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BSN (bassoon) and PRKN/parkin in concert control presynaptic vesicle autophagy

Carolina Montenegro-Venegas [a,b,c*], Anil Annamneedi [a,b,d*], Sheila Hoffmann-Conaway[e], Eckart D. Gundelfinger [a,b,f], and Craig C. Garner [e,g,h]

[a]Department of Neurochemistry and Molecular Biology, Leibniz Institute for Neurobiology, Magdeburg, Germany; [b]Center for Behavioral Brain Sciences (CBBS), Magdeburg, Germany; [c]Institute for Pharmacology and Toxicology, Medical Faculty, Otto Von Guericke University, Magdeburg, Germany; [d]Institute of Biology, Otto Von Guericke University, Magdeburg, Germany; [e]German Center for Neurodegenerative Diseases (DZNE), Berlin, Germany; [f]Molecular Neurobiology, Medical Faculty, Otto Von Guericke University, Magdeburg, Germany; [g]Charité – Universitätsmedizin Berlin, Institute of Neurobiology, Berlin, Germany; [h]NeuroCure Cluster of Excellence, Charité – Universitätsmedizin Berlin, Berlin, Germany

ABSTRACT

Maintaining the integrity and function of the presynaptic neurotransmitter release apparatus is a demanding process for a post-mitotic neuron; the mechanisms behind it are still unclear. BSN (bassoon), an active zone scaffolding protein, has been implicated in the control of presynaptic macroautophagy/autophagy, a process we recently showed depends on poly-ubiquitination of synaptic proteins. Moreover, loss of BSN was found to lead to smaller synaptic vesicle (SV) pools and younger pools of the SV protein SV2. Of note, the E3 ligase PRKN/parkin appears to be involved in BSN deficiency-related changes in autophagy levels, as shRNA-mediated knockdown of PRKN counteracts BSN-deficiency and rescues decreased SV protein levels as well as impaired SV recycling in primary cultured neurons. These data imply that BSN and PRKN act in concert to control presynaptic autophagy and maintain presynaptic proteostasis and SV turnover at the physiologically required levels.

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The maintenance of chemical synapse functionality over long time periods is critical for proper brain performance. Synapses are complex cell-cell contacts that allow a fast and efficient communication between neurons through the regulated fusion of synaptic vesicles (SVs) at the active zone membrane of presynaptic boutons. This process requires a coordinated effort of hundreds of proteins, making synapses particularly susceptible to stress and the potential accumulation of damaged proteins and subsequent neurodegeneration. To keep this under control, local degradative pathways, including the proteasome, the endo-lysosomal system and autophagy, are essential. Pathway specificity is determined by the attachment of ubiquitin tags, which can vary in length and position, although the different pathways can also compensate for each other. It has been established that ubiquitination is important for the generation of autophagic structures and that reactive oxygen species-damaged SV proteins undergo clearance through autophagy; but how these proteins are tagged for degradation, and which E3 ubiquitin ligases are involved remains still unclear. For instance, the presynaptic proteins RIMS1/RIM1 and UNC13/Munc-13 undergo proteasomal degradation in an E3 ubiquitin ligase-dependent manner, and our previous work has shown that loss of the active zone scaffolding proteins BSN and PCLO/piccolo increases ubiquitination levels of SV proteins and leads to synapse degeneration, partially through the activation of the RING-type E3 ubiquitin ligase SIAH1A. Moreover, BSN controls presynaptic autophagy, in part via its interactions with ATG5.

In our recent study [1], we explore the underlying mechanism of how BSN modulates presynaptic autophagy. This was accomplished by using different manipulations to interfere with BSN-deficiency induced autophagy. For example, we overexpressed a BSN fragment containing the two N-terminal zinc fingers (BSN609), that interacts with and suppress the activity of SIAH1A, in neurons from BSN-deficient mice (referred to as BSN^{GT}). Co-transduction of hippocampal neurons with BSN609-eGFP and the autophagy reporter RFP-LC3 results in a reduction of axonal RFP-LC3 puncta and an increase in SV pools as shown by staining for the SV marker SYP (synaptophysin) in BSN^{GT} neurons. Furthermore, expression of a recombinant ubiquitin, with all lysine residues mutated to arginine (UbKO), thus blocking poly-ubiquitination, decreases the number of RFP-LC3 puncta and their colocalization with SYP in BSN^{GT} cells down to wild-type (WT) levels. This indicates that presynaptic autophagy induced by the absence of BSN depends on ubiquitination.

