Unraveling what it means to be alive

# B D R TIMES

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RIKEN Center for
Biosystems Dynamics Research

# Dive into BDR's intriguing research

**RIKEN BDR researchers are carrying out many intriguing and interesting research projects, but it can sometimes be difficult to understand what they are actually doing. Hideki Yakushiji meets with researchers to delve behind the scenes of their research.**

# In between Technology and Science

**The person I interviewed this time was Dr. Takashi Niwa, who is a member of the Laboratory for Chemical Biology. I assumed his research is chemistry oriented, but at BDR where a lot of the research centers around biology, I wondered what he actually did. So I decided to talk to him to appease my curiosity.**

## What chemistry can do for biology

**Yakushiji (HY)** ▸The name of your lab is "Laboratory for Chemical Biology", but what exactly is it that your team is working on?

**Niwa (TN)** ▸Basically, our mission as a team is to create molecules to study particular proteins in our body. My basic love is chemistry, but as the Center is mostly focused on biological research, my focus is basically on what chemistry can do for biology. When we think about it from this angle, it means developing methodologies and molecular tools.

**HY**▸Does that mean that you are someone who develops tools?

**TN**▸The Center has a large emphasis on biology in general, so we work on developing tools and methods to aid biological studies.

I've realized, after talking to many of the biologists here, that they often face many struggles that fall within the scope of chemistry. Since I interact with many biologists on a daily basis, I thought I could find a way for chemistry to aid the advancement of biological studies.

**HY**▸I suppose the biologists would like to have some kind of probe for tracking a molecule of interest?

**TN**▸It's basically a probe, but more precisely, it's transforming a molecule within a living organism into something like a probe or labeling specific proteins with a tiny molecule which can help us to visualize them. If we are successful in these developments, we can use them in diagnostics and treatments. But that would just end up in the scope of engineering. My personal passion is still chemistry, so while I'm creating the probes, I'm also thinking of ways to design new molecules.

## Human brain over computer

**HY**▸How do you go about designing a molecule?

**TN**▸I've been studying the development of new molecular reactions since I was an undergrad. It's an area in chemistry where a Nobel Prize is awarded about once every five years or so. Developing organic reactions means finding a way to link molecules together that don't normally attach to each other.

**HY**▸How do you attach the molecules that don't normally attach to each other?

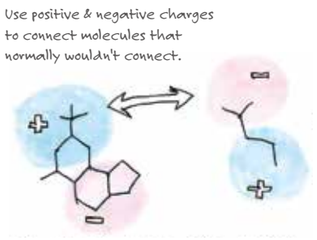
**TN**▸There are many ways of doing this but the simplest way is to basically use their electrostatic properties, where negatives and positives attract each other. It's similar to the way that sodium ions ($Na^+$) and chloride ions ($Cl^-$) can make sodium chloride (NaCl) because of their positive and negative charges. When you take a closer look at organic compounds, we can see that there are fluctuations in the electric charge distribution. Organic chemistry starts by linking the chains that we suspect have negative and positive charges.

**HY**▸I can imagine that if it's a small molecule, but if it's a big molecule, would you be using simulations?

**TN**▸Absolutely. Based on my experience and knowledge from reading a number of textbooks and research papers, I can usually tell which part might be positive or negative from looking at the shape of the molecule. But in order to have a rigorous discussion, we can't just rely on our instincts, so we use supercomputers to simulate reactions.

**HY**▸Does that mean nowadays you can design molecules using simulation?

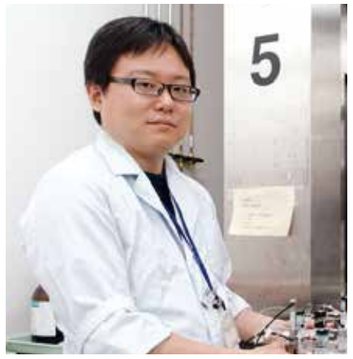
**TN**▸Yes and no. There are unlimited ways that chemical reactions can happen. There are countless paths to crossing the activation energy barrier. This means that it'll require too much time to calculate even using supercomputers. Furthermore, even if we think linking A and B will create C, we also have to consider the possibility that A is really reacting to something other than B to create D, and that D and B are reacting to create C. If we try to calculate all of these possibilities using a supercomputer, the calculations will take way too long so we have to use our brains to narrow it down to more plausible scenarios before starting the simulations.




