（別紙様式５）

変革を駆動する先端物理・数学プログラム（FoPM）

国外連携機関長期研修　報告書

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受入先	Shvartsman Lab, Lewis Sigler Institute for Integrative Genomics, Princeton University (Department: Quantitative and Computational Biology)
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Background story

In Ozawa lab at the University of Tokyo, I have always been interested in the dynamics of signaling molecules, especially how molecules temporally regulate their downstream pathways and affect to cellular responses. In my master' s course, I investigated the temporal dynamics of a kinase, Akt. I utilized an optogenetic tool named PA-Akt [1] to control Akt activity by light illumination. By using the PA-Akt strategy, I measured the temporal phosphorylation patterns of three Akt isoforms (Akt1, Akt2, and Akt3) and found that the isoforms have different temporal properties. Additionally, I measured temporal phosphorylation level changes of Akt1 and Akt2 isoforms and their downstream molecules on mouse myotube, and the results were published in Science Signaling [2]. As the next step, I was interested in elucidating the physiological meanings of the Akt temporal dynamics in various biological phenomena and contexts. I was also eager to learn various research that applied mathematical models to quantitatively investigate biological phenomena.

Prof. Shvartsman has been working on developmental biology, especially on ERK kinase roles on *Drosophila* (fruit fly) development. I was fascinated by Shvartsman lab's research which aims to quantitatively investigate ERK roles, cell cluster formation, and developmental abnormalities using optogenetics and computational modeling in cells and tissues. I got interested in learning his lab's techniques to examine the relation of kinase dynamics to the signaling in biological phenomena.

I contacted to Prof. Shvartsman in April 2023, and he kindly accepted me as a host for this program. Around May, I started to prepare to go to the U.S. to do research at Shvartsman lab, which is located at Lewis-Sigler Institute for Integrative Genomics (LSI) in Princeton University. I applied to a non-degree graduate student program (visiting student research collaborators, VSRC) in the Quantitative Computational Biology (QCB) department, while applying for a F-1 visa. I was initially set my stay as 3 months from September to November, but in the middle of October, Prof. Shvartsman kindly offered me to extend my stay so that I could obtain data for publishing my results as a short paper. By receiving this wonderful opportunity, my stay finally became 4 months in total.

Figure 1 The beautiful campus of Princeton University.
Upper: Firestone Library,
Lower: Princeton University Chapel

My research theme and outcomes in Shvartsman lab

Since I have been working on optogenetics and interested in kinase signal transduction, Prof. Shvartsman and his lab's post-doc, Dr. Robert Marmion, offered me a research topic that employs optogenetic kinase manipulation in *Drosophila*. My task was to observe a change in *Drosophila* larva to pupa transition (pupation) timing when ERK signaling accelerated in a specific organ that regulates larva development.

An organ named the prothoracic gland (PG), one part of the ring gland which consists of three distinct parts adjacent to the brain, regulates *Drosophila* development by secreting a hormone called ecdysone [3,4]. Ecdysone is secreted

when the PG receives signals from other organs, such as prothoracicotropic hormone (PTTH) from neurons [3,4]. Ecdysone temporally regulates *Drosophila* larvae metamorphosis, that are, L1, L2, and L3 instar, by showing pulse-like increase at each stage [3]. Also, ecdysone titer largely increases when pupation happens [3]. PTTH silenced larvae showed slower pupation and larger pupa cases than the wild type, because of the extended duration of L3 instar larvae eating many foods before pupation [5].

Figure 2 Lewis-Sigler Institute of Integrative Genomics.

It is reported that ERK signaling (the ERK signaling cascade: RAS→SOS→RAF→MEK→ERK) plays roles in ecdysone biosynthesis and secretion in the PG [6, 7]. For example, the larvae that has constitutively active RAS within the PG was smaller than the normal size [6,7]. It was suggested that altered RAS signaling in the PG reduced body size by changing ecdysone secretion [6].

In my research in Shvartsman lab, I focused on the temporal association of ERK signaling and ecdysone biosynthesis in the prothoracic gland and how it regulates larvae pupation. To investigate ERK signaling in timing- and organ- specific manner, we employed optogenetics system that functions only in the prothoracic gland. We utilized optogenetics for MEK kinase called photoswitchable MEK (psMEK) [8-10]. MEK only has ERK as its substrate. By using psMEK, we can directly activate ERK without concerning signal divergence to other pathways. psMEK contains two Dronpa fluorescent proteins, which dimerizes and hinders the active site of MEK in the dark state. When we apply 500 nm green light, dimerized Dronpa dissociates and MEK can activates ERK.

We introduced psMEK to *Drosophila* larva only in their PG by using Gal4-UAS system with a PG-specific Gal4 driver. Gal4-UAS system is a widely used techniques in *Drosophila* genetics that enables organ specific expression of a target gene [11]. By using a *Drosophila* larva expressing psMEK in the PG, I performed pupation assay to observe the effect of timing- and organ- specific ERK signaling activation on pupation. Also, I visualized ERK activation states in the larva PG by conducting dpERK (dually phosphorylated ERK, activated ERK) immunostaining. We aim to publish the outcomes within a short paper in near future.

