Exploring retrotransposon regulation mechanisms via piRNA

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Dr. Saito graduated from the Department of Material and Biological Chemistry, Faculty of Science, Yamagata University and completed his Ph.D. (Science) course at the Graduate School of Natural Sciences, Kobe University. He worked as a postdoctoral fellow at the Institute for Genome Research, Tokushima University, and Assistant Professor and Associate Professor at the Department of Molecular Biology, Keio University School of Medicine. In 2017, he was appointed as a Professor at the National Institute of Genetics.

Saito took a new position at NIG in 2017 and has launched many projects, such as the production mechanisms of small RNAs, formation and maintenance of germ cells, regulatory mechanism of transposons that move within the genome, and the structure of *Drosophila* chromosomes. Not that he had a long-standing interest in all of these topics; he stated that these subjects began to interest him as he conducted a careful analysis of one particular topic. Saito told us about the trajectory of his research and his future visions.

It all started with research on the production mechanisms of small RNA

The original research that led to my current studies goes back to a research on small RNAs. During the period between the 1990s and the beginning of 2000, genome projects were conducted where large-scale genomic analyses were performed, covering everything from nematodes and *Drosophila* to mice and humans. As a result, the gene number of each species was precisely counted and the function of each gene began to become clear. At the same time, it was found that there was a large number of RNAs (non-coding RNAs, ncRNAs) that do not code for proteins after being transcribed from DNA (a process called transcription). Although it was considered at the time that these ncRNAs were junk and waste with no function, they were numerous. Therefore, researchers suspected that they must have some function, and so functional analyses were initiated. While the functional analyses were ongoing, it was revealed that small RNAs with lengths of 20–30 bases tended to suppress the expression of genes. For example, a single-stranded small RNA (called a microRNA or miRNA), with a length of approximately 20 bases, anneals with its complementary sequence in messenger RNA (mRNA) to regulate the expression of genes involved in developmental timing and cell growth. Abnormalities in such regulations have a connection with various diseases, such as cancer.

In 2004 when I obtained Ph.D. and started working as a postdoc, microRNAs were known to be synthesized from a long precursor with a hairpin shape, and subsequently processed to form a short, mature microRNA. I was interested in the mechanism of microRNA synthesis, so I decided to study the molecular mechanism of the precise systematic processing of long precursor RNA. For example, it was known at the time that a precursor of microRNA is cleaved by a specific protein called “Dicer”. However, I discovered that Dicer does not function alone, but functions via interactions with a different protein called “Loquacious”. Later, it was found that double-stranded RNA created by Dicer/Loquacious is denatured to produce a single-stranded RNA, and single-stranded RNA binds to a protein called “Argonaute” to become a functional microRNA, which can regulate the expression of genes.

Progression to the piwi protein, piRNA, and transposons

While conducting my research, I noticed that five proteins with an amino acid sequence and structure (called a domain) similar to the Argonaute protein had been reported in *Drosophila*, and furthermore seven proteins had been reported in mammals. Among these proteins, there was an interesting protein called Piwi (P-element induced wimpy testis). *piwi* gene had been identified in *Drosophila* in 1997. Mutations in this gene were reported to cause abnormalities in the formation and maintenance of reproductive cells in both males and females, resulting in an inability to produce offspring (i.e., sterility).

Later, *Aubergine* (*Aub*) and *AGO3*, which were similar to *piwi*, were identified; proteins coded by these three genes are grouped together and are now called the PIWI subfamily group. Based on findings that these three proteins possess a structure similar to that of the argonaute protein and that AGO1, one of argonaute family proteins, binds to microRNA, I hypothesized that the PIWI subfamily group can also bind to small RNA. At the time, I was working at the Institute for Genome Research at Tokushima University where I reported that the PIWI protein binds to small RNA in *Drosophila* ovary.

However, contrary to my expectation, the small RNA was found to be anywhere between 25 and 30 bases, which is a little longer than microRNA. After careful consideration, I began to think that this small RNA was different from microRNA, and so I decided to study its sequence and genomic origin. As a result, I discovered that the PIWI-associated small RNAs possessed a sequence complementary to a part of a retrotransposon. Later, Aub and AGO3 proteins were also found to bind to the retrotransposon-derived small RNA. As a result, these small RNAs are now being called piRNA (PIWI-interacting RNA) as the small RNA group that binds to the PIWI subfamily proteins. This means a new player to my research: retrotransposon biology.

