SUMOylation, Arc and the regulation homeostatic synaptic scaling
Implications in health and disease

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Neurons compensate for changes in network activity by altering the sensitivity of transmission across collections of synapses by up or downregulating the number of synaptic AMPA receptors. We recently reported that, in parallel to increasing AMPA receptor surface expression, suppression of network activity with TTX increases protein SUMOylation by decreasing levels of the deSUMOylating enzyme SENP1. SUMOylation of the immediate early gene product Arc is required for synaptic scaling. These results reveal a previously unsuspected role for protein SUMOylation in activity-dependent AMPA receptor trafficking and the regulation of neuronal network activity, processes which play important roles in neurodegenerative disease.

SUMOylation is an important regulator of neuronal function and dysfunction. SUMO is attached to lysine residues in target proteins by a three-enzyme pathway analogous to, but distinct from, ubiquitination. A major difference is
expressing Arc-ΔKK did not exhibit any TTX-induced scaling of AMPAR surface expression. While other, as yet unidentified, SUMO substrates are almost certainly involved, these biochemical results suggest that decreased SENP1, which leads to increased SUMOylated Arc, is permissive for synaptic scaling and reveal a previously unsuspected regulatory mechanism for the control of AMPAR trafficking, long-term homeostatic plasticity and cell responsiveness.

The discovery that SUMOylation can regulate Arc function may have far reaching implications for the understanding of AMPAR trafficking and mechanisms of plasticity. Further, the roles of Arc, SUMO and synaptic scaling in the development and expression of neurological and neurodegenerative diseases hold exciting potential. For example, synaptic scaling is impaired in a FMRP mouse model of fragile X syndrome and defective synaptic scaling has been strongly implicated in Alzheimer disease. Intriguingly, Arc is required for activity-dependent generation of β-amyloid (Aβ) and binds to amyloid precursor protein (APP), β-site APP cleaving enzyme 1 (BACE1) and presenilin1 to regulate activity dependent γ-secretase.

Arc deletion reduces Aβ in mouse models and unlike for ubiquitin, where there are many E2 ligases, Ubc9 is the only E2 for SUMOylation. There are three validated SUMO isoforms in mammals, designated SUMO-1-3 although SUMO-2 and -3 differ by only three amino acids, and are thus collectively known as SUMO-2/3. SUMO is removed from substrates via the actions of a family of SUMO-specific deconjugating enzymes, SENPs.

We found that, in parallel to the increase in AMPAR surface expression, TTX caused a reduction in levels of the deSUMOylating enzyme SENP1 (Fig. 1). Further, we showed that overexpression of the catalytically active catalytic domain of SENP1 prevents the TTX-induced increase in surface AMPARs but had no effect on AMPAR surface expression in the absence of TTX treatment. To confirm the scaling effects we observed by confocal imaging and surface biotinylation assays, we used hippocampal slice electrophysiology to measure AMPAR-mediated excitatory postsynaptic responses (EPSCs) in CA1 neurons. Sindbis virus overexpression of the catalytic domain of SENP1 decreased AMPAR EPSCs only in cells treated with TTX. In contrast, the control catalytically inactive SENP1 mutant had no effect on AMPAR EPSCs in control slices or those treated with TTX. These functional data indicate that the TTX-induced increase in AMPAR surface expression is synaptic. Thus, we conclude that SUMOylation of one or more protein targets is required for the forward trafficking of AMPAR to synapses under conditions of activity suppression.

What Might these SUMO Targets be?

Arc contains two consensus SUMOylation sites at lysine residues K110 and K268, which are located close to the binding sites for endophilin-3 and dynamin-2 respectively. Mutation of these sites disrupts Arc localization in dendrites and this has been interpreted to suggest that Arc SUMOylation plays a role in structural changes required for some forms of LTP consolidation. Thus, SUMOylation of K110 and/or K268 might disrupt the interaction of Arc with endophilin-3 and dynamin-2 to inhibit AMPAR endocytosis, which could explain the dependence of synaptic scaling on SUMOylation. We therefore compared the effects of expressing wild-type and a non-SUMOylatable double lysine mutant of Arc (Arc-ΔKK). In contrast to control cells, neurons expressing Arc-ΔKK did not exhibit any TTX-induced scaling of AMPAR surface expression. While other, as yet unidentified, SUMO substrates are almost certainly involved, these biochemical results suggest that decreased SENP1, which leads to increased SUMOylated Arc, is permissive for synaptic scaling and reveal a previously unsuspected regulatory mechanism for the control of AMPAR trafficking, long-term homeostatic plasticity and cell responsiveness.
of Alzheimer disease and can be present in abnormally high levels in human Alzheimer patients.\textsuperscript{17} APP is also a SUMO substrate and SUMOylation of APP reduces Aβ production whereas mutagenesis of the SUMO target lysines in APP enhances Aβ production.\textsuperscript{16,20} Thus, we anticipate that SUMOylation will prove to be a fundamental factor in several neurological disorders and may provide a therapeutic target for drug development.

In summary, we believe that the activity-dependent regulation of SENP1 levels, which in turn regulates SUMOylation of Arc is a novel and exciting observation. We expect future work will reveal in greater detail how these regulatory mechanisms control AMPAR trafficking, homeostatic plasticity and network activity in normal and diseased brain.

Disclosure of Potential Conflicts of Interest
The authors declare that they have no conflict of interest.

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