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Spotlight

Beyond housekeeping: autophagy regulates PKA signaling at synapses

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Autophagy modulates synaptic function and plasticity, but the molecular basis for this process is largely unknown. A recent tour de force study by Overhoff and colleagues identifies a novel role for autophagy in regulating PKA signaling at synapses to modulate the organization of the postsynaptic proteome and neuronal excitability.

To mitigate proteotoxic stress, neurons execute macroautophagy (hereafter autophagy), which captures cargo (i.e., components of the cytoplasm) within autophagosomes that fuse with lysosomes to enable cargo degradation (Figure 1). Canonically, autophagy is viewed as a homeostatic pathway that protects against neurodegeneration by eliminating aged and damaged proteins and organelles. Emerging evidence suggests that beyond these housekeeping functions, autophagy has specific roles in neuronal excitability and synaptic plasticity [1]. The precise cargos targeted by autophagy to facilitate these synaptic functions, however, remain largely unknown. In a new study published in *The European Molecular Biology Organization Journal*, Overhoff and colleagues identify a novel role for autophagy in modulating neuronal excitability by regulating protein kinase A (PKA) activity at the synapse via selective degradation of its regulatory subunits (R1α/β) [2].

To study the function of autophagy in neurons, Overhoff *et al*. selectively knocked out (KO) the gene encoding ATG5, a protein required for autophagosome formation, in excitatory or inhibitory neurons and performed a quantitative proteomic analysis of brain lysates. Intriguingly, among the most highly upregulated proteins in both KO models were the R1α/β subunits of PKA. PKA is a heterotetrametric holoenzyme comprised of two regulatory subunits (R1α, R1β, R2α, R2β) and two catalytic subunits (Cα, β, γ) (Figure 1). PKA signaling is activated when cAMP binds to the regulatory subunits to induce their dissociation from the catalytic subunits. The catalytic subunits are then able to phosphorylate substrates at serine or threonine residues. Confirming their proteomic data, the authors used a combination of immunofluorescence, 3D reconstruction, and electron microscopy to demonstrate that the accumulation of R1α/β in the absence of ATG5 occurred specifically in neurons (and not in astrocytes or oligodendrocytes), and at synapses. This accumulation appears specific to R1α/β, as they did not observe changes in the expression of R2α/β or Cα in the absence of ATG5 in their proteomic data or by immunoblotting. Moreover, knockout of ATG5 arrested R1α/β in inclusions positive for the selective autophagy receptors p62 and NBR1. Finally, the authors show that under baseline conditions, R1α is present within purified autophagic organelles and is targeted for lysosomal degradation. Collectively, these data implicate the autophagy–lysosome axis in the turnover of synaptic R1α/β.

Since R1α/β sequesters PKA catalytic activity, the authors speculated that reduced R1α/β turnover would globally dampen PKA activity. Indeed, ATG5 KO neurons exhibited reduced PKA responses to cAMP, as measured by downstream pCREB transcriptional activity. Furthermore, a FRET-FLIM-based reporter of PKA activity targeted to PSD-95 revealed reduced interactions between PKA and postsynaptic substrates in ATG5 KO neurons. Thus, loss of ATG5 stabilizes R1α/β and reduces PKA signaling. Hence, the authors next evaluated the impact of ATG5 deficiency on the neuronal phosphoproteome using quantitative phosphoproteomics. Substrates that were hypophosphorylated in the ATG5 KO background were enriched in the postsynaptic density (PSD) of glutamatergic synapses and included PKA targets, consistent with a reduction in PKA activity. The authors also observed a corresponding change in the localization of PSD proteins and an enlargement of the PSD in ATG5 KO neurons compared with wild-type controls. These alterations in the PSD in ATG5-deficient neurons are consistent with prior observations that R1β preferentially localizes to dendrites versus axons [3]. Combined, these results suggest that autophagy can indirectly regulate the organization of the synaptic phosphoproteome by controlling PKA signaling.

The phosphorylation status of different cytoskeletal scaffolding proteins, receptors, and ion channels within the PSD can profoundly impact synaptic transmission. Moreover, enlarged PSDs can correlate with increased insertion of AMPA receptors (AMPARs) at the plasma membrane. Interestingly, Overhoff *et al*. found that phospho-targets downregulated in ATG5-deficient neurons included cytoskeletal-associated PSD proteins implicated in trafficking the GluR1 subunit of AMPARs. Thus, the authors asked how the loss of ATG5 would impact AMPAR distribution and function. They found that AMPARs containing the GluR1 subunit accumulated in ATG5-deficient dendritic spines compared with controls. As GluR1-containing AMPARs are calcium permeable, ATG5 KO neurons also show enlarged AMPAR currents and higher baseline excitability. These molecular changes at the synapse may explain the increase in neuronal network activity and startle-evoked seizures observed in ATG5 KO mice.

