perpendicular to the that of the force applied, similar to what occurs during elongation of the eyes. When we inhibit the function of the gene described earlier and perform the same experiment, the patch does not elongate even with application of force. This means that the cells cannot sense the stretching force applied to the tissue. In other words, the cells cannot respond to forces without that gene.

HY ▶ Then that gene is like a 'force sensor'?

DO ▶ Right. This action of cells sensing and responding to force is called 'mechanosensation' and the function of this gene is linked to the regulation of this mechanosensory ability. This means that the function of this gene is no longer associated with the bones or brain.

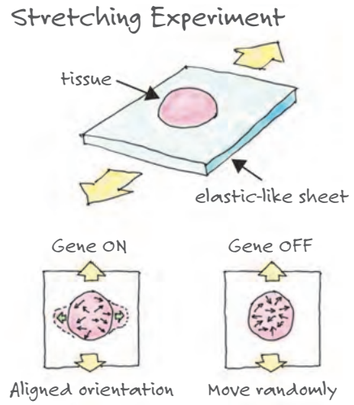
HY ▶ So, the function of that gene is to sense forces and convert that into directional information; not to give commands like, "make a finger".

DO ▶ I am hoping to reach this kind of generalized understanding of the genes. While we know that loss of function of certain genes leads to the lack of eye formation or finger formation, that is of course the outcome. I don't think that we have connected all the dots yet on how a gene is involved in the formation of a specific part or how this process can be impaired. That is what I am interested in understanding.

HY ▶ I guess it's like updating annotations or information on the significance of the gene.

DO ▶ Incidentally, I recently published a new paper reporting on what I just explained about the why in organisms displaying cyclopia, the eyes don't elongate into two, so please take a look at that as well. We also put out a press release for that paper!

HY ▶ Wow, that work is really hot off the press!



Postscript

When shapes undergo change, I thought that the cells were probably just proliferating, but it's true that the arrangement of the cells can change. One might think, "Can cells really move that much?" but I guess in fact they do move. It's oddly curious. Since 2000, we have seen a lot of work on labeling gene functions, and I am sure we will see a lot more advancements in the future. This field seems like it is an extremely exciting one.

Read other interviews ▶

BDR Research Highlights

Research highlights articles and press releases between March to June 2022.
Read these and other articles on the BDR website.

01

Two-part tango triggers signal activation of key regulatory protein

Signaling proteins known as G-protein-coupled receptors (GPCRs) are found in the membranes of cells, and they convert signals from outside a cell into responses within a cell. They are responsible for reacting to signals from hormones, neurotransmitters and sensory signals from the eyes, nose and mouth. The critical role they play in cell biology is highlighted by the fact that roughly half of all drugs on pharmacy shelves target GPCRs. There has been much research into how GPCRs activate their scaffolding partners, known as β-arrestins, by binding to them. For a long time, β-arrestins were thought to be activated only by the tail end of GPCRs. But now, using nuclear magnetic resonance spectroscopy, Ichio Shimada and Yutaro Shiraishi in the Lab for Dynamic Structure of Biomolecules and their collaborators discovered that β-arrestins bound to the tail of an interacting GPCR are only partially activated—subsequent binding with the core region is needed to push the β-arrestins into full activation mode. This finding highlights a path forward for therapeutically biasing the signaling activities of β-arrestins.

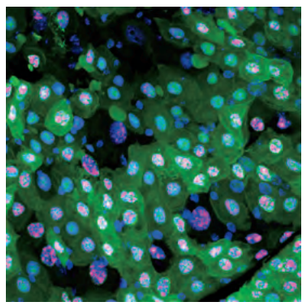
Shiraishi Y, Kofuku Y, Ueda T, et al. *Nat Commun* 12, 7158 (2021)

02

Death in darkness: a new type of cell death discovered in fly guts

Like the skin, cells that make up the intestines are constantly dying and being replaced by new cells. This process, called turnover, helps maintain the balance, or homeostasis, between tissue growth and tissue renewal. The conventional theory for turnover in the intestines is that aging or damaged cells die through a process called apoptosis. Also called "programmed cell death", apoptosis is one of three types of cell death that are currently recognized. A research group led by Sa Kan Yoo (Lab for Homeodynamics) has discovered a completely unknown type of cell death that takes place in the guts of the common fruit fly that they tentatively named erebosis, based on the Greek 'erebos' meaning 'darkness', because the dying cells looked so dark under the microscope. The new process is thought to play a role in gut metabolism. The findings necessitate a revision of the conventional concept of cell death, and at the same time, overturn the previously established theory of tissue homeostasis in the gut.

