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Previews

Condensing our understanding of endocytosis

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In this issue of *Neuron*, Imoto et al. report that a splice variant of dynamin (Dyn1xA) interacts with syndapin to form a molecular condensate at the edge of the presynaptic active zone. This enables rapid recruitment of proteins to endocytic sites essential for powering ultrafast endocytosis.

Neural activation causes fusion of synaptic vesicles with the plasma membrane, releasing neurotransmitters into the synapse. To replenish the supply of vesicles within the presynaptic compartment and maintain communication between neurons, the cell must locally retrieve membrane and synaptic proteins through a process termed endocytosis (Heuser and Reese, 1973). Several studies have worked to understand the kinetics and molecular components of endocytosis and how it can support neurotransmission. Original studies of endocytosis mainly from non-neuronal cells identified clathrin-mediated endocytosis, which takes up to 20 s. This puzzled many neuroscientists; how could such a slow process keep up with vesicle fusion, which can occur several times per second during rapid neural activity? A combination of rapid freezing and optogenetic stimulation of neurons allowed a type of *temporally defined* electron microscopy, which identified a new form of endocytosis unique to synapses that occurs in less than 300 ms after vesicle fusion. This novel membrane retrieval process was dubbed ultrafast endocytosis (Watanabe et al., 2013a, 2013b) and effectively laid down a gauntlet to find new molecular pathways that can support rapid retrieval.

All endocytosis depends on dynamin, which forms helical oligomers at the base or "neck" of invaginating membrane. Dynamin hydrolyzes guanosine-5′-triphosphate (GTP) to constrict at the membrane neck, enabling membrane scission to complete endocytosis. The functional importance of dynamin in the nervous system is exquisitely seen in *Drosophila* shibire mutants, which contain a temperature-sensitive dynamin mutation. At elevated temperatures, the shibire flies quickly paralyze without functional dynamin due to an inability to replenish the synaptic vesicle pool (Poodry and Edgar, 1979). Dynamin's recruitment to membrane necks has been defined in clathrin-dependent endocytosis as a gradual process taking seconds (Merrifield et al., 2002), which is not temporally compatible with supporting ultrafast endocytosis. Using an impressive and rare combination of temporally defined electron microscopy, fluorescent imaging, and genetic manipulations, in this issue of *Neuron* Imoto and colleagues from the Watanabe lab (Imoto et al., 2022) discovered a new localization mechanism for a splice variant of dynamin in nerve terminals. This new mechanism involves a molecular condensate of dynamin and is essential to support ultrafast membrane scission (Figure 1).

There are three mammalian isoforms of dynamin (Dyn) that share 80% homology yet have distinct cellular expression profiles: Dyn2 is ubiquitously expressed, while Dyn1 and Dyn3 are concentrated in the brain. Endocytosis is eliminated in presynaptic nerve terminals of Dyn1,3 knockout animals (Raimondi et al., 2011), although shallow endocytic pits still form, which cannot separate from the plasma membrane. To determine if one dynamin was necessary for ultrafast endocytosis, Imoto et al. explored different dynamin isoforms including splice variants and identified a critical splice variant of Dyn1, Dyn1xA. Interestingly, solely expressing Dyn1xA (but not Dyn1xB) perfectly restores ultrafast endocytosis in Dyn1,3 knockout animals. The question remained, however, how Dyn1xA is able to be recruited to the plasma membrane efficiently to support ultrafast endocytosis. To explore this question, Imoto et al. fluorescently labeled Dyn1xA and Dyn1xB, showing clear localization differences between the splice variants. Contrary to Dyn1xB, which has a uniform distribution throughout the axon, Dyn1xA has a punctate localization at the edge of presynaptic active zones where ultrafast endocytosis is known to occur. Imoto et al. go on to rigorously show evidence for phase separation of Dyn1xA into droplet condensates in neurons and heterologous cells that is dependent on phosphorylation state and critical to its function in ultrafast endocytosis.

