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 heterotypic adhesions with presynaptic neurexins 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

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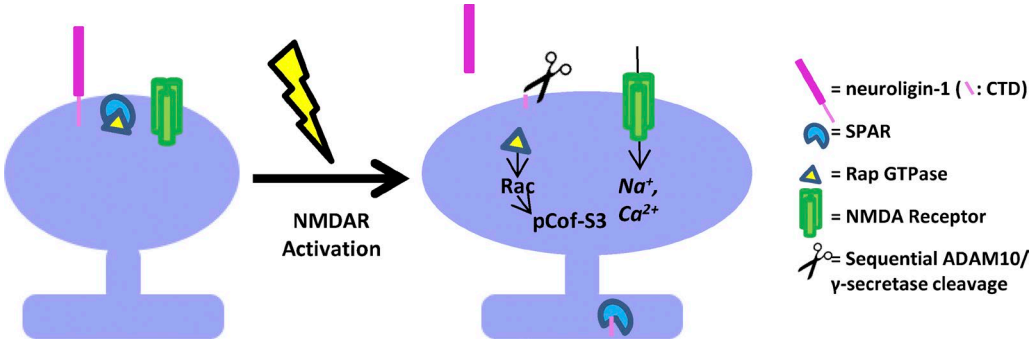

Figure 1. **NLG's CTD strengthens the synapse from within through dynamic actin remodeling.** Excitatory activation of NMDA receptors (NMDAR) results in sequential cleavage of NLG1 (Suzuki et al., 2012). Liu et al. (2016) describe how the CTD of NLG1 interacts with SPAR, a negative regulator of Rap GTPase 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.

currents, which was inhibited by a peptide that blocked cofilin phosphorylation. Together, these results establish that the NLG1-CTD requires cofilin phosphorylation to strengthen synaptic connections, prompting Liu et al. (2016) to investigate the mechanism underlying NLG1-induced cofilin phosphorylation.

SPAR is a known regulator of the actin cytoskeleton that is hypothesized to bind to NLG1 (Craig and Kang, 2007). Using brain lysates and HEK293 cells expressing both NLG1 and SPAR, Liu et al. (2016) demonstrated that SPAR interacts with NLG1-CTD via its PBD domain. In brain slices, KCl-mediated excitation, which induces proteolytic cleavage of endogenous NLG1, increased the association of NLG1 and SPAR, suggesting that the interaction occurs in response to activity-dependent release of an intracellular CTD. To test whether this interaction regulates cofilin phosphorylation, Liu et al. (2016) expressed SPAR in HEK293 cells, where it decreased cofilin-S3 phosphorylation. However, incubation with a recombinant NLG1-CTD containing an intact PBD restored cofilin phosphorylation, demonstrating that this interaction alleviates SPAR-mediated repression of cofilin phosphorylation. In neurons, NLG1-CTD reduced the levels of synaptic SPAR, as assessed by both immunofluorescence and Western blotting of purified synaptosomes. SPAR is known to negatively regulate Rap1 signaling, and Rap1 signaling is important for Rac1 activation and spine morphogenesis (Pak et al., 2001; Maillet et al., 2003). In cultured neurons, a Rap1 inhibitor prevented NLG1-CTD–induced cofilin phosphorylation, whereas treatment with recombinant NLG1-CTD without Rap1 inhibition activated Rac1 signaling, leading to phosphorylation of its downstream targets, LIMK1 and cofilin. The results demonstrate that the CTD of NLG1 binds and displaces SPAR from the spine, alleviating its inhibition on Rap1 signaling. In turn, increased Rap1 signaling promotes Rac1 activation, leading to LIMK-1 and cofilin phosphorylation (Fig. 1). Lastly, these NLG-driven changes in actin assembly were found to simultaneously inhibit long-term depression, an activity-dependent reduction in the efficacy of synapses, and facilitate LTP, as determined by whole-cell patch clamping of brain slices incubated with NLG1-CTD.

This work provides important insights into the mechanism by which NLG1 impacts synapse development and function by highlighting a critical role for SPAR in the regulation of actin assembly mediating synaptic strengthening. Interestingly, the temporal delay between the release of the NLG1-CTD and the subsequent sequestration of SPAR from the PSD could serve to distinguish an early disassembly phase following excitatory stimulation from later LTP consolidation, which is known to rely on both Rac1 activation (Rex et al., 2009) and cofilin phosphorylation (Bosch et al., 2014). Furthermore, it will be of interest to determine whether NLG1's CTD affects the localization of other proteins known to bind its PBD, such as PSD95 (Irie et al., 1997), and whether these dynamic rearrangements at the postsynaptic scaffold also serve to simultaneously promote actin assembly while alleviating SPAR-mediated negative regulation of actin remodeling. For example, NLG1 has been shown to interact with Kalirin-7 (Owczarek et al., 2015), an activator of Rac1 that binds to PSD95 at the synapse; however, binding to PSD95 reduces Kalirin-7–mediated activation of Rac1 (Penzes et al., 2001). It is therefore attractive to speculate that the activity-dependent release of protein fragments, such as the CTD of NLG1, might alter postsynaptic density interactions that further promote localized Rac1-driven F-actin assembly. Consistent with this hypothesis, adhesion disassembly triggered by the extracellular domain of NLG1's binding partner (β-neurexin) increases Rac1 activation (Owczarek et al., 2015). Ultimately, more work is necessary to determine how the strengthening effects of the intracellular CTD compete with the destabilizing effects of the extracellular domain (Suzuki et al., 2012). Recent research demonstrates that CAMKII phosphorylates and increases NLG1 surface expression in response to NMDA receptor activation (Bemben et al., 2014). If this phosphorylation event protects NLG1 from cleavage, it could serve to stabilize an adhesive pool of NLG1 while allowing for the release of the CTD from an unprotected population. Alternatively, this phosphorylation event could serve to recruit new NLG1 proteins to the synapse later in the LTP process when adhesions are re-established. Further research is needed to understand how the adhesive and intracellular signaling capabilities of NLG1 are balanced at discrete stages of synaptic plasticity, and in particular how phosphorylation of NLG1 regulates both its surface expression as well as its cleavage.

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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