Lab life & research environment

We had lab meetings on Mondays and developmental biology colloquium on Fridays. I was inspired by various research topics every time. Moreover, there were many events inside and outside the lab. At the beginning of September, I participated LSI retreat outside the campus. I was surprised to learn a wide range of research, from experimental ones using diverse model animals (such as mouse, *C. elegans*, and flies) to theoretical ones. In October, I got my first chance to attend the international conference called DICE (Dynamics in Cells and Embryos) conference at Flatiron institute in New York, where Prof. Shvartsman has his another lab. At the end of November, there was another retreat which held only for the Shvartsman lab members. All the members had presentations about their research. At these events, not only I deepened my knowledge on developmental and quantitative biology, but I also interacted with many researchers and graduate students and was inspired by their thoughts on science. It was meaningful for me to learn various biological research, which will lead me to broaden my doctoral research in Japan.

Other activities

In front of the campus, there was Nassau Street that has good food and shops. After the holiday season began on the Thanksgiving Day, I enjoyed beautiful illumination around there like by attending Christmas tree lighting up event. Also, I enjoyed home parties at the lab mates' house. They invited me to the "Fall formal" as well, which is one of the biggest parties held by graduate students. I enjoyed the atmosphere such as I was always watching in movies. On some weekends, I visited New York several times by taking New Jersey transit for about 1 and half hours from Princeton. I enjoyed many wonderful food and museums such as Metropolitan Museum and American Museum of Natural History. During my stay, I felt that the climate was nice overall and suitable for these activities and everyday life in both Princeton and New York, if I was taking care of adjusting my body temperature because it was colder than Tokyo. These experiences became good topics to talk about with my lab mates. I got to know how they spend their weekends and think about their lives and leisure time around Princeton.

Figure 4 Times Square in New York.

Acknowledgement

I would like to show my deepest appreciation to Prof. Stanislav.Y Shvartsman for having me as a visiting research student in his lab. I also would like to offer my special thanks to Dr. Robert A. Marmion, who kindly guided me on all the experiments and discussions throughout my stay. All the members of the Shvartsman lab warmly welcomed me, and I am grateful to each one of them. I hope to keep in touch with them in the future. Also, I thank Dr. Kara Dolinski, Ms. Laura Gallagher-Katz, and all the administrative staffs in LSI and Princeton University for their quick and kind support.

I also thank Prof. Takeaki Ozawa for continuously supporting me from when preparing for this program until complete it and coming back to Japan. My supervisor in Japan, Dr. Genki Kawamura, gave me warm encouragement throughout my stay. The secretary in Ozawa lab, administrative staffs in WINGS Desk and the School of Science in UTokyo have always offered me kind help, and I could not have realized this program without their support.

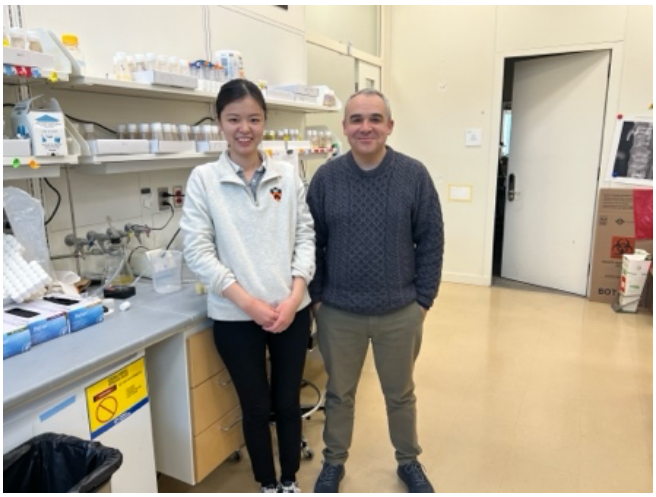

Figure 5 Photo with Prof. Shvartsman in the end of my stay.

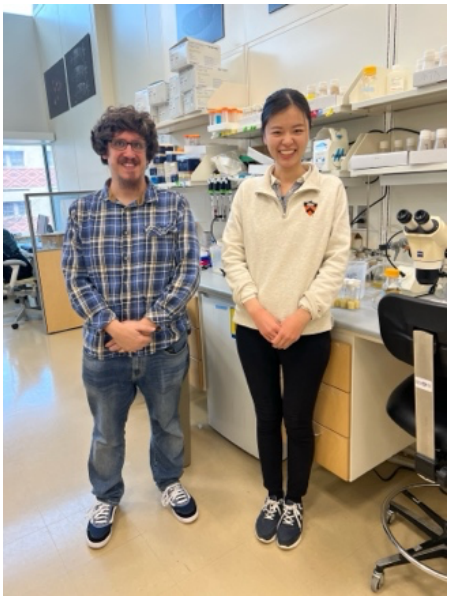

Figure 6 Photo with Dr. Robert Marmion as well.

References

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