At rest, Dyn1 is predominantly dephosphorylated; however, synaptic activity increases phosphorylation of a proline-rich motif and reduces dynamin interaction with proteins including syndapin 1 and endophilin A. To test the role of phosphorylation state in dynamin localization and function, Imoto et al. expressed wild-type, phosphomimetic, and phospho-deficient forms of Dyn1xA-GFP in cultured neurons. Compared to wild-type and phospho-deficient Dyn1xA, which were discretely concentrated at the edge of active zones in presynaptic terminals, phosphomimetic Dyn1xA was found diffusely throughout the axon. Beyond localization, phosphorylation state was also found to control endocytic function. Overexpressing phospho-deficient Dyn1xA was sufficient to restore ultrafast endocytosis in Dyn1,3 knockout animals, while phosphomimetic Dyn1xA could not. Thus, dephosphorylation of Dyn1xA is required for the subsynaptic enrichment and condensate formation critical for maintaining synaptic vesicle recycling at the millisecond timescale.

To further demonstrate the impact of phosphorylation state on Dyn1 function, Imoto et al. investigated the interaction between Dyn1 and its regulatory partner syndapin 1, which is disrupted by Dyn1

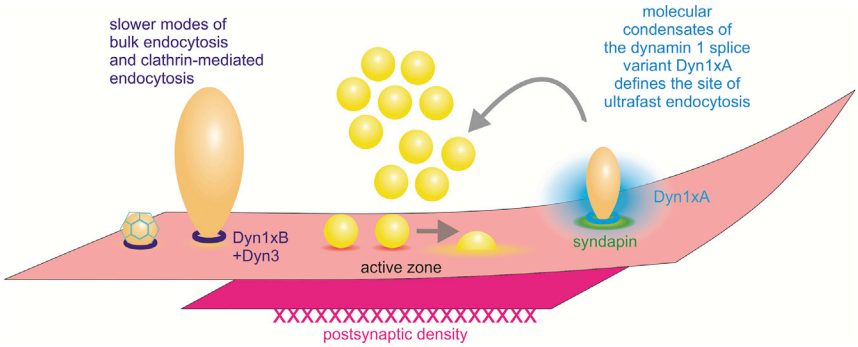

Figure 1. Molecular condensates of the dynamin 1 splice variant (Dyn1xA) localize with membrane bound syndapin at the edge of the active zone in presynaptic terminals to support ultrafast endocytosis in hippocampal neurons

Several isoforms of dynamin exist in neurons, all supporting separate forms of endocytosis. While dynamin is typically recruited from the cytosol over a timescale of seconds for membrane scission in slower forms of clathrin-mediated and bulk endocytosis, the unique neuronal splice variant Dyn1xA was found to exist in a phase-separated condensate to support rapid (<200 ms) endocytosis. Other isoforms of dynamin such as Dyn3 and Dyn1xb support slower modes of endocytosis.

phosphorylation. Loss of syndapin 1 expression by shRNA caused axonal Dyn1xA-GFP to become diffusely localized outside endocytic sites, suggesting syndapin 1 is necessary for concentrating Dyn1xA at the edge of active zones. Given that syndapin 1 binds to both dynamin and membranes, Imoto et al. investigated the role of these interactions by overexpressing either membrane-binding or dynamin-binding syndapin 1 mutants. Consistent with the role of syndapin 1 in regulating Dyn1 function, preventing membrane or dynamin binding abolished both subsynaptic Dyn1xA enrichment and ultrafast endocytosis.

Collectively, Imoto et al. present a novel role where syndapin helps localize condensates of Dyn1xA necessary for ultrafast endocytosis. Contrary to cytosolic dynamin, which is gradually recruited over timescales of seconds during clathrin-mediated endocytosis, condensates of Dyn1xA are available for on-demand membrane fission at defined sites to support ultrafast endocytosis. Rapid and immediate recruitment may also allow Dyn1xA to act not only in membrane scission but earlier in vesicle formation during neck cinching. Interestingly, the number and size of condensates seems to vary between synapses, suggesting Dyn1xA-dependent ultrafast endocytosis may have variable endocytic capacities between synapses, while bulk and clathrin-mediated endocytosis may act as a backup during large bouts of neural activity through other dynamin variants such as Dyn1xB (Figure 1).