## Easily transforming molecules

**HY**▸If you get a request from a biologist to help them look at something specific, how do you go by designing a molecule?

**TN**▸If there is a molecule that I can use as a model, I just use that. An instrument called PET, which is often used in diagnosing cancers, allows us to track the molecules of interest inside the body. However, using PET to trace the molecule requires the incorporation of a radioisotope into the original molecule.



**Takashi Niwa**

Deputy Team Leader in the Laboratory for Chemical Biology. After graduating from The University of Tokyo, he earned his Ph.D. at Kyoto University. His career as a researcher then took him to Harvard University, Waseda University, and eventually in 2013, to RIKEN. Niwa came to RIKEN through a chance introduction to his current boss by a friend from graduate school who works in his boss' other lab at the Tokyo Medical and Dental University. He currently works at the RIKEN campus in Kobe, which means he's away from his family that live in Tokyo whom he misses. In his spare time, he enjoys drinking craft beer.

**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 widerange of activities to assist in commercializing technologies and ideas born from academia, including setting up opportunities for idea sharing, finding investors, and strategic planning.

**HY**▸You mean something like $^{18}F$ (radioisotope of fluorine)?

**TN**▸Yes, exactly. Fluorine is often used to stabilize molecules in pharmaceuticals. It's efficient if we can substitute fluorine which is normally not radioactive normally to one that is radioactive.

**HY**▸Are you saying that you remove the original fluorine (F) and then attach a radioactive fluorine (F*)?

**TN**▸Exactly. What we actually do is to first exchange the F to a boron (B), and then change the B to an F*. The nice thing about this approach is that the F* of the end product can be anything. Removing an F is a cumbersome process, but B can be removed relatively effortlessly. Thus, we can construct various molecules based on the structure of the original molecule. We call this "molecular renovation."

## The world of craftsmen and trace impurities...

**TN**▸They hone their skills as they undertake undergraduate, masters, and doctoral research, but their proficiency can vary.

**HY**▸Do you mean that the collected data can be dramatically different for the same reaction?

**TN**▸Yes, it's actually true. There are cases when it works for one person and doesn't work for another. The reason for that is that there is still know-how missing in the procedures for an experiment, no matter how detailed we try to write them.

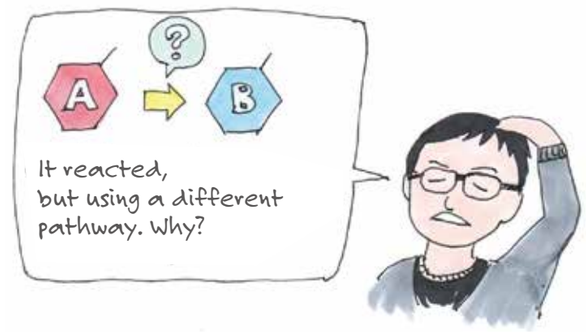
**HY**▸You mean like how you shake the test tube would make a difference? (Laughing)

**TN**▸It's literally like things like that. Things like the speed at which you add a reagent, the amount of solvent used in the purifying process, the amount of water or the method of purification when a column is used can make a significant difference. Also the differences in people's instincts as well as methods between labs can make a difference.

**HY**▸I'm surprised to hear that there are situations where another lab can't reproduce the same results in the realm of chemistry.

**TN**▸The differences between those labs could just be the size of the container, the container used when heating, the purity of the reagent, etc. It really could be anything, so we usually don't know until we go and take a look in person.

**HY**▸Do you mean purity of the reagent?




**TN**▸There is this well-known story that happened in the early 2000s. The metals used as catalysts can be quite expensive, but there were multiple publications that came out reporting that iron can be used instead. Then a few years after that another report was published which contradicted the earlier publications, showing that reactions can't be observed when using high-purity iron. In the end, it was determined that the copper found in ppm order in the iron, was acting as the catalyst.

**HY**▸That means the purity of the iron was 99.999%. Are you saying that the less than 0.0001% of copper found in the iron was reacting?

