JCB Journal of Cell Biology

SPOTLIGHT

Neurexin nanoclusters: A novel structure at presynaptic terminals

Daichi Kawaguchi[1] and Yukiko Gotoh[1,2]

The trans-synaptic cell adhesion molecule neurexin regulates synaptic functions but its high-resolution subcellular localization and dynamics were unknown. Trotter et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201812076) describe previously unrecognized nanoscale clusters of neurexin-1 in presynaptic terminals and their regulation by ADAM10-mediated proteolysis.

Synapses are specialized subcellular interfaces between neurons that transmit electrochemical signals and serve as fundamental information processing units of the brain. Changes in synapse structure and function are the basis of learning and memory, while aberrant synaptic development underlies a variety of neuropsychiatric disorders. Trans-synaptic cell adhesion molecules are crucial to confer specificity between synaptic partners during synapse formation and control their mature properties and dynamics. Neurexins (Nrxns) are one family of presynaptic molecules that bind to postsynaptic ligands such as neuroligins, leucine-rich repeat transmembrane proteins, neurexophilins, dystroglycan, $GABA_A$ receptors, and GluD2-cerebellin-1 and mediate the proper assembly and functional maturation of synapses in an isoform-specific manner (1). In mice, Nrxns are encoded by three genes, *Nrxn1–3*, with numerous spliced isoforms, and the genetic deletion of Nrxns impairs the regulation of presynaptic Ca^{2+} channels and neurotransmitter release and reduces the trans-synaptic recruitment of postsynaptic AMPA-type glutamate receptors (AMPARs; 2, 3). Recent studies with the use of super-resolution microscopy techniques unveiled trans-synaptic molecular nanocolumns within the active zone that axially align presynaptic release sites and postsynaptic receptors for efficient signal transmission (4–6). Related to these observations, overexpressed Nrxn1β tagged with a biotin acceptor peptide was found to cluster at presynaptic terminals of cultured mouse hippocampal neurons (7). However, the precise location and dynamics of endogenous Nrxns at the synaptic junction have remained elusive.

In this issue, Trotter et al. describe super-resolution 3D stochastic optical reconstruction microscopy (STORM) imaging in mouse hippocampal neurons and report an unexpected localization pattern of Nrxn1, namely, a nanoscale cluster-like presynaptic organization (Fig. 1). To investigate the precise localization of Nrxn1, the authors generated conditional knockin mice that express HA-tagged Nrxn1 for anti-HA immunostaining (8). Each Nrxn1 nanocluster covered 15–20% of the Homer1-covered synaptic cleft in cultured hippocampal neurons and hippocampal sections. Only a fraction (~40%) of excitatory synapses contained Nrxn1 nanoclusters in hippocampal neurons, and most of these synapses harbored only a single nanocluster that comprised more than four Nrxn1 molecules. These surprising results suggest that Nrxn1 nanoclusters are not associated with many trans-synaptic nanocolumns at a given time in a given synapse. Moreover, Nrxn1 nanoclusters showed localization and clustering patterns distinct from SynCam and N-cadherin, two other trans-synaptic cell adhesion molecules that form ring-like structures surrounding the active zone. Therefore, Nrxn proteins appear to show a unique localization and clustering pattern that is distinct from any other known synaptic molecules.

To rule out the possibility that Nrxn1 nanoclusters were artifacts due to the insertion of the HA tag into the Nrxn1 protein, Trotter et al. (8) confirmed that endogenous wild-type Nrxn proteins detected by anti-pan-Nrxn antibodies also show similar nanoclusters at excitatory synapses in hippocampal neurons. Importantly, these signals were eliminated by a triple knockout of the *Nrxn1–3* genes but not by a single knockout of the *Nrxn1* gene, suggesting that Nrxn2 and/or Nrxn3 also form nanoclusters. Moreover, they confirmed that conditional knockin mice expressing an HA-tagged schizophrenia-related mutant of Nrxn1 that lacks the transmembrane and intracellular domains do not show HA-Nrxn1 nanoclusters. Together, these results validate the existence of Nrxn nanoclusters.