Ciesielski HM, Nishida H, Takano T, et al. *PLoS Biol* 20, e3001586 (2022)

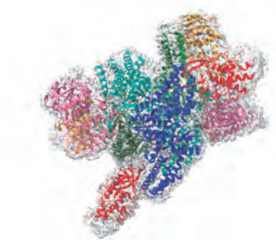


03

A viral inhibitor of cellular stress response shows therapeutic potential

When eukaryotic cells experience stressors such as infection by viruses, oxidative stress or insufficient nutrients, they activate the integrated stress response (ISR)—a signaling network in cells that helps cells to remain healthy under challenging circumstances. The ISR dramatically reconfigures the physiology of cells, largely shutting down protein production while activating genes that help cells respond appropriately to stressful stimuli. On the other hand, in many neurodegenerative diseases, prolonged ISR in neurons contributes to neuronal cell death for reasons that are unclear. Takuhiro Ito and Kazuhiro Kashiwagi in the Lab for Translation Structural Biology and their colleagues have focused on the fact that certain viruses, like the sandfly Sicilian virus, can disrupt the host cell ISR by producing a protein called NSs. They generated a high-resolution structure of the NSs and host translation machinery complex using cryo-electron microscopy and elucidated the molecular mechanism for disrupting ISR. The team then confirmed that NSs can protect cultured rat and mouse neurons from damage or death resulting from chemically-induced stress. Their findings are expected to lead to clinical applications such as the development of treatments for neurodegenerative diseases in which ISR has been implicated in their pathogenesis.

Kashiwagi K, Shichino Y, Osaki T, et al. *Nat Commun* 12, 7102 (2021)



04

Rapid, single-cell analysis of microbiotas now possible

There has been an explosion in awareness of the various critical roles that bacteria in our guts play in health and disease. Biologists want to explore how the make-up of the gut microbiota affects their hosts, but methods for classifying bacteria according to species provide only very rough results. Now, Jianshi Jin and Katsuyuki Shiroguchi in the Lab for Prediction of Cell Systems Dynamics and their co-workers have devised a truly single-cell method that can accurately and rapidly characterize communities consisting of hundreds of thousands of bacteria. They also demonstrated the power of their technique by using it to see how diets deficient in vitamin A affected the gut microbiota of mice, which revealed that only one species of gut bacteria was significantly affected. Their method holds the potential to assess the effects of recently developed bacterial therapies on the gut microbiome.

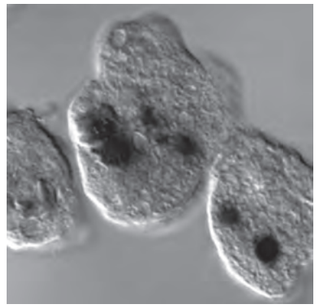
Jin J, Yamamoto R, Takeuchi T, et al. *Nat Commun* 13, 863 (2022)

05

Iron-snatching compound effective against the parasitic amoeba *Entamoeba histolytica*

The parasitic amoeba *Entamoeba histolytica* infects about 50 million people a year, mostly in developing countries. It causes amebiasis, which has symptoms such as diarrhea, dysentery and colitis and is fatal in just over one in ten cases. Amebiasis is usually treated with the anti-protozoal metronidazole, but this drug is a potential carcinogen, and there have been several issues with its use during pregnancy and lactation. Since *E. histolytica* takes in a lot of iron ions, which are essential for maintaining the life activities of the amoeba, Akira Wada (Lab for Nonnatural Amino Acid Technology) and his collaborators investigated whether compounds that target iron ions could be used to combat the infection. They found that an iron-targeting polypyridine compound could curb the proliferation of the amoeba, and importantly, because it did not appear to affect human cells, it is not expected to have significant side effects. The team is now looking at ways to enhance their iron-targeting compound.

Wada A, Umeki Y, Annoura T, Saito-Nakano Y. *ACS Infect Dis* 8, 457-462 (2022)



06

Imaging how a light-driven chloride pump works

Many bacteria and single-cell algae ferry ions into and out of their cells using pumps that work by altering their shape when activated by light. Such light-driven pumps allow cells to regulate their contents relative to their environment and are not just of interest to biochemists; neuroscientists use them to probe brain circuits in animals by turning neurons on and off in response to light. Learning about how these pumps work will enable brain researchers to tailor them for this application. In a recent study, Mikako Shirouzu and Toshiaki Hosaka in the Lab for Protein Functional and Structural Biology, and their co-workers at the RIKEN SPring-8 Center used a powerful x-ray laser to visualize how the shape of a light-driven pump of chloride ions from a marine bacterium that is based on the light-sensitive proteins rhodopsin changes during operation. They discovered that the pump had an intriguing mechanism for preventing chloride ions from returning the way they came.