**TN**▸We just couldn't achieve the radioactivity that we were expecting. We thought that the normal, non-radioactive carbon ($^{12}C$) of methane gas could be the reason for this. But, we later ruled it out because we use an air-tight reactor called a "hot cell" for the synthesis reactions so there is no way for methane gas to get mixed in.

We then investigated whether purity of the elements could be a cause, and found out the culprit was ammonia gas. Ammonia gas is a source material used to produce the $^{11}C$-cyano group and its purity was 99.999%, which is not bad at all. But we suspected that a minuscule amount of $^{12}C$-methane gas may be mixed in, so we switched to using "ultra-high purity" ammonia which has a purity of 99.9999% and the problem was solved.

It may sound simple, but 99.999% purity is still categorized as "high-purity" so it took us a while to figure out what to look into.

## We actually don't know what's really happening

**HY**▸I pictured chemistry to be more simple and sophisticated, but it sounds like you can be thrown a lot of curveballs.

**TN**▸Definitely. "Molecule renovation" sounds cool, but in reality, we often don't know why the reaction was successful. I mentioned earlier that the electron distribution diagram is rendered after the reaction is completed, but often the reaction pathway is different from what we had anticipated.

**HY**▸Oh, is that so?

**TN**▸When we are developing reaction pathways, it's basically engineering rather than doing science. I think that it becomes science when we go beyond that scope of why an experiment was successful. I enjoy doing both, but I must admit that there are some experiments that haven't reached the realms of science yet.

For example, we use nickel for certain reactions based on past experience and publications. While there are a number of scientific explanations, it's hard to describe it in layman's terms or difficult to evaluate quantitatively as we partly rely on our instincts. I've been told it's like I have the instinct of a craftsman when they asked me why I used nickel.

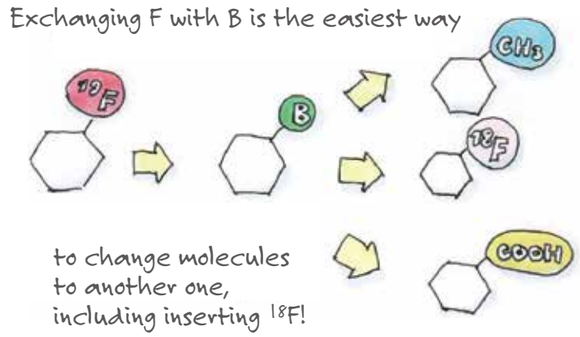
**HY**▸So it's like you have to elucidate the reason behind the successful reaction.

**TN**▸On one hand, it's sufficient to just say fluorine was converted to boron, but from a scientific perspective, we are still lacking in explanations. It'd still make a great thesis though.

**HY**▸I imagine that you will keep on developing many things since you'll be involved in biological research?

**TN**▸Yes. Since I'm already involved in biological research, I'd like to continue contributing to molecule transformations that can be useful for biology. But again, this is more an engineering approach, rather than science.

What, then, is science I wonder? (Laughing)





## Postscript

I hear a lot about the difficulties of producing the results in biology, but I didn't realize the same is true in chemistry. I was amazed to hear that the way a flask is shaken or the way the reagent is added can make a difference in synthetic efficiency. I'm still dumbstruck by the fact that purity of 99.999% wasn't considered pure enough and they had to use higher grade of purity of 99.9999%.

Read other interviews ▶

# BDR Research Highlights

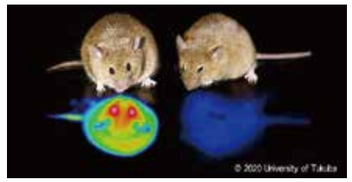
Research highlights articles and press releases between August to November 2020

## 01

### A neural circuit that makes rodents go into a hibernation-like state found

Many mammals, from hamsters to bears, survive the harsh temperatures and food scarcity of winters by hibernating. Hibernation is essentially an energy-saving mode since it lowers the metabolic rate, allowing an animal to expend much less energy than normal. BDR's Genshiro Sunagawa and Prof. Takeshi Sakurai's group at the University of Tsukuba have found that activating a certain brain circuit causes mice and rats—non-hibernating animals—to enter a hibernation-like state. This finding could have implications for other non-hibernating mammals, including humans, and it might eventually find application in space travel and the transportation of seriously injured patients.

