BIOGRAPHICAL SKETCH

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NAME: Watanabe, Shigeki

eRA COMMONS USER NAME (credential, e.g., agency login): SHIGEKIWATANABE

POSITION TITLE: Assistant Professor of Cell Biology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Utah	B.A.	05/2004	Biology
University of Utah	Ph.D.	05/2013	Biology
University of Utah	Postdoctoral	12/2015	Neuroscience
Charité – Universitätzmedizin, Berlin	Postdoctoral	12/2015	Neuroscience

A. Personal Statement

I was trained in genetics, molecular biology and cell biology in *C. elegans* and mouse with a special focus on imaging neuronal functions. In addition, I have developed two novel techniques in electron microscopy that allows visualization of proteins and membrane dynamics at synapses. One technique induces membrane movement using optogenetic stimulation of neurons and captures the subsequent events at a millisecond temporal resolution using a rapid high-pressure freezing method. Another technique pinpoints the locations of proteins within their subcellular context by coupling super-resolution imaging with electron microscopy. Using these techniques, I have been studying cellular and molecular mechanisms underlying synaptic transmission and synaptic plasticity. Moreover, I have collaborated with many scientists across the world and worked with various model organisms including zebrafish. Many of these collaborations have led to peer-reviewed publications, demonstrating my ability to carry out collaborative research. I have mentored many students including two post-doctoral fellows, two graduate students, five undergraduate researchers, and four summer interns.

a. Watanabe, S., T. Trimbuch, M. Camacho-Pérez, B.R. Rost, B. Brokowski, B. Söhl-Kielczynski, A. Felies, M.W. Davis, C. Rosenmund, and E.M. Jorgensen. 2014. Clathrin regenerates synaptic vesicles from edosomes. Nature 515, p228-33, DOI 10.1038/nature13846. PMCID: PMC4291189.
b. Watanabe, S., B. Rost., M. Camacho, M. W. Davis, B. Söhl-Kielczynski, A. Felies, C. Rosenmund and E.M. Jorgensen. 2013. Ultrafast endocytosis at mouse hippocampal synapses. Nature. 504, 242-7. doi: 10.1038/12809. PMCID: PMC3957339.
c. Watanabe, S., Q. Liu, M.W. Davis , N. Thomas, J. Richards, G. Hollopeter, M. Gu, N.B. Jorgensen and E.M. Jorgensen. 2013. Ultrafast endocytosis at the C. elegans neuromuscular junction. eLife 2:e00723. PMCID: PMC3762212.
d. Watanabe, S., A. Punge , G. Hollopeter , K.I. Willig, R.J. Hobson , M.W. Davis , S.W. Hell , and E.M. Jorgensen. 2011. Protein localization in electron micrographs using fluorescence nanoscopy. Nature Methods 8, p80-84. PMCID: PMC3059187.

B. Positions and Honors

Positions and Employment

2003-2005 Research assistant, Department of Ophthalmology, University of Utah, UT
2004-2007 Lab specialist, Department of Biology, University of Utah, UT
2005-2006 Research assistant, Department of Ophthalmology, University of Utah, UT
2013 -2015 Post-doctoral research fellow, Department of Biology, University of Utah, UT
2013 -2015 Post-doctoral research fellow, Charité – Universitätzmedizin Berlin, Berlin, Germany
2016 - Assistant professor, Department of Cell Biology, Johns Hopkins University, MD

Other Experience and Professional Memberships

2015- Member, Society for Neuroscience
2015- Member, American Society for Cell Biology
2015- Member, Biophysical Society

Honors

2010 Poster award. 1st place. Bioscience symposium, University of Utah, UT
2010 Poster award. 1st place. New optical methods in cell physiology conference, MA.
2011 EMBO short-term fellowship (EMBO ASTF 485-2010)
2011 Stringfellow fellowship, University of Utah, UT
2012 EMBO short-term fellowship (EMBO ASTF 443-2012)
2013 Riser award for outstanding research, University of Utah, UT
2013 Nemko Prize in cellular or Molecular Neuroscience, the Society for Neuroscience
2014 Grass fellowship, Marine Biological Laboratories, Woods Hole, MA
2015 Emil du Bois-Reymond prize, the German Physiological Society
2015 Eppendorf and Science Prize for Neurobiology
2015 Merton Bernfield Award, the American Society for Cell Biology

C. Contribution to Science

My major contributions to the scientific community are in 1) discovering a novel mechanism for synaptic vesicle endocytosis and 2) exocytosis, 3) developing cutting-edge methods in electron microscopy, and 4) collaborating with scientists from all over the world.