Although modulatory roles for phosphorylation in protein localization and activity are well established, that phosphorylation state controls phase separation is also not without precedent. Many disordered proteins that have been found to undergo phase separation are regulated by phosphorylation, allowing cells to tune condensate formation based on activity. Indeed, the synaptic vesicle protein synapsin 1 forms liquid condensates via five SH3 domains that are dispersed by synapsin 1 phosphorylation (Milovanovic et al., 2018). Interestingly, the neuronal Dyn1 splice variants xA and xB differ in their C termini, with xA containing two additional SH3-binding motifs. It remains to be seen whether the SH3 domains also modulate phase separation for Dyn1xA. Further, interfering with Dyn1-SH3 domain interactions has been shown to impair endocytosis (Shupliakov et al., 1997), suggesting that perhaps Dyn1 also recruits other SH3 domain-containing interactors such as amphiphysin or endophilin into liquid condensates. Dynamin and both endophilin and amphiphysin reciprocally affect localization of the protein pairs (Meinecke et al., 2013), further pointing to co-recruitment of these proteins into liquid condensates. Thus, phosphorylation-dependent phase separation of Dyn1 allows neurons to position Dyn1, and potentially other protein interactors, to allow for rapid vesicle retrieval during ultrafast endocytosis. Given dynamin's enzymatic activity, local enrichment or production components of GTP may also be recruited into these domains.

Interestingly, *Caenorhabditis elegans* has also been shown to engage in ultrafast endocytosis in nerve terminals but does not contain an equivalent form of Dyn1xA (Watanabe et al., 2013a). Thus, the mechanism presented here by Imoto et al. that enables the efficient recruitment and localization of dynamin to support ultrafast endocytosis is likely not the only mechanism proteins can utilize at the synapse. Other condensates have also recently been proposed to help build specialized signaling domains within both pre- and post-synaptic compartments. Likely a collection of mechanisms exists and may be shared to regulate the formation, size, and localization of condensates with regard to synaptic function and plasticity. The combination of approaches used here provides a template for future work to unravel molecular regulation of condensates to better understand synaptic transmission.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Heuser, J.E., and Reese, T.S. (1973). Evidence for recycling of synaptic vesicle membrane during transmitter release at the frog neuromuscular junction. J. Cell Biol. *57*, 315–344. https://doi.org/10.1083/jcb.57.2.315.

Imoto, Y., Raychaudhuri, S., Ma, Y., Fenske, P., Sandoval, E., Itoh, K., Blumrich, E.M., Matsubayashi, H.T., Mamer, L., Zarebidaki, F., et al. (2022). Dynamin is primed at endocytic sites for ultrafast endocytosis. Neuron *110*. https://doi.org/10.1016/j.neuron.2022.06.010.

Meinecke, M., Boucrot, E., Camdere, G., Hon, W.C., Mittal, R., and McMahon, H.T. (2013). Cooperative recruitment of dynamin and BIN/amphiphysin/Rvs (BAR) domain-containing proteins leads to GTP-dependent membrane scission. J. Biol. Chem. *288*, 6651–6661. https://doi.org/10.1074/jbc.m112.444869.

Merrifield, C.J., Feldman, M.E., Wan, L., and Almers, W. (2002). Imaging actin and dynamin recruitment during invagination of single clathrin-coated pits. Nat. Cell Biol. *4*, 691–698. https://doi.org/10.1038/ncb837.

Milovanovic, D., Wu, Y., Bian, X., and De Camilli, P. (2018). A liquid phase of synapsin and lipid vesicles. Science *361*, 604–607. https://doi.org/10.1126/science.aat5671.

Poodry, C.A., and Edgar, L. (1979). Reversible alteration in the neuromuscular junctions of

Drosophila melanogaster bearing a temperature-sensitive mutation, shibire. J. Cell Biol. *81*, 520–527. https://doi.org/10.1083/jcb.81.3.520.

Raimondi, A., Ferguson, S., Lou, X., Armbruster, M., Paradise, S., Giovedi, S., Messa, M., Kono, N., Takasaki, J., Cappello, V., et al. (2011). Overlapping role of dynamin isoforms in synaptic vesicle endocytosis. Neuron *70*, 1100–1114. https://doi.org/10.1016/j.neuron.2011.04.031.

