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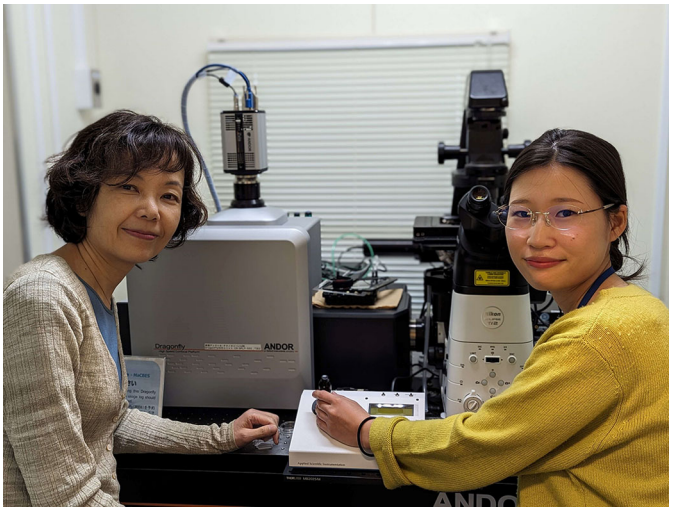
INTERVIEW

The people behind the papers – Akane Hatsuda and Mineko Kengaku

Calcium signals evoked by neuronal activity play an important role in dendritic development and establishment of neuronal circuit connectivity. A new study demonstrates a link between activity-dependent dendritic development and AMP-activated protein kinase (AMPK)-dependent mitochondrial homeostasis in rodent hippocampus. We caught up with first author Akane Hatsuda and corresponding author Mineko Kengaku, Professor at Kyoto University.



Mineko Kengaku (left) and Akane Hatsuda (right)

Mineko, can you give us your scientific background and the questions your lab is trying to answer?

MK: I was fascinated by the diversity and functionality of animal morphology and majored in Zoology at the University of Tokyo. I studied primary axis formation in the *Xenopus* brain for my PhD. In fact, my PhD study was published as two articles in Development. Then I went on to do a postdoc in morphogenesis with Cliff Tabin at Harvard Medical School. It was the golden era of morphogen signalling, and in Cliff's lab I had the privilege of witnessing various groundbreaking discoveries up close. After that experience in Boston, I moved back to Japan and began studying the dynamics of cortical formation in the developing mouse brain. My lab is particularly interested in the mechanisms of position and shape control of growing neurons, and we primarily employ live imaging as a key research approach.

Akane, what brought to you join Mineko's lab, and what drives your research today?

AH: I was interested in brain development and curious about what creates individual differences in neural circuits and brain function. When I joined the Kengaku Lab as a Master's student, I learned various techniques of live imaging and was really fascinated by the dynamic movement of mitochondria, such as fission and fusion. Hundreds of mitochondria seem to move randomly in a single neuron, but their movement is actually precisely regulated. My research is largely driven by a desire to understand the mechanism behind the precise regulation.

What was known about the role of mitochondrial homeostasis in developing neurons before your work?

MK: Mitochondrial biogenesis and transport are essential for neuronal development to meet increased energy needs during the rapid outgrowth of dendrites and axons. Vanderhaeghen's group has recently demonstrated that mitochondrial homeostatic control is an important determinant of the timing of differentiation and maturation of cortical neurons (Iwata et al., 2020, 2023). Molecular mechanisms of mitochondrial transport in neurons are also well understood by many excellent previous studies. However, less is known about what triggers mitochondrial biogenesis and degradation in neurons, and how they affect neuronal development.

Can you summarise the key findings in a paragraph?

MK: It is known that calcium influx by neuronal activity induces extension of dendritic processes in immature neurons. Besides the already identified molecular signals regulating transcription and cytoskeletal remodelling, we demonstrate that neural activity regulates mitochondrial fission/fusion balance, thereby adapting to elevated energy demands in emerging dendrites. We identify AMPK as an important mediator of activity-dependent neuronal growth, which induces mitochondrial fission and the downstream pathways regulating mitophagic clearance of damaged mitochondria. AMPK function has been highlighted in prevention and progression of neurodegenerative diseases such as Alzheimer's disease (AD). AMPK activation in early pathogenesis of AD improves mitochondrial functions to restore energy balance. However, disruption of Ca^{2+} homeostasis overactivates AMPK in advanced AD, inducing mitochondrial fragmentation and degeneration. Despite the accumulating evidence for AMPK function in pathological conditions, however, the physiological function of AMPK during brain development is less understood. Our results suggest that maintaining proper levels of AMPK activity is crucial for maintaining healthy mitochondria in growing dendrites. Also, this is the first study demonstrating that the regulation of mitophagy is important for neuronal development.

Were you surprised to observe periodic fluctuation of AMPK activity in synchrony with calcium influx triggered by neuronal activity?

