BIOGRAPHICAL SKETCH

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NAME: Watanabe, Shigeki

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

POSITION TITLE: Associate 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 several novel techniques in electron microscopy to follow membrane and protein dynamics at synapses with a millisecond temporal resolution. I have been studying cellular and molecular mechanisms underlying synaptic transmission and synaptic plasticity. I have discovered a novel clathrin-independent endocytic pathway in mouse hippocampal neurons and *C. elegans* neuromuscular junctions. In the last few years, my lab has been studying molecular mechanisms underlying this novel pathway as well as other membrane trafficking events at synapses that govern synaptic transmission and plasticity. Moreover, my lab has been collaborating with many scientists across the world. Many of these collaborations have led to peer-reviewed publications, demonstrating my commitment to share both resources and expertise. I have successfully competed for several government-funded grants and published manuscripts describing the results from the experiments proposed in these grants, demonstrating my ability to conceive and oversee projects, perform experiments of my own, and train students and postdoctoral fellows to conceive and carry out their projects. In addition to the research-related skills, my training programs for students and postdoctoral fellows include attendance to annual ethics course and mentoring undergraduate research fellows. I also participate in and also encourage my trainees to participate in local diversity and inclusion programs.

a. Kusick, G.F., Chin, M., Lippmann, K., Adula, K.P., M. Wayne, Davis, Jorgensen, E.M., and Watanabe, S., (2018) BioRxiv. doi: https://doi.org/10.1101/509216

b.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.

c. 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.

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.

For all other publications,
https://www.ncbi.nlm.nih.gov/sites/myncbi/shigeki.watanabe.1/bibliography/47664889/public/?sort=date&direction=ascending

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
2016-2019 Assistant professor, Department of Cell Biology, Johns Hopkins University, MD
2020- Associate 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
2015-present Instructor, Neurobiology course, Marine Biological Laboratory
2016-2017 Topic Editor, Frontiers in Cellular Neuroscience
2018-present Associate editor, FASEB journal
2018-present Instructor, Cajal School of Neuroscience
2019 NSF GRFP review panelist
2019 NIH SYN reviewer
2019 HFSP *ad hoc* reviewer

Honors

2011 Stringfellow fellowship, University of Utah, UT
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
2016 Kazato Prize, the Kazato Research Foundation, Japan
2017 Morphological Sciences Award, American Association of Anatomists
2017 Ministry Prize, Ministry of Science, Technology, and Education, Japan
2017 FEI Young Investigator Award, Finalist, FEI
2018 Alfred P. Sloan Foundation fellow
2018 NIH Director's New Innovator Award
2019 National Academy of Science, Kavli Frontiers of Science symposium invitee
2019 McKnight Foundation Scholar
2019 Klingenstein and Simons Foundation Scholar
2019 NYSCF Neuroscience investigator award, Finalist

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., Mamer, L.E., Raychaudhuri, S., Luvsanjav, D., Eisen, J., Trimbuch, T., Söhl-Kielczynski, B., Fenske, P., Milosevic, I., Rosenmund, C., and Jorgensen, E.M. (2018) Synaptojanin and endophilin mediate neck formation during ultrafast endocytosis. Neuron 98, 1184-1197. PMCID: PMC6086574
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, 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. 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.

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. 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. It was the first demonstration that docking of vesicles to the membrane is mediated by SNAREs. Using a novel approach Zap-and-freeze that couples electrical stimulation of neurons with high-pressure freezing, we have now shown that these docked vesicles are not static at the membrane: they undock and redock transiently following an action potential. This time course parallels paired-pulse facilitation. In addition, we visualized the site of asynchronous fusion for the first time. Our R01 (1R01 NS105810-01A1) is supporting the follow-up experiments to figure out the molecular underpinnings of fusion site organization at mammalian central synapses.
a. Kusick, G.F., Chin, M., Lippmann, K., Adula, K.P., M. Wayne, Davis, Jorgensen, E.M., and Watanabe, S., (2018) BioRxiv. doi: https://doi.org/10.1101/509216
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. 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 have been developing 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., Flash-and-freeze: coordinating optogenetic stimulation with rapid freezing to visualize membrane dynamics at synapses with millisecond resolution, *Front. Synaptic Neurosci*. 8:24. doi: 10.3389/fnsyn.2016.00024. PMID:27594835
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 also contributed to understanding the role of glia in synaptogenesis, and I have current collaboration to investigate the role of microglia in brain functions. 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. Itoh, K., Murata, D., Kato, T., Yamada, T., Araki, Y., Saito, A., Adachi, Y., Igarashi, A., Li, S., Pletnikov, M., Huganir, R.L., Watanabe, S., Kamiya, A., Iijima, M., and Sesaki, H. (2019) Brain-specific Drp1 regulates postsynaptic endocytosis and dendrite formation independently of mitochondrial division. eLife DOI: 10.7554/eLife.44739.
b. Nakamura, H, Lee, A, Afshar, A, Watanabe, S., Rho, E, Razavi, S, Suarez, A., Lin, Y., Tanigawa, M., Huang, B., DeRose, R., Bobb, D., Hong, W., Gabelli, S.B., Goutsias, J., Inoue, T. (2017). Intracellular Production of Hydrogels and Synthetic RNA Granules by Multivalent Interactions. Nature Materials 17, 79-81.
c. 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.
d. 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.

