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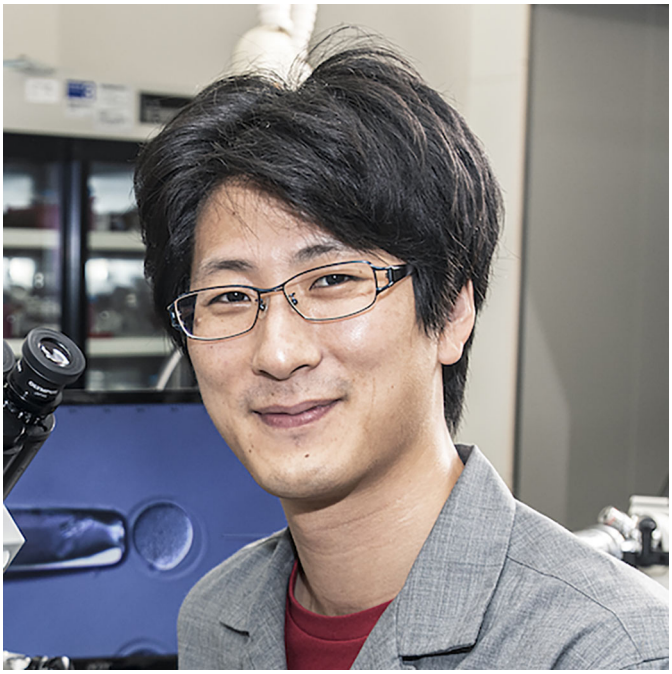
INTERVIEW

Transitions in development – an interview with Azusa Inoue

Azusa Inoue is a Team Leader at RIKEN Center for Integrative Medical Sciences (IMS) in Japan. His lab studies oocyte-mediated epigenetic inheritance by understanding the mechanisms of epigenome establishment and reprogramming. Last year, Azusa was awarded the Ministry of Education, Culture, Sports, Science and Technology (MEXT) Young Scientists' Award. We spoke to Azusa over Zoom to learn more about his career so far and how, since becoming a principal investigator, he still spends most of his time at the bench.



Let's start at the beginning, when did you first become interested in science?

Since I was a kid, I loved catching insects and I was interested in biology because my father was a high school biology teacher. He brought rare animals home, including silkworms, that I just loved to handle. When I was a high school student, my biology teacher – who was not my dad – was previously in research. He showed us his research articles written in English, which was very cool, and it was at that time I decided to become a researcher in biology. I wanted to be a postdoc because I wanted to spend all my time doing research – my dream has already come true!

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You then went on to obtain your Master's and PhD from the University of Tokyo, Japan. Could you tell me a bit about what you researched during that time?

I was with Fugaku Aoki and the project for my Master's thesis was about the mysterious correlation between developmental competence and the chromatin structure of germinal vesicle (GV) mammalian oocytes. There are two types of GV oocytes: surrounded nucleolus (SN), which have chromatin surrounding the nucleolus, and non-SN (NSN), which have a different chromatin structure. Only SN oocytes can develop whereas NSN oocytes cannot even mature. I wanted to know about the tight coordination between the chromatin structure and developmental competence in mice. I developed a non-harmful technique that can distinguish between SN and NSN without any DNA staining, instead using the perivitelline space as a marker (Inoue et al., 2007), which allowed me to transfer nuclei from SN to NSN and from NSN to SN to determine whether the cytoplasm or nucleus influences competence. Eventually, I found that the cytoplasmic materials of SN oocytes are important for meiotic competence, i.e. the maturation of oocytes. Meanwhile, nuclear materials, but not chromatin itself, are important for developmental competence, the ability of the oocyte to form an embryo after fertilization (Inoue et al., 2008).

Azusa Inoue's contact details: RIKEN-IMS, Suehirocho 1-7-22, Tsurumi-ku, Yokohama City, Kanagawa 230-0045, Japan.
E-mail: azusa.inoue@riken.jp

My PhD was carried out in the same lab. During my Master's experiments, I became curious about the identity of the nucleolus-like body (NLB), a huge structure in the nucleus of GV oocytes where there's no ribosomal synthesis. At that time, the components of the NLB were totally unknown. I decided to identify the components by isolating the NLB from GV oocytes and doing mass spectroscopy analysis. I identified nucleoplasmin 2 (NPM2) as a major component of the NLB (Inoue and Aoki, 2010). NPM2 is essential for NLB formation and for facilitating sperm DNA decondensation shortly after fertilisation (Inoue et al., 2011a). Interestingly, one of these papers was rejected by Development!