What is the physiological function of Nrxn nanoclusters? Trotter et al. (8) found that Nrxn nanocluster-positive synapses show higher levels of presynaptic vesicle exocytosis and postsynaptic surface AMPAR expression than nanocluster-negative synapses, suggesting that Nrxn nanocluster-positive synapses are more active. However, Nrxn1 deletion did not affect excitatory synapse formation or basic properties of synaptic transmission such as AMPAR currents, at least in cultured hippocampal neurons, which may be due to the compensatory effects of Nrxn2 and Nrxn3. It will be necessary to manipulate all Nrxn nanoclusters to investigate their synaptic functions, specifically by disrupting their clustering feature.

Are Nrxn1 nanoclusters stable or dynamically regulated? Trotter et al. (8) found that the proportion of excitatory synapses that contain Nrxn1 nanoclusters, the Nrxn1 content per nanocluster, and the relative nanocluster size all greatly increase in association with the maturation of hippocampal neurons during development. Furthermore, localization of Nrxn1 nanoclusters appear to be influenced by neuronal activity, as pharmacological activation of cultured hippocampal neurons did not significantly change the

[1]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan; [2]International Research Center for Neurointelligence (WPI-IRCN), The University of Tokyo, Tokyo, Japan.

Correspondence to Yukiko Gotoh: ygotoh@mol.f.u-tokyo.ac.jp.

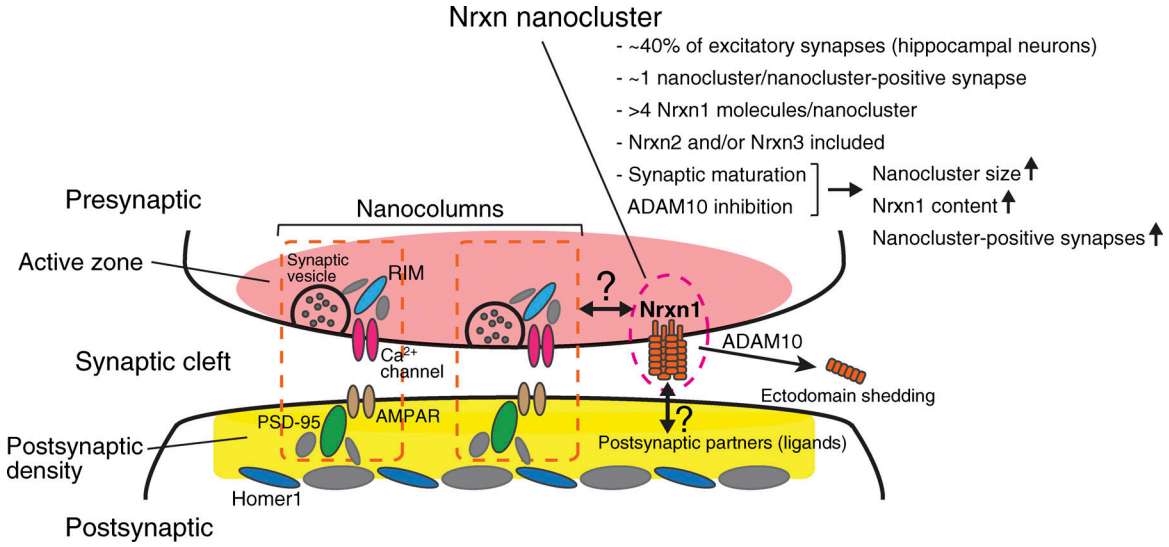

Figure 1. **Nrxn nanoclusters at presynaptic terminals and their dynamic regulation by ADAM10-mediated ectodomain cleavage.** Two distinct presynaptic nanoscale organizations, nanocolumns and Nrxn nanoclusters, are shown. Nanocolumns are observed across the synaptic cleft that axially align presynaptic release sites and postsynaptic receptors. In contrast, only a fraction (~40%) of excitatory synapses contained Nrxn1 nanoclusters in hippocampal neurons in vitro and in vivo, and most of these synapses harbored only a single nanocluster that was comprised of more than four Nrxn1 molecules. Nrxn nanoclusters are dynamically regulated during synaptic maturation and Nrxn1 ectodomain shedding by ADAM10.