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

1DP2NS111133-01(Watanabe PI) 9/30/18-6/30/23 3.0 calendar months
NIH/NINDS $300,000 Annual Direct Costs
Reviving electron microscopy for synaptic cell biology
The goal of this grant is to develop novel approaches in electron microscopy to study cell biology of the synapse with unprecedented spatial and temporal resolution.

1R01 NS105810-01A1 (Watanabe PI) 9/30/18-7/31/23 4.2 calendar months
NIH/NINDS $218,750 Annual Direct Costs
Spatial and molecular determinants of fusion probability and timing
The goal of this grant is to study the functional and structural organization of synaptic vesicle fusion sites and gain insight into the mechanisms underlying transsynaptic arrangement of calcium channels and vesicles.

McKnight Foundation Scholar 7/1/19-6/30/22 1.0 calendar month
McKnight Foundation $75,000 Annual Direct Costs
Mechanistic insights into membrane remodeling at synapses
The goal of this project is to develop new approaches to study the molecular details of membrane remodeling at millisecond temporal resolution.

MJFF Grant (Watanabe PI/Edwards PI) 6/1/19-5/31/21 1 calendar month
Michael J. Fox Foundation $175,000 Annual Direct Costs
Presynaptic mechanisms in Parkinson’s Disease

The goal of this project is to investigate the functions of two Parkinson's Disease-related proteins, alpha-synuclein and LRRK2, in synaptic membrane trafficking.

Research Fellowship in Neuroscience (Watanabe PI) 9/15/18-9/14/20 1.0 calendar month
Alfred P. Sloan Foundation $32,418 Direct Costs in Yr 1
Mechanisms of synaptic vesicle recycling
The goal of this project is to develop new approaches to study the molecular details of membrane remodeling at millisecond temporal resolution.

R01 2R01DC011099-06A1 (Pereda PI) 4/1/17-1/31/19 1 calendar month
NIH/NIDCD $50,549 Annual Direct Costs
Plasticity of auditory electrical synapses
The goal of this grant is to study the mechanisms of plasticity of electrical synapses using zebrafish as a model system.

NSF 1727271 (Agrawal PI/Watanabe PI) 9/1/2017-8/31/2020 0.9 calendar months
NSF (CMMI) $94,859 Annual Direct Costs
Collaborative research: Biophysical and Molecular mechanisms of ultrafast endocytosis at neuronal synapses
The goal of this grant is to use experimental and computer simulation approaches to study the role of tension in ultrafast endocytosis.

Whitman fellowship (Watanabe PI) 6/10/2018-8/10/2019 0.1 calendar month
Marine Biological Laboratory
Role of synaptic ribbons in synaptic transmission
The goal of this study is to investigate the role of synaptic ribbons in sensory signal transmission from cone photoreceptors of Zebrafish.

Klingenstein Foundation (Watanabe PI) $75,000 Annual Direct Costs 0.8 calendar month
The cellular and molecular basis of short-term plasticity
The goal of this project is to study how the strength of synaptic transmission is controlled on a millisecond time scale using time-resolved electron microscopy.

R01 (Waites PI) 7/1/19-6/30/21 0.1 calendar month
NIH/NINDS $10,794
Uncovering the roles of ubiquitination and the ESCRT pathway in degradative sorting of SV proteins
The goal of this project is to localize ESCRT proteins to organelles at synapses using correlative super-resolution and electron microscopy imaging.

<u>Completed Research Support</u>
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.

Discovery funds, Johns Hopkins University Watanabe (PI) 7/1/2016-6/30/2017
Synaptic lipidology at a millisecond temporal resolution
The goal of this study is to develop novel tools to study synaptic lipid organization and trafficking at a millisecond temporal resolution.

Start up funds, Johns Hopkins University Watanabe (PI) 01/01/2016-12/31/2018
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.