Studies examining piRNA brought big changes

At the time, we had only biochemically identified piRNA as a factor that bound to the PIWI subfamily group; there was no extensive information regarding the biological functions of piRNAs, how they were distinguished from other small RNAs and specifically loaded onto PIWI proteins, and how *piwi* mutations caused sterility. Therefore, we decided to move away from microRNA research and [begin a new field]. At the time, research on small RNA, including microRNA, was extremely competitive worldwide. Thus, I wanted to move toward a more challenging field, one with great mystery.

Retrotransposons are transposable elements that are integrated throughout the genome, and some retrotransposons are long, approximately 10,000 bp. A characteristic of retrotransposons is that they first transcribe their own DNA into RNA, and then, while replicating DNA from the RNA through reverse transcription, they integrate (i.e., move around) within the genome. It is easy to understand if you imagine copying and pasting words using a word processor. Retrotransposons became integrated into the genome during the development of organism following a retroviral infection, and retrotransposons contribute to the increasing size and genome diversity. For example, in cases where a retrotransposon integrated into a gene, a new function may be added to the gene or, inversely, the existing function of the gene may be deleted. According to genomic analysis, 45% of the human genome and 20% of the *Drosophila* genome is occupied by retrotransposons.

Going back to my experiments, we had already discovered that the argonaute protein suppress the expression of target genes by binding to small RNAs. Therefore, we thought that piRNA also suppressed the function of retrotransposons by binding to the piwi protein. That is, we predicted that the copy and paste function in variants with abnormal *piwi* will be increased. Therefore, we examined such variants using individual or cultured *Drosophila* cells. As predicted, the expression of retrotransposons was increased in *piwi* mutant.

We are beginning to understand the relationship between the piwi protein, piRNA, and retrotransposons

After further analyses, it was discovered that *piwi* is localized in the nucleus of germline stem cells and in the ovarian somatic cells surrounding the germ cells in the testis and ovaries of *Drosophila.* In contrast, Aub and AGO3 were localized in the cytoplasm of germ cells of both the testis and ovaries. Therefore, we conducted an experiment to suppress the expression of *piwi* and found that suppression caused the overexpression of retrotransposons and caused abnormalities in the development of germ cells. Phenomena such as overexpressed retrotransposons damaging genes important

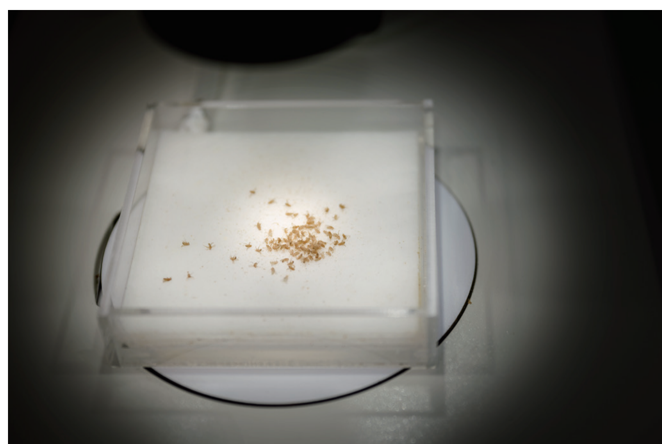
for the production and maintenance of germ cells are suspected, but additional studies are required to elucidate the details.

Meanwhile, I discovered that the PIWI protein interacts with histone H1, a protein that regulates the tightness of the chromatin structure. I believe that interactions between target retrotransposon locus and histone H1 are increased by the PIWI protein, causing a condensed chromatin structure, and that the retrotransposon activity is suppressed as a result. Thus, we believe that piRNAs are also required and that the interaction between the PIWI protein and piRNA suppresses the expression of retrotransposons. However, the presence of other proteins that interact with the PIWI protein has been discovered, so we need to more closely examine these proteins for better understanding.

A system wherein RNAs derived from retrotransposons suppress the activity of retrotransposons is a conflict system. Whereas, the amplification of retrotransposons can be a driving force for genomic diversity, integration of retrotransposons can cause the destruction of genes. Therefore, we believe that this system was acquired during the earliest stages of genomic diversification on Earth.

NIG is a privileged environment

It has only been approximately 6 months since I have started working at NIG, so I am currently constructing various *Drosophila* constructs in which specific genes are tagged with epitopes, with the help of an assistant professor, Dr. Shu Kondo, as well as preparing the experimental environment. I have consistently used *Drosophila* for my research because it is easy to handle because of its small size, has a short cycle of maturation and reproduction (i.e., alternation of generations), introducing and inactivating its genes is relatively easy, and results obtained may be easily applied to higher organisms, such as mice and humans. NIG is equipped with a wonderful environment, where studies using *Drosophila* can reach their full potential. In our laboratory, various *Drosophila* strains are separately stored, and there is an RNAi library that can artificially suppress more than 9,000 of the total 14,000 *Drosophila* genes.