Shupliakov, O., Low, P., Grabs, D., Gad, H., Chen, H., David, C., Takei, K., De Camilli, P., and Brodin, L. (1997). Synaptic vesicle endocytosis impaired by disruption of dynamin-SH3 domain interactions. Science *276*, 259–263. https://doi.org/10.1126/science.276.5310.259.

Watanabe, S., Liu, Q., Davis, M.W., Hollopeter, G., Thomas, N., Jorgensen, N.B., and Jorgensen, E.M. (2013a). Ultrafast endocytosis at Caenorhabditis elegans neuromuscular junctions. Elife *2*, e00723. https://doi.org/10.7554/elife.00723.

Watanabe, S., Rost, B.R., Camacho-Perez, M., Davis, M.W., Sohl-Kielczynski, B., Rosenmund, C., and Jorgensen, E.M. (2013b). Ultrafast endocytosis at mouse hippocampal synapses. Nature *504*, 242–247. https://doi.org/10.1038/nature12809.

Spike timing-based regulation of thalamocortical signaling

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For decades, thalamic burst and tonic spiking modes have been theorized to regulate sensory signaling in the thalamocortical circuit. In this issue of *Neuron*, Borden et al. demonstrate a timing-based mechanism by which thalamic spiking mode controls sensory responses in the awake cortex.

The natural world presents the nervous system with a constant stream of sensory inputs that must be prioritized and triaged based on an organism's current state and immediate goals. Most incoming sensory information passes through the thalamus to reach the cortex. This influential position in the sensory processing stream endows the thalamus with the potential to modulate or gate cortical responses to the outside world.

A prominent theory about thalamic sensory gating is articulated by the "wake-up call" hypothesis (Sherman, 2001). This proposes that the ability of thalamic relay neurons to spike in two distinct modes—tonic mode, in which normal Na+/K+-driven spikes occur in a relatively linear relationship with the stimulus, and burst mode, in which Ca2+ currents cause short, high-frequency bursts of spikes—regulates the information content of the ascending thalamocortical signal. These two spiking modes allow the thalamus to provide either an accurate relay of stimulus information or a less accurate, but stronger, signal useful for stimulus detection (Sherman and Guillery, 2002). In tonic mode, a higher spontaneous spike rate and relatively linear stimulus response help convey detailed information about stimulus features. In burst mode, neurons transmit sensory information in a more "all-or-nothing" manner, sacrificing nuance for an enhanced efficacy of transmission at the thalamocortical synapse (Swadlow and Gusev, 2001). A transition from tonic to burst mode transforms the ascending sensory signal from faithful copy to a distorted but loud wake-up call. Both signaling modes are valuable: at the airport, while standing around waiting to board a flight, one wants to hear the details of a boarding announcement; having fallen asleep at the gate, one simply wants to hear it at all.

The transition between burst and tonic thalamic firing modes must be controllable to be useful for sensory gating. A dependence on the membrane potential of the thalamic neurons confers such control via a voltage-dependent inactivation or de-inactivation of T-type calcium channels (Suzuki and Rogawski, 1989). Under hyperpolarized conditions, T-type calcium channels are de-inactivated, allowing the neuron to fire short, high-frequency bursts while tonic firing is suppressed. This bursting is in turn suppressed by sustained, more depolarized membrane potentials that cause tonic spikes but inactivate the T-type calcium channels. In this way, the response mode of a thalamic relay neuron is determined by the various hyperpolarizing and depolarizing influences on its membrane potential. Massive synaptic inputs from the cortex and brainstem provide ample sources for achieving these voltage changes and thus possible substrates for a signal to control the burst/tonic transition.

While these physiological and anatomical findings support the wake-up call hypothesis, how changes in thalamic response mode impact sensory processing in the cortex of intact and non-anesthetized animals has remained elusive. This is due in part to the technical difficulty of acquiring both cortical and thalamic electrophysiological data during precisely controllable and rapidly reversible perturbations of the thalamus in awake animals.

In this issue of *Neuron*, Borden, Wright, et al. use a novel combination of optogenetic hyperpolarization of the thalamus,