abundance of HA-Nrxn1 nanoclusters but shifted their location from the center to the periphery of the active zone, which was also observed during neuronal maturation. Most intriguingly, the authors also found that Nrxn1 undergoes extensive ectodomain shedding with a dominant contribution of ADAM10-mediated cleavage at an extracellular juxtamembrane site. Indeed, under physiological conditions, ~4% to ~6% of HA-Nrxn1α was present as soluble ectodomain protein in the adult mouse brain. Inhibition of ADAM10-mediated proteolysis in cultured hippocampal neurons suppressed ectodomain shedding of Nrxn1 and dramatically increased the number of synapses containing Nrxn1 nanoclusters from ~40% to ~80% of excitatory synapses and the Nrxn1 content per nanocluster by twofold. ADAM10 may thus disassemble Nrxn1 nanoclusters by cleaving Nrxn1 to generate soluble ectodomains.

The unexpected discoveries of Nrxn nanoclusters and their dynamic shedding in hippocampal excitatory neurons raise many exciting questions. How are the Nrxn nanoclusters spatially and functionally related to known active zone structures, most notably the trans-synaptic nanocolumns composed of many molecules including RIM1/2, AMPARs, and PSD95 that are repeatedly distributed within the active zone? Nrxn nanoclusters might provide a selective dynamic regulation of nanocolumn-based synaptic machinery given the existence of only a single Nrxn nanocluster per synapse. Then, what molecules do Nrxn nanoclusters interact with? Nrxn proteins have been implicated in the regulation of synaptic maturation, possibly via their interaction with CASK (Ca^{2+}/calmodulin-dependent serine protein kinase), and it would therefore be interesting to determine if Nrxn proteins interact physically with CASK-associated active zone proteins including RIM and RIM-BP, which are also known to associate with Ca^{2+} channels and have recently been shown to induce liquid–liquid phase separation (9). Do Nrxn nanoclusters interact with specific postsynaptic partners or ligands? Given that neuroligin-1 forms postsynaptic nanodomains with AMPARs and plays an essential role in alignment of pre- and postsynaptic machinery (4), it will be important to determine whether Nrxn nanoclusters interact with the neuroligin-1–mediated nanodomains. Further investigation of the nature and interacting partners of Nrxn nanoclusters may provide clues to understanding their functions.

Finally, human genetic studies suggest that mutations in Nrxn genes are linked to several psychiatric disorders including autism and schizophrenia (10). Further characterization of Nrxn nanoclusters and determination of how they are disrupted or altered by disease-associated mutations might provide unexpected insights into the pathogenesis of these disorders.

Acknowledgments

We thank Michisuke Yuzaki, Heike Blockus, Taisuke Tomita, and Charles Yokoyama for helpful suggestions. We apologize to all researchers whose work could not be cited because of space limitation.

The authors are supported by Ministry of Education, Culture, Sports, Science and Technology/Japan Society for the Promotion of Science KAKENHI (JP18K06477 for D. Kawaguchi; JP16H06479, JP16H06481, and JP15H05773 for Y. Gotoh), by Japan Science and Technology Agency AMED-CREST (JP18gm0610013), and by the Uehara Memorial Foundation.

The authors declare no competing financial interests.

1. Südhof, T.C. 2018. *Neuron.* https://doi.org/10.1016/j.neuron.2018.09.040
2. Zhang, W. 2005. *J. Neurosci.* https://doi.org/10.1523/JNEUROSCI.0497-05.2005
3. Missler, M., et al. 2003. *Nature.* https://doi.org/10.1038/nature01755
4. Haas, K.T., et al. 2018. *eLife.* https://doi.org/10.7554/eLife.31755
5. Tang, A.-H., et al. 2016. *Nature.* https://doi.org/10.1038/nature19058
6. MacGillavry, H.D., et al. 2013. *Neuron.* https://doi.org/10.1016/j.neuron.2013.03.009
7. Chamma, I., et al. 2016. *Neurophotonics.* https://doi.org/10.1117/1.NPh.3.4.041810
8. Trotter, J.H., et al. 2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201812076
9. Wu, X., et al. 2019. *Mol. Cell.* https://doi.org/10.1016/j.molcel.2018.12.007
10. Südhof, T.C. 2008. *Nature.* https://doi.org/10.1038/nature07456