You moved to the University of North Carolina at Chapel Hill and then Harvard Medical School, USA, for your postdoc with Yi Zhang. What was that experience like for you?

I wanted to continue research on mammalian oocytes and early embryos. At that time, almost nothing was known at the molecular level about oogenesis, fertilisation and pre-implantation development because the material was too limited to study using molecular biology. Therefore, there was a huge black box and I like studying black boxes! Meanwhile, I also wanted to extend my research field because Fugaku's lab was focused, but I wanted to look more broadly. Yi Zhang's lab was good for this. The lab has studied various biological contexts, including early development, induced pluripotent stem cells, and islet cells. It also had many cutting-edge technologies and was one of the top labs in the world. I learned a lot there, including how to manage a lab, how to sell a scientific story, and how to work together with lab mates. My colleagues were very smart, so it was encouraging, inspiring and exciting to be there.

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About half of the lab members were working on TETs (ten-eleven translocation proteins), which mediate DNA demethylation, in different biological contexts. For my first project, I worked on the role of TET3 in mouse zygotes. There was a famous paper showing that sperm DNA methylation is erased shortly after fertilisation (Mayer et al., 2000) and, because TET3 was reported to convert 5-methyl-cytosine to 5-hydroxylmethyl-cytocine (5hmC) in zygotes, I was working on how 5hmC is further converted to cytosine. Chromosome spread followed by immunostaining analysis revealed that 5hmC is just diluted in a DNA replication-dependent manner in every cell division of pre-implantation development (Inoue and Zhang, 2011). Furthermore, I also found that some of 5hmC was further processed into 5-formyl-cytocine (5fC) and 5-carboxyl-cytosine (5caC) in zygotes, and that both 5fC and 5caC are also lost by the DNA replication-coupled dilution mechanism (Inoue et al., 2011b).

While I was working on TET, I was also working on sperm chromatin remodelling following fertilisation, so in zygotes. Sperm chromatin is packaged by protamines so that sperm DNA can be very tightly packaged into a small space. So, after fertilisation, the sperm DNA has to be decondensed, which involves the release of protamines and the incorporation of maternal histones. I was interested in this protamine–histone exchange, which is crucial for functionalisation of the paternal genome. I could identify the histone chaperone, HIRA, responsible for the maternal histone incorporation. Very curiously, protamine can be erased while histones are not deposited in *Hira* knockdown mouse zygotes and, as a result, the paternal DNA became nearly free from nucleosomes, which are surrounded by a nuclear envelope without any nuclear pore complexes. Somehow, nucleosome assembly is required for nuclear pore complex assembly. I studied the molecular link between nucleosomes and the nuclear pore complex. I found a key molecule, ELYS, which can interact with the nucleosome and is a component of the nuclear pore complex as well as a master regulator of the nuclear pore complex assembly (Inoue and Zhang, 2014).

How was your experience of moving from Japan to the USA?

Oh yeah, there's a huge culture shock! In Japan, there's a well-established public transportation system and it is Japanese culture to have many small convenience stores, where I can get anything I need. But the USA is different. In North Carolina, I had to have a car, even just to buy a single bottle of milk because there were no little stores nearby. After I moved to Boston, many things changed again. It was very easy to live there and get around the city, although rent is very expensive. Also, many top researchers from around the world come to Boston, and there are also many Japanese researchers and scientific communities in Boston where we can exchange information, which was really helpful for my career.

When did you start applying for independent positions?

About four years after I joined Yi Zhang's lab, I started to explore opportunities to become a principal investigator (PI) in Japan, but positions for young group leaders are extremely limited there – it's very different from the situation in the USA. I only applied to about two places a year and it took more than two years to get a position.

Why did you want to go back to Japan to start your own group at RIKEN IMS, Japan?

The biggest reason is that I love Japan! I love Japanese people, atmosphere and culture. Another reason is that researchers in Japan are really leading the germ cell field. It's easier for me to do collaborative work here in Japan where germ cell research is really strong, whereas, in the USA, the community is much smaller.

I only got offered this position because I didn't apply to many, as I said, so I didn't compare institutes and make a choice. But the bottom line is that RIKEN is probably the best research institute in Japan and one of the best in the world, in terms of internal funding and research environment. RIKEN is quite famous, so there's really no reason *not* to accept a position at RIKEN!

How was the transition to becoming a group leader?