For further insight, we performed a mass spectrometry analysis of a remnant motif (K-e-GG) from ubiquitination from immunoprecipitated synaptic peptides from WT and BSN^{GT} synaptosomes. BSN loss of function results in an increase in the ubiquitination levels of numerous presynaptic proteins, including some active-zone proteins, the SV proteins SV2 and VAMP2, as well as the ubiquitin conjugation enzyme

CONTACT Craig C. Garner craig.garner@dzne.de; Carolina Montenegro-Venegas cmontene@lin-magdeburg.de German Center for Neurodegenerative Diseases (DZNE), Charité Medical University, Leibniz Institute for Neurobiology, Magdeburg 39118, Germany

*These authors contributed equally.

UBE2N/E2N, among others, without any major changes in postsynaptic proteins. These data point to a selective ubiquitination of presynaptic proteins in the absence of BSN. Using quantitative immunofluorescence, we could confirm the reduction of SV2B and VAMP2 levels in BSN^{GT} neurons, an effect that is reversed by treating neurons with the autophagy inhibitors wortmannin or SAR405.

Considering the increased formation of autophagosomes at presynaptic sites along with increased ubiquitination of presynaptic proteins in BSN^{GT} cells, we further explored the local formation of autophagosomes with SV-related cargos. Cryo-electron microscopy (EM) analysis revealed a significant increase in double-membraned autophagic vacuoles (AVs) per presynaptic bouton in BSN^{GT} compared to WT boutons, which can be rescued by treating neurons with wortmannin. Additionally, we find AVs containing SV-like structures. No significant increase of the number of multivesicular bodies (MVBs), a hallmark of the endo-lysosomal pathway, in BSN^{GT} boutons is observed. This indicates that autophagy rather than the endo-lysosomal system mediates the degradation of ubiquitinated presynaptic proteins within BSN^{GT} boutons. Consistent with observed lower SV protein levels, EM data also confirm a reduced number of SVs per presynaptic area in BSN^{GT} terminals. The treatment with wortmannin increases the SV numbers per presynapse in both BSN^{GT} and WT neurons, supporting a direct role of autophagy in the maintenance of the SV pool size, although the exact mechanism is not known yet. We further investigated whether increased autophagy in BSN^{GT} neurons can lead to an overall younger pool of SV proteins. To this end, we fused a medium fluorescence timer tag/mFT, which changes its emission from blue (maximum intensity after 21 h) to red (maximum intensity after 197 h) with time, to SV2. An observed decrease in red:blue ratio in BSN^{GT} neurons indicates a shift toward younger SV2 proteins in comparison to WT neurons.

What is the E3 ubiquitin ligase responsible for BSN-deficiency induced autophagy? Is it SIAH1A that can physically interact with BSN? Knockdown of SIAH1A moderately reduces the percentage of RFP-LC3 puncta colocalizing with SYP in BSN^{GT} neurons, indicating a possible role for SIAH1A in this phenotype. We further tested a role for the E3 ubiquitin ligase PRKN, a RING-between-RING-type enzyme, reported to be involved in neurodegenerative processes and known to ubiquitinate a number of synaptic proteins. ShRNA-mediated PRKN knockdown results in a reduction of both the number of RFP-LC3 puncta and colocalization of RFP-LC3 with SYP puncta back to WT levels. PRKN knockdown also rescues SV2B and VAMP2 levels in BSN^{GT} neurons. These data demonstrate that PRKN facilitates presynaptic autophagy, which removes SV proteins in a ubiquitin-dependent manner in BSN^{GT} neurons.

Our study shows that SV proteins and potentially whole SVs are substrates for enhanced autophagy in BSN^{GT} neurons and that the E3 ligases SIAH1A and in particular PRKN promote their clearance. These observations contribute to our understanding of how BSN is involved in the regulation of presynaptic proteostasis. They also underscore the need to better define how the different degradation pathways contribute to proper presynaptic function, which specific E3 ligases are involved, what are their substrates and how they interact to control the different pathways. Future studies in this direction are needed to better understand the development of neurological disorders with impaired proteostasis such as Parkinson disease.

Disclosure statement

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ORCID

Carolina Montenegro-Venegas http://orcid.org/0000-0002-2293-2023
Anil Annamneedi http://orcid.org/0000-0002-6743-8627
Eckart D. Gundelfinger http://orcid.org/0000-0001-9377-7414
Craig C. Garner http://orcid.org/0000-0003-1970-5417

Reference

[1] Hoffmann-Conaway S, Brockmann MM, Schneider K, et al. Parkin contributes to synaptic vesicle autophagy in Bassoon-deficient mice. Elife. 2020 May 4;9:pii: e56590.