Takahashi TM, Sunagawa GA, Soya S, et al. *Nature* 583, 109-114 (2020)




## 02

### Coordination of hormonal signaling and nutrient metabolism drives critical life-cycle transition

Steroid hormones regulate many developmental transitions in animals, from metamorphosis in insects to puberty in people. Yet the compounds that determine the energy metabolism in these biological events have long been overlooked. Now, BDR's Takashi Nishimura (former Lab for Growth Control Signaling) has discovered how steroid hormone signaling regulates glucose metabolism to drive the transition from larvae to pupal stages in the fruit fly. His work could have much wider implications that may extend to life-stage changes in people.

Nishimura T. *Curr Biol* 30, 3624-3632.e5 (2020)

## 03

### Humans develop more slowly than mice because our chemistry is different

In the early stages of vertebrate development, the embryo develops into a series of "segments" that eventually differentiate into different types of tissues such as muscles or ribs. This process is known to be governed by an oscillating biochemical process known as the "segmentation clock". Mitsuhiro Matsuda and Miki Ebisuya of the former Lab for Reconstitutive Developmental Biology, now both at European Molecular Biology Laboratory (EMBL) Barcelona, and their colleagues have found that this segmentation clock progresses more slowly in humans than in mice because the biochemical reactions are slower in human cells. The differences in the speeds of biochemical reactions may underlie differences between species in the tempo of development.

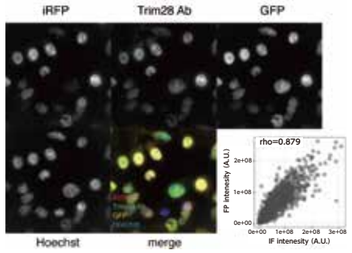
Matsuda M, Hayashi H, Garcia-Ojalvo J, et al. *Science* 369, 1450-1455 (2020)

## 04

### Scientists identify the molecules responsible for transcriptional bursting

When viewing lots of cells en masse, DNA transcription seems smooth and continuous. But on a single-cell level, DNA transcription proceeds in fits and starts. This erratic nature of transcription, which is dubbed transcriptional bursting, is partly why cells with identical DNA in the same environment differ from each other. Now, by analyzing the mRNA produced by single embryonic stem cells from mice, Itoshi Nikaido and others in the Lab for Bioinformatics Research as well as collaborators at Hiroshima University have identified some of the proteins that play a key role in regulating the kinetics of transcriptional bursting. Their findings and techniques may help to establish efficient methods for inducing the differentiation of pluripotent stem cells, such as human iPS cells, into specific cell types, and to study the origin and evolution of cancer since cellular heterogeneity that is possibly caused by transcriptional bursting occurs when cancer cells grow in the body.

Ochiai H, Hayashi T, Umeda M, et al. *Sci Adv* 6, eaaz6699 (2020)




## 05

### New artificial skin functions like natural skin

Our skin provides a barrier and physical cushion that protects the body from the external environment. The outer layer of the skin maintains a stable and steady tension through collagen fibers. In new work led by Takashi Tsuji (Lab for Organ Regeneration), in collaboration with ROHTO Pharmaceutical Co., Ltd., the team has developed an improved human-skin equivalent from cultured skin cells, which reproduces the tension balance of natural skin. This artificial skin will enhance in-depth analyses of physiological skin functions, provide solutions to skin problems caused by diseases or aging, and reduce the need for animal testing.

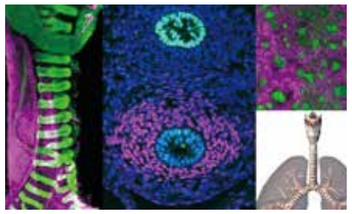
Kimura S, Tsuchiya A, Ogawa M, et al. *Commun Biol* 3, 637 (2020)

## 06

### Reconstructing the cellular signaling pathways that shape trachea development

Keishi Kishimoto and Mitsuru Morimoto of the Lab for Lung Development and Regeneration, together with Aaron Zorn's group at Cincinnati Children's Hospital and other colleagues, examined tracheal mesodermal development and established a protocol for inducing embryonic stem cells into the tracheal cartilage and smooth muscles. The procedure can lead to a biologically accurate organoid model that could offer both explanations and potential therapeutic options for life-threatening conditions such as malformation of the trachea.