One of the exciting moments was that I could decide which instrument to buy and how much to spend on what, such as hiring staff and housing mice. That freedom is very different from being a postdoc. Another exciting moment is when one of my graduate students gets awarded a prize, which makes me very happy.

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Can you summarise the research themes of your group now?

We have several projects in the lab investigating the functions of maternally deposited Polycomb repressive complexes. Previously, researchers in the mammalian Polycomb field, which is a big field within chromatin biology, have been studying late stages of development or using embryonic stem cells as a model, but there hasn't been much attention paid to the earliest developmental period. One of our important findings was the discovery of maternal Polycomb-dependent genomic imprinting (reviewed by Inoue, 2023). Previously, the genomic imprinting community believed that imprinting is achieved by DNA methylation. However, Polycomb complexes deposit histone modifications in oocytes to achieve germline DNA methylation-independent imprinting. We are studying how this imprinting is established by maternal Polycomb complexes and its functions, as well as the other roles Polycomb complexes play in pre-implantation development. We are also interested in the environmental effects on the oocyte epigenome, such as the environmental cues that can trigger or moderate histone modifications, including Polycomb, and what impact these changes can have on the next generation. This has been quite a challenge!

What advice would you give to someone starting their own lab?

Don't stop working at the bench! It's not easy to find a good postdoc as a junior PI. Initially, you're your best postdoc.

How have you approached hiring new team members?

My recent strategy is to approach people and ask their interests and career plans at meetings, etc. If I think they fit well, I ask if they want to join my lab. I think this is the best way to recruit good people.

Do you think mentorship is important for an academic career?

I welcome ideas from lab members, but I think negative selection is an important mentorship skill. By this, I mean that if an idea is too small or too focused on the details I have to reject it. I think it's important for young people to be conscious of the big picture and not to spend too much time looking for something small. Yi Zhang carried out this process of negative selection

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for me. At the time, I was a bit frustrated by it, but now I think he was right!

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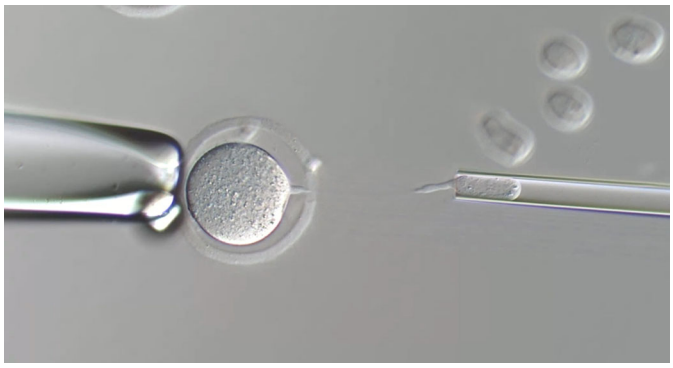
Did you ever consider an alternative/non-academic career path? What and why?

When I started learning micromanipulation, which is a cool technology to inject something such as sperm into the oocytes, I found it very fun. I love micromanipulation, maybe too much! For this reason, I did consider becoming an embryologist and performing assisted reproduction technology (ART).



Chromosomes being removed from a mouse oocyte.

Earlier this year, you attended the EMBO workshop celebrating the 60th anniversary of the discovery of X-chromosome inactivation (Moyano Rodriguez and Borensztein, 2023). How was it?

It was a fantastic meeting! Initially, I was surprised to be invited to it, because I had never attended an XCI meeting before, but I found in the meeting that my research about maternal Polycomb-dependent imprinting was well appreciated by the community. In addition, it was exciting to finally meet some researchers whom I previously knew by only name. Actually, it was also the first time that I had been in Europe. I felt that European research culture is closer to the Japanese one in that both appreciate researchers who focus on a specific problem. I like that approach, too.

Last year, you received the Ministry of Education, Culture, Sports, Science and Technology (MEXT) Young Scientists' Award. What did this achievement mean to you?

That prize, I think, means that I may be on the right scientific path, at least for now, but the future is unpredictable.

Finally, is there anything Development readers would be surprised to learn about you?

I don't know if it will be surprising or not, but I still spend about 70% of my time at the bench, for two reasons. First, micromanipulation is a difficult skill to master; even now, with >17 years of experience already, I am better than a year ago. So, it makes sense for me to do micromanipulation experiments to help things move quickly and efficiently. Also, I like to find answers to my questions by myself. I don't like to push lab members, but I can push myself to deliver the results as quickly as possible!

Azusa Inoue was interviewed by Alex Eve, Senior Editor at Development. This piece has been edited and condensed with approval from the interviewee.

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