1) Synaptic vesicle endocytosis. To sustain neurotransmission, synaptic vesicles must be recycled locally at synapses. Two models for synaptic vesicle endocytosis have been put forward based on the morphological studies in frog neuromuscular junctions. Heuser and Reese proposed that endocytosis occurs via a slow mechanism using clathrin scaffolds. Ceccarelli and his coworkers proposed a fast mechanism, kiss-and-run. Since then, many studies have sought to identify the mechanism for synaptic vesicle endocytosis. However, instead of resolving the issue, conflicting evidence has accumulated over the years.
To investigate how endocytosis takes place, I developed a method, 'flash-and-freeze' fixation that couples optogenetic stimulation with rapid high-pressure freezing and captures endocytosis at millisecond temporal resolution. I have demonstrated that an alternative, ultrafast mechanism is at work. Ultrafast endocytosis retrieves membrane in a large vesicle within 100 ms after exocytosis both in *C. elegans* and mouse hippocampal synapses. I have further demonstrated that these large endocytic vesicles are then delivered to an endosome. Clathrin then regenerates synaptic vesicles from endosomes. At non-physiological temperature, however, ultrafast endocytosis is blocked, and clathrin regenerates synaptic vesicles directly from the plasma membrane. These results likely alter the current dogma in the field.

a. Watanabe, S., T. Trimbuch, M. Camacho-Pérez, B.R. Rost, B. Brokowski, B. Söhl-Kielczynski, A. Felies, M.W. Davis, C. Rosenmund, and E.M. Jorgensen. 2014. Clathrin regenerates synaptic vesicles from edosomes. Nature 515, p228-33, DOI 10.1038/nature13846. PMCID: PMC4291189.
b. Watanabe, S., B. Rost., M. Camacho, M. W. Davis, B. Söhl-Kielczynski, A. Felies, C. Rosenmund and E.M. Jorgensen. 2013. Ultrafast endocytosis at mouse hippocampal synapses. Nature. 504, 242-7. doi: 10.1038/12809. PMCID: PMC3957339.

c. Watanabe, S., Q. Liu, M.W. Davis , N. Thomas, J. Richards, G. Hollopeter, M. Gu, N.B. Jorgensen and E.M. Jorgensen. 2013. Ultrafast endocytosis at the C. elegans neuromuscular junction. eLife 2:e00723. PMCID: PMC3762212.
d. Gu, M., Q. Liu, S. Watanabe, L. Sun, B. Grant, and E.M. Jorgensen. 2013. AP2 hemicomplexes contribute independently to synaptic vesicle endocytosis. eLife 2, p00190. PMCID: PMC3591783.

2) Synaptic vesicle exocytosis. SNAREs mediate fusion of two opposing membranes. Unlike the constitutive exocytosis of vesicles in other cells, neurons must regulate fusion of vesicles based on the activity. The calcium influx through voltage-gated calcium channels act on calcium-sensing molecule, synaptotagmin, to regulate fusion of synaptic vesicles. My colleagues and I have shown that another molecule, complexin, is also required for the regulated fusion. When calcium enters the terminal, a set of vesicles fuses with the plasma membrane for transmitter release by the SNAREs-mediated process. These vesicles are called release-ready vesicles and are thought to be those vesicles in the close proximity of the plasma membrane. Our work demonstrated that the SNAREs are likely already engaged to bring vesicles in direct contact with the membrane before the calcium influx and that complexin likely acts as a brake for fusion. It was the first demonstration that docking of vesicles to the membrane is mediated by SNAREs.

a. Watanabe, S., B. Rost., M. Camacho, M. W. Davis, B. Söhl-Kielczynski, A. Felies, C. Rosenmund and E.M. Jorgensen. 2013. Ultrafast endocytosis at mouse hippocampal synapses. Nature. 504, 242-7. doi: 10.1038/12809. PMCID: PMC3957339.
b. Watanabe, S., Q. Liu, M.W. Davis , N. Thomas, J. Richards, G. Hollopeter, M. Gu, N.B. Jorgensen and E.M. Jorgensen. 2013. Ultrafast endocytosis at the C. elegans neuromuscular junction. eLife 2:e00723. PMCID: PMC3762212.
c. Hobson, R.J., Q. Liu, S. Watanabe and E.M. Jorgensen. 2011. Complexin maintains vesicles in the primed state in C. elegans. Current Biology 21, p106-113. PMCID: PMC348763.
d. Hammarlund, M., Palfreyman, M. T., Watanabe, S., Olsen, S., and Jorgensen, E. M. (2007). Open syntaxin docks synaptic vesicles. PLoS Biol. 5, e198. PMCID: PMC3048763.

