Breaking down to build up: Neuroligin’s C-terminal domain strengthens the synapse

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The mechanisms by which neuroligin adhesion molecules modulate synaptic plasticity remain unclear. In this issue, Liu et al. (2016. J. Cell Biol. http://dx.doi.org/10.1083/jcb.201509023) demonstrate that neuroligin 1 promotes actin assembly associated with synaptic strengthening independent of adhesion, suggesting additional ways for neuroligins to contribute to neuronal development and disease pathology.

Spines are actin-enriched dendritic protrusions that serve as the major site of excitatory neurotransmission, underlying learning and memory formation (Lynch et al., 2007). Spines associate with presynaptic axon terminals through diverse adhesion molecules to form synapses (Siddiqui and Craig, 2011). Dynamic rearrangements of these synaptic adhesions and of the underlying actin cytoskeleton lead to either strengthening or weakening of particular synaptic connections. Synaptic strengthening, or long-term potentiation (LTP), is initiated by excitation of glutamate N-methyl-d-aspartate (NMDA) receptors, which promotes cleavage of synaptic adhesion molecules and disassembly of actin filaments (Lynch et al., 2007). Actin disassembly is mediated in part by recruitment of the actin-severing protein cofilin into the spine (Bosch et al., 2014). After the breakdown of the existing synaptic architecture, the actin cytoskeleton is stabilized again via Rac1-driven actin polymerization (Rex et al., 2009) and phosphorylation-mediated cofilin inactivation (Bosch et al., 2014). In parallel, recruitment and anchoring of synaptic adhesion molecules, including neuroligin 1 (NLG1; Schapitz et al., 2010) and glutamate receptors, increases the size of the postsynaptic signaling scaffold (PSD) across from the presynaptic terminal. In the final stage of LTP, the changes in synaptic morphology are consolidated by stabilization of actin filaments through actin capping and cross-linking together with the insertion of newly synthesized synaptic proteins (Lynch et al., 2007). Although the different steps of LTP shaping spine morphology and stability are generally understood, the signaling events that coordinate the initial disassembly of the existing synaptic architecture with reassembly of a stronger synaptic connection remain unclear.

Neuroligins (NLGs) are a family of four transmembrane postsynaptic adhesion molecules (NLGs 1–4) that form hetero-typic adhesions with presynaptic neuromuscular junctions via an extracellular acetylcholinesterase-like domain (Südhof, 2008). Of the four NLG family members, NLG1 localizes predominantly to excitatory glutamatergic synapses (Song et al., 1999). Both in vitro and in vivo evidence demonstrate that the NLG–neurexin binding interaction is sufficient to promote synapse formation (Südhof, 2008; Chen et al., 2010). However, NLG knockout mice exhibit normal spine density but impaired synaptic transmission, suggesting that NLGs may regulate synaptic function independent of adhesion (Südhof, 2008). In addition to trans-synaptic adhesion mediated by the extracellular domain of NLGs, their short intracellular C-terminal domain (CTD) contains a PDZ binding domain (PBD) that facilitates binding and recruitment of postsynaptic density scaffold proteins, such as PSD95 (Irie et al., 1997; Dresbach et al., 2004). NLG1 is cleaved in an activity-dependent manner, leading to the release of an extracellular fragment that destabilizes synaptic adhesion and of the intracellular CTD (Suzuki et al., 2012).

In this issue, Liu et al. focused on how activity-dependent cleavage of NLG1 and the subsequent release of its CTD affect actin organization and spine stability at excitatory synapses. They first observed that NLG1 knockout mouse brains, as well as cultured neurons infected with an shRNA targeting NLG1, exhibit decreased cofilin-S3 phosphorylation when compared with wild-type levels. Cofilin-S3 phosphorylation functions as a marker of mature dendritic spines, as cofilin inactivation results in F-actin assembly and is associated with the later stages of LTP (Calabrese et al., 2014). In addition, the absence of NLG1 prevented dynamic regulation of cofilin phosphorylation in response to KCl-induced neuronal excitation of brain slices, suggesting that cofilin phosphorylation depends on NLG1 both basally and in an activity-dependent manner. Remarkably, incubation with recombinant NLG1-CTD increased spine-associated cofilin phosphorylation in cultured neurons and rescued cofilin phosphorylation in NLG1 knockout mouse brain slices. Using full-length or truncated NLG1 constructs with a wild-type or mutated PDB sequence, Liu et al. (2016) demonstrated that NLG1-induced cofilin phosphorylation depends on both NLG1 cleavage and an intact PBD sequence within the released CTD. As the NLG1-CTD alone induced spine-associated cofilin phosphorylation, the researchers investigated its impact on actin assembly associated with synapse formation and function. In cultured neurons, recombinant NLG1-CTD increased F-actin levels together with spine and synapse formation. Similarly, intravenous injection of NLG1-CTD increased spine density in the CA1 region of the mouse hippocampus. This increased spine and synapse formation resulted in a corresponding increase in the frequency of excitatory postsynaptic events.
Subsequent sequestration of SPAR from the PSD could serve a temporal delay between the release of the NLG1-CTD and the regulation of actin by which NLG1 impacts synapse development and function by facilitating LTP, as determined by whole-cell patch clamping of brain slices incubated with NLG1-CTD.

Rac1 activation, leading to LIMK-1 and cofilin phosphorylation, increases F-actin filament assembly within spines. These changes in actin organization ultimately result in increased spine density and promote LTP.

NLG's CTD strengthens the synapse from within through dynamic actin remodeling. Excitatory activation of NMDA receptors (NMDAR) results in sequential cleavage of NLG1 (Suzuki et al., 2012). Liu et al. (2016) describe how the CTD of NLG1 interacts with SPAR, a negative regulator of Rap GTPase activity. This activity-dependent interaction displaces SPAR and alleviates the local inhibition of Rap activity within the dendritic spine. Rap drives a corresponding increase in Rac activation and phosphorylation of its downstream target, the actin regulator cofilin, thereby increasing F-actin filament assembly within spines. These changes in actin organization ultimately result in increased spine density and promote LTP.

Consistent with the multiple roles of NLGs in modulating synaptic architecture, it is not surprising that NLG mutations have been implicated in diverse cognitive and neurodevelopmental disorders, such as Alzheimer’s disease and autism (Südhof, 2008; Tristán-Clavijo et al., 2015). In light of this study, it will be interesting to determine how disease-associated NLG mutations contribute to both synaptic adhesion as well as stabilization of the actin cytoskeleton that supports synaptic strengthening.
This is particularly important because both Alzheimer's disease and autism-associated NLG mutant proteins exhibit decreased surface expression (Chubykin et al., 2005; Tristán-Clavijo et al., 2015), although the autism-associated mutant NLG proteins present at the cell surface still promote synapse formation (Chubykin et al., 2005). However, the decreased postsynaptic NLG pool could impair subsequent activity-dependent synaptic strengthening. Likewise, understanding whether binding of the postsynaptic scaffolding protein Shank3 to the CTD of NLG1 (Arons et al., 2012) affects NLG1 cleavage could provide insights into the mechanism by which Shank3 affects activity-dependent synaptic remodeling in autism pathogenesis. The work by Liu et al. (2016), demonstrating that adhesion disassembly coordinates subsequent actin assembly underlying synaptic strengthening, takes an important step toward shedding light on the altered synaptic plasticity underlying both complex neurodevelopmental and neurodegenerative pathologies.

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