AH: Yes, I was. It was also surprising that the pattern of AMPK fluctuation varies from cell to cell. I assumed that the pattern of AMPK activity may cause differences in mitochondrial status and determine individual dendritic morphology. The periodic

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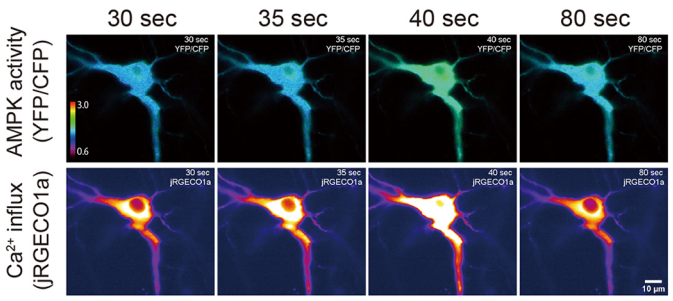

Dynamic AMPK activation (measured by fluorescence resonance energy transfer imaging) synchronized with spontaneous neuronal firing (detected using the red fluorescent Ca^{2+} indicator jRGECO1a). CFP, cyan fluorescent protein; YFP, yellow fluorescent protein.

fluctuation is reasonable to prevent overactivation of AMPK, because the signalling pathway must be turned off in unwanted conditions. Spatiotemporal activity of kinases is difficult to capture, and much remains unclear. FRET probes are an effective way to elucidate this, but currently only those for major kinases have been developed. The development of various FRET probes and other tools to analyse the spatiotemporal activity of kinases would be important to understand how various signalling pathways are differentially regulated depending on the cellular situation.

Why do you think the AMPK-dependent mitochondrial changes are only seen in dendrites, not axons?

MK: There are multiple AMPK-related kinases expressed in the axon that are known to regulate mitochondrial fission/fusion balance. These include SAD and NUAK kinases, which have been shown to be constitutively activated by the upstream kinase LKB1 (STK11) in the axon. By contrast, AMPK activity is independent of LKB1 and we found that it dynamically oscillates as a result of Ca^{2+} influx and CaMKK2 activity. Dendritic structures are more sensitively regulated by neuronal activity than axons. We surmise that AMPK dynamically regulates dendritic mitochondria via an activity-dependent pathway, whereas other AMPK-related kinases are involved in the more static regulation of mitochondrial homeostasis in the axon.

Akane, did you have any particular result or eureka moment that has stuck with you?

AH: I was so excited when I first observed mitochondrial dynamics in neurons. I didn't expect that mitochondria were such dynamic organelles. Interestingly, the mitochondrial dynamics correlate with dendritic dynamics. And mitochondrial fission, fusion and transport are particularly active during early development, when dendritic elongation and retraction occur significantly. I felt that there should be a link between cellular status and mitochondrial dynamics, and I am glad to reveal one of the regulatory mechanisms.

I was so excited when I first observed mitochondrial dynamics in neurons

And the flipside: were there any moments of frustration or despair?

AH: I had a hard time when the experiment stalled during the COVID-19 pandemic. However, I was able to perform image analysis during lockdown and found important phenomena that served as a starting point for my research. This experience taught me that sometimes it is important to allow enough time to stare at data throughout the day!

Where will this story take the Kengaku lab?

MK: The *in vivo* function of AMPK in the brain has yet to be elucidated. We aim to explore it, including its role in circuit formation processes.

In the paper, you used many imaging techniques to answer your questions – how do you think the advancement of imaging techniques will impact the future of developmental biology?

MK: Developmental processes are dynamic, and I believe the importance of live imaging has already been widely accepted. Technological innovations are ongoing, with improvements not only in spatiotemporal resolution, but also in deep imaging and multiplex labelling techniques, and we can expect new discoveries of developmental phenomena in the future.

Why did you choose to submit your paper to Development?

MK: To be honest, we initially submitted to more general cell biology journals, but they did not recognize AMPK activity in normal neuronal differentiation as novel enough, as it has been identified in pathological mechanisms. I personally consider Development as my home journal, and I thought it would be great for Akane's academic career if her work could be published in Development. She will do her postdoc study on honeybee caste development with Gene Robinson.

Finally, let's move outside the lab – what do you like to do in your spare time?

MK: Cooking and exploring new restaurants.

AH: I like to watch anime and to read. It refreshes my brain by forgetting about the research and entering into another world.

Reference

Hatsuda, A., Kurisu, J., Fujishima, K., Kawaguchi, A., Ohno, N. and Kengaku, M. (2023). Calcium signals tune AMPK activity and mitochondrial homeostasis in dendrites of developing neurons. *Development* **150**, dev201930. doi:10.1242/dev.201930

Iwata, R., Casimir, P. and Vanderhaeghen, P. (2020). Mitochondrial dynamics in postmitotic cells regulate neurogenesis. *Science* **369**, 858-862. doi:10.1126/science.aba9760

Iwata, R., Casimir, P., Erkol, E., Boubakar, L., Planque, M., Gallego López, I. M., Ditkowska, M., Gaspariunaite, V., Beckers, S., Remans, D. et al. (2023). Mitochondria metabolism sets the species-specific tempo of neuronal development. *Science* **379**, 6632. doi:10.1126/science.abn4705