Hosaka T, Nomura T, Kubo M, et al. *Proc Natl Acad Sci U S A* 119, e2117433119 (2022)

Peek-a-LABoo

The Laboratory for Neuroepitranscriptomics was launched at the BDR in April 2021 and is headed by Team Leader Dan Ohtan Wang (front row, left in photo). Originally from China, Dr. Wang first came to Japan as a high school student on a short-term exchange program, and then later returned to attend the Tokyo Institute of Technology for her bachelor's and master's degrees in bioengineering and biosciences, respectively. She moved to the United States for her PhD studies at the University of Southern California, where she decided to pursue molecular and cellular neuroscience research. Following a post-doc at University of California, Los Angeles, she returned to Japan to work at the former RIKEN Advanced Science Institute (ASI), where she began working on RNA imaging technologies for brain neural networks, specifically for understanding dynamics at neuronal synapses, which she continued to expand on in her following post as an assistant professor at the Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University. We sat down with Dr. Wang (or "Ohtan" to many of her colleagues) to hear about the Laboratory for Neuroepitranscriptomics.

Laboratory for Neuroepitranscriptomics

Q What are the main research themes of the laboratory?

A The two keywords related to our research are the "brain" and "RNA". RNA is molecule capable of storing genetic information like DNA as well as functioning like an protein enzyme. Over the past decade, chemical modifications to RNA, reminiscent of those seen on DNA to turn genes on or off (i.e. epigenetics), have emerged as a possible new layer of regulation of gene expression for fine-tuning a wide range of biological functions including neural network formation, and this has spurred a new field called epitranscriptomics (or RNA epigenetics), the study of RNA modifications.

Our laboratory is focusing on a novel RNA neuroepigenetic mechanism associated with synapse function in the brain. Neural synapses in the brain are dynamic and undergo life stage-dependent changes in response to learning, memory, and other cognitive input. We are particularly interested in revealing the role and regulation of mRNA modifications occurring at neuronal synapses, as well as understanding how mRNA modifications may influence gene networks for experience-based behavioral changes and diseases over the lifespan of an organism. To address these topics, we are adopting a diverse range of approaches and technologies, including quantitative and omics technology, fluorescence imaging, cell and molecular biology, and the use of genetic animal model systems.

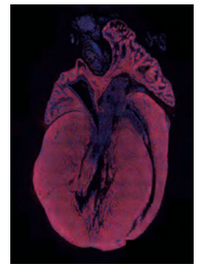
Q What are some strengths of the laboratory?

A We have been developing new experimental approaches to meet each of the objectives of our research. This has resulted in the development of a novel live-imaging method of RNA in a brain ex vivo to monitor RNA localization, the development of a highly sensitive method for the comprehensive analysis of RNA chemical modifications using the smallest amount of RNA as well as the development of a photo-switchable translation reporter-system in the synaptic region. We are currently in the process of developing a large-scale analytical method of the synaptic structure, which is the basic structural unit for computation within the brain; this would help tie together our understanding of synaptic function with behavioral output. We also have researchers with varied backgrounds in the laboratory from specialists in cell and molecular biology, electrophysiology, chemistry as well as psychology, which will allow us to tackle questions related to chemical modifications of RNA, their roles and regulatory mechanisms related to synapse formation spanning all biological scales from molecular and cellular levels through to individual levels, including looking at influences on behavior.

Q What are your long-term goals for your research?

A As our particular interest lies in the neural synapses of the brain, we hope to reveal the regulatory mechanisms of RNA modifications in relation to synaptic functions, including synapse formation in response to environmental or experience-based input. Until recently, the structure, modifications, movements and translational dynamics of RNA molecules had been largely unexplored in molecular neurosciences because of their small numbers and the lack of tools available. By collaborating with many specialists in different fields working at BDR and adopting the various tools available here, we hope to be able to develop methods that will enable us to achieve a comprehensive understanding of the dynamics and function of the RNA regulatory mechanism, eventually leading us to establish a new model for explaining environment-genome interactions in humans.

On the cover!



A maze and a cave?

This is a heart of a newborn opossum (red). Opossums are marsupials, like kangaroos. Their hearts retain the ability to regenerate for at least two weeks after birth, which is the longest reported to date in mammals. The green cells are those currently proliferating.

◎Credit:
Lab for Heart Regeneration

BDR TIMES vol.11
2022 SUMMER
Issued August 1, 2022
Published by RIKEN
Center for Biosystems
Dynamics Research (BDR)
E-mail: bdr-riken@ml.riken.jp
https://www.bdr.riken.jp/en/

RIKEN 2022-037