In addition, I can tell that researchers in NIG are passionate about their research; there are several inquisitive people here. It has only been 6 months since I moved here, and I am already very pleased to be a part of this laboratory. Before I came here, an associate at the Keio University told me a story about a researcher at NIG who continued conducting his experiment on a table at a faculty meeting room during discussions. This story describes the passion and devotion that scientists here have for their research.

I also like how naturally English education is being conducted here. For example, NIG has its own textbook with audio for English education, called the NIG Method, by Dr. Tatsumi Hirata at the Brain Function Research Lab, Dr. Yasushi Hiromi who is head of office for research development at NIG and professor emeritus at the Graduate University for Advanced Studies, SOKENDAI, and others together with an assistant professor in English. This text book is also available to the public. It is said that the NIG Method was developed based on the idea that researchers need to be able to think logically and conduct discussions in English. The method offers excellent tips, such as describing unusable English that researchers and students tend to use, as well as describing how to prepare effective slides and presentations.

Furthermore, a journal club run without a barrier between labs is very meaningful in terms of improving both research and English. There are currently 22 journal clubs, each with roughly 15 researchers and addressing a different research area and theme; I belong to a journal club for germ cells and developmental biology. At meetings of the journal club, the speaker of the day introduces a published paper written in English that is suitable to their field. After the introduction of the paper by the speaker, everybody discusses the paper. Speakers are rotated, with each individual speaking about once every 6 months. Staff and students all take turns in speaking.

I want students to know that research is fun

I do not have any students in my lab at the moment; however, I plan to have one student in April 2018. If anyone is interested in my research as I have described it, I invite them to join my lab. I think that anybody who is interested in performing experiments who can come up with their own hypothesis and plan experiments to support or deny the hypothesis can join us, regardless of their background. In the first place, the SOKENDAI entrance exam, despite being considered an entrance exam, is not a typical exam but tests the quality of individuals. Therefore, I would encourage students to consider joining us.

At the beginning of a doctoral program in my lab, students will notice that there are many occasions where they will learn from observation. However, I think that they will gradually be able to design their own experiments. One more assistant professor is scheduled to join us in January 2018, so I am anticipating that we will have enough researchers to conduct various experiments.

More opportunities lie ahead as we steadily move forward, step by step

People think that scientific research should be practical and return a benefit to the society. However, I try to focus on the present work. I think that results are obtained if you are focused on a proper target, have trust in yourself, and are devoted to research. Collaborators and rivals will naturally appear, and discussions with the same will lead your research to a more advanced stage. For example, I will not conduct research involving humans, but I would be happy if other researchers were to apply our results from *Drosophila* to mammals and humans.

An example of the application of good research is the development of the CRISPR/Cas9 system, which is now an indispensable tool in

genome editing. CRISPR/Cas9 is a system where a targeted DNA sequence is clipped using DNA endonuclease (Cas9) guided by RNA. This system originated in bacteria and protects them from attack by foreign materials, such as bacteriophages. The CRISPR/Cas9 mechanism is very similar to the retrotransposon regulation mechanism of the PIWI proteins and piRNA. I believe it is likely that the origin of both mechanisms has something in common. I once encountered an occasion where an argonaute researcher changed his research area to CRISPR/Cas9 research. This happened several years ago prior to a report suggesting that the CRISPR/Cas9 system can be used as a gene manipulation tool. Both the CRISPR/Cas9 system and argonaute protein suppress the specific gene expressions via small RNA. Therefore, I think that the overseas researcher thought that the CRISPR/Cas9 system is useful as a gene manipulation tool. Thus, my research at the time was very similar to the CRISPR/Cas9 research, which is now a possible nominee for the Nobel Prize. I should have moved on to CRISPR/Cas9 research at that time; however, I expect that a similar opportunity will appear if I diligently continue my research.

I would like to expand my research to unknown fields

Competition in piRNA research is becoming intense. The group of Julius Brennecke and his colleagues in Austria published an article in Nature stating that they identified an important factor involved in the regulation of piRNA transcription. The research was accomplished using next generation sequencing, which can determine nucleotide sequences at a high speed and accuracy, and I feel that they have solved the issue that I was interested in. I do not want to change my interests or publish papers without having confirmed evidence and conclusions. Research begun with the production mechanism of small RNAs led to the field of developmental biology such as the formation and maintenance of germ cells and has further expanded to the regulation of piRNA, retrotransposons, and chromosomes. I have no idea where my research will lead in the future, but I want to advance onward, enjoying research in unknown fields.

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