Kishimoto K, Furukawa KT, Luz-Madrigal A, et al. *Nat Commun* 11, 4159 (2020)



## 07

### Experimental evolution reveals how bacteria gain drug resistance

Counteracting multidrug-resistant bacteria is becoming a critical global challenge. It seems that every time we develop new antibiotics, novel antibiotic-resistant bacteria emerge during clinical use. Tomoya Maeda and Chikara Furusawa of the Lab for Multiscale System Dynamics and their colleagues have succeeded in experimentally evolving the common bacteria *Escherichia coli* (*E. coli*) under pressure from a large number of individual antibiotics. In doing so, they were able to identify the mechanisms and constraints underlying evolved drug resistance. Their findings can be used to help develop drug-treatment strategies that minimize the chance that bacteria will develop resistance.

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

# Peek-a-LAB

At the RIKEN BDR-DAIKIN Collaboration Center, one of two collaboration centers established at BDR with a partnering company, BDR scientists researching human fatigue and Daikin Industries, Ltd., a global manufacturer of air conditioning systems, have teamed up to share and exchange their respective knowledge and technologies with the goal of creating an "anti-fatigue" environment—a healthy and comfortable environment that can reduce or prevent fatigue in people. We talked to unit leaders Kei Mizuno (Laboratory for Health Estimation ; photo on right) and Yasuyoshi Watanabe (Laboratory for Health Solutions; concurrently, director of BDR-DAIKIN Collaboration Center ; photo on left) who lead the two laboratories of the BDR-DAIKIN Collaboration Center to hear more about their research activities.

## RIKEN BDR-DAIKIN Collaboration Center

**What kinds of activities are being conducted under this Collaboration Center?**

In our efforts to create an "anti-fatigue" environment, we are first looking into the relationship between different environmental factors, such as temperature and humidity, and human fatigue. For example, we often move between areas with large temperature differences in our daily lives, which can place a burden on our autonomic nervous system as the body adapts to the changes, thereby contributing to fatigue. Many studies to date have only evaluated temperature and humidity effects based on subjective measures such as comfort. But in our studies, we focused on clarifying the effects of these environmental factors on health and fatigue by including analyses of quantitative measurements of autonomic nervous system activity and work efficiency in addition to psychological evaluations.

**Can you explain how the studies are carried out?**

A special research facility with four rooms, in which the temperature and humidity settings in each room can be finely controlled in increments of 0.1℃ for temperature and 1% for humidity, was built in the RIKEN Integrated Innovation Building (IIB) in Kobe. For example, in tests for gauging ideal summer workplace conditions to reduce fatigue while maintaining work efficiency, we selected 12 different combinations of temperature and humidity settings. Study volunteers were then asked to perform tasks requiring concentration under six randomly assigned conditions. Besides evaluating their work efficiency and psychological states under each condition, we also measured their health parameters, such as changes in heart rate variability to calculate autonomic nerve activities, using monitoring devices worn by volunteers. Comprehensive analyses of these data indicated that in room temperatures of 28℃, lowering humidity was effective to reduce fatigue levels. These findings were reported in a media release from DAIKIN in May 2020*1.

In future studies, we plan to examine other environmental factors such as how airflow or odors may affect human health, including fatigue.

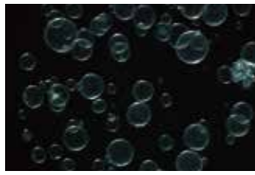
*1 Media release from DAIKIN (in Japanese) "Lowering humidity is effective in reducing fatigue even at room temperature of 28℃" (May 28, 2020)

**What is important for you when collaborating with DAIKIN?**

It's important for us that the collaboration does not end up being like commissioned research. Whenever possible, we have our research collaborators at DAIKIN actually come to our labs and conduct research here, and in doing so, learn and experience firsthand about how research is carried out in our labs as well as RIKEN in general. The collaborating researchers from partnering companies can benefit by developing new skills through their experiences at RIKEN which in turn will become an asset for their company in the long run.

Read full column and past columns

On the cover!



**Floating bubbles?**

These are 3D alveolar (lung) organoids or mini-organs generated from culturing mouse alveolar stem cells in a culture dish. The organoids can produce different types of alveolar cells.

◎Image:
Lab for Lung Development and Regeneration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