3) Novel methods in electron microscopy. I have developed two novel techniques in electron microscopy: “flash-and-freeze” and nano-resolution fluorescence electron microscopy. There are two major problems in electron microscopy: lack of temporal information and lack of molecular information. As discussed in 1), the “flash-and-freeze” adds temporal information in electron micrographs by stimulating physiological changes in neurons with channelrhodopsin and capturing the subsequent cellular dynamics. This technique can be widely used to study other cellular events that can be triggered by light stimulation. Many optogenetic tools are or will be available, and these tools are compatible with the ‘flash-and-freeze’ approach.
Nano-resolution fluorescence electron microscopy adds molecular information to electron micrographs by coupling super-resolution imaging with electron microscopy. I found a method that preserves fluorescence through harsh fixation and plastic embedding and successfully performed super-resolution imaging on plastic sections. This technique can be used to pinpoint the locations of proteins within their subcellular context, providing cell biologists a way to map molecular topology of a cell.

a. Watanabe, S. M.W. Davis, and E.M. Jorgensen. 2014. Flash-and-freeze electron microscopy: coupling optogenetics with high-pressure freezing. In Imaging nanoscale dynamics at synapses. Ed. V. Nägerl and A. Triller. Springer Verlag. Neuromethods 84, p43-57. Book chapter.
b. Watanabe, S., M. Lehmann, E. Hujber, R.D. Fetter, J. Richards, B. Söhl-Kielczynski, A. Felies, C. Rosenmund, J. Schmoranzer, and E.M. Jorgensen. 2014. Nanometer-resolution fluorescence electron microscopy (nano-fEM) in cultured cells. Ed. J. Kuo. Humana Press. In Methods in Molecular Biology: Electron Microscopy 1117, p. Book chapter.
c. Watanabe, S., Richards, J., Hollopeter, G., Hobson, R.J., Davis, M.W., and Jorgensen, E.M. 2012. Nano-fEM: protein localization using correlative photo-activated localization microscopy and electron microscopy. Journal of Visual Experiments 3, e3995. doi: 10.3791/3995. PMCID: PMC3566706
d. Watanabe, S., A. Punge , G. Hollopeter , K.I. Willig, R.J. Hobson , M.W. Davis , S.W. Hell , and E.M. Jorgensen. 2011. Protein localization in electron micrographs using fluorescence nanoscopy. Nature Methods 8, p80-84. PMCID: PMC3059187.

4) Collaborative research. In addition to the described contributions, I have elucidated cellular and molecular mechanisms underlying synaptic stability and plasticity. I have developed ultrastructural assays to characterize mutants defective in these processes. The tools and protocols I developed have been widely distributed and adapted by many laboratories across the world.

a. Shao, Z., Watanabe, S., Christensen, R., Jorgensen, E.M., and Colón-Ramos, D.A., (2013). Synapse location during growth depends on glia location, Cell 154, 337-350. PMCID: PMC3808971.
b. Oikonomou, G., Perens, E. A., Lu, Y., Watanabe, S., Jorgensen, E. M., and Shaham, S. (2011). Opposing activities of LIT-1/NLK and DAF-6/patched-related direct sensory compartment morphogenesis in C. elegans. PLoS Biol. 9, e1001121. PMCID: PMC3153439.
c. Ou, C.-Y., Poon, V. Y., Maeder, C. I., Watanabe, S., Lehrman, E. K., Fu, A. K. Y., Park, M., Fu, W.-Y., Jorgensen, E. M., Ip, N. Y., et al. (2010). Two cyclin-dependent kinase pathways are essential for polarized trafficking of presynaptic components. Cell 141, 846–858. PMCID: PMC3168554.
d. Pellettieri, J., Fitzgerald, P., Watanabe, S., Mancuso, J., Green, D. R., and Sánchez Alvarado, A. (2010). Cell death and tissue remodeling in planarian regeneration. Dev. Biol. 338, 76–85. PMCID: PMC2835816.

D. Research Support

Ongoing Research Support

Start up funds, Johns Hopkins University Watanabe (PI) 01/01/2016-
Cellular and molecular characterizations of rapid changes during synaptic plasticity
The goal of this study is to investigate novel endocytic pathway and gain insight into the molecular mechanisms underlying learning and memory in our brain.
Role: PI

Completed Research Support

Grass fellowship, Marine Biological Laboratory Watanabe (PI) 05/26/2015-08/30/2015
Mechanisms of synaptic plasticity in mouse hippocampal synapses
The goal of this study was to establish a role of ultrafast endocytosis in AMPA receptor trafficking and link the endocytic pathway with synaptic plasticity.
Role: PI

EMBO ASTF 443-2012 Watanabe (PI) 10/15/2012-01/15/2013
Synaptic vesicle endocytosis at hippocampal synapses
The goal of this study was to elucidate the mechanisms underlying endocytosis of synaptic vesicle endocytosis in mouse hippocampal cultures.
Role: PI

Stringfellow fellowship, University of Utah Watanabe (PI) 06/01/2011-5-31/2012
Synaptic vesicle endocytosis at *C. elegans* neuromuscular junctions
The goal of this study was to elucidate the mechanisms underlying endocytosis of synaptic vesicle endocytosis in *C. elegans* neuromuscular junctions.
Role: PI

EMBO ASTF 485-2010 Watanabe (PI) 03/21/2010-06/20/2010
Nanoscale localization of AMPA-type glutamate receptors
The goal of this study is to localize AMPA receptors within the molecular architecture of the synaptic spine using correlative fluorescence nanoscopy and electron microscopy.
Role: PI