In sum, Overhoff *et al*. propose that autophagy is a key modulator of PKA

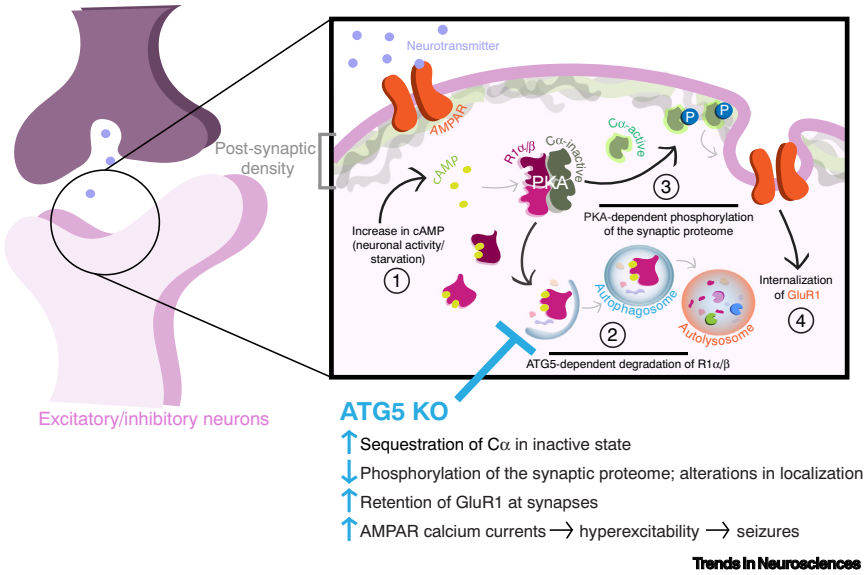

Figure 1. Regulation of protein kinase A (PKA) signaling by autophagy at postsynaptic compartments. Proposed pathway outlined by Overhoff *et al*. [2] in which (1) cAMP enhances PKA catalytic activity by promoting the (2) selective degradation of the inhibitory subunits R1α/β via autophagy. (3) Catalytically active PKA phosphorylates multiple postsynaptic density substrates, whose phosphorylation status impacts the (4) trafficking and function of GluR1 containing AMPA receptors (AMPARs). Disruptions in autophagy result in the accumulation of inactive PKA, hypophosphorylation of the postsynaptic proteome, and aberrant AMPAR trafficking and calcium currents. Ultimately, these molecular alterations in the postsynaptic density (PSD) may explain the increased neuronal network excitability and startle-evoked seizures observed in ATG5 knockout (KO) mice.

signaling to regulate the organization and excitability of the synapse (Figure 1). This work expands our understanding of how autophagy may select specific cargos to modulate synaptic transmission. An outstanding question is: how are R1α/β selectively recognized by the autophagy machinery and not other subunits of PKA? Interestingly, R1β localizes to dendrites whereas R2β localizes to axons, emphasizing the compartment-selective nature of this process [3].

The study by Overhoff *et al*. joins a flurry of publications aiming to identify the specific cargos degraded by autophagy in neurons, yielding a diversity of substrates. Proteomic profiling of autophagic organelles isolated from whole mouse brain preparations identified an enrichment of mitochondrial components and synaptic proteins [4,5]. By contrast, proteomic analysis of cultured cerebellar neuron lysates deficient for ATG5 revealed a selective enrichment of endoplasmic reticulum (ER)-associated proteins, suggesting ER as a major substrate for neuronal autophagy [6]. Lastly, several studies propose that synaptic vesicles may be substrates for autophagy in presynaptic terminals (reviewed in [1]). Perhaps the diversity of cargos reflects specialized functions for autophagy in distinct populations of neurons. Further, contributions from non-neuronal cells, including glia, need to be considered in the analysis at baseline and in response to cellular stress. In fact, Yang *et al*. found that upon nutrient starvation, many of the cargos enriched in autophagic organelles isolated from mouse brain originated from glial and endothelial cells; neuronal cargos were relatively unchanged [5]. This finding is consistent with enhanced induction of autophagy in astrocytes versus neurons in response to starvation [7].

Accumulating evidence further suggests that subpopulations of neurons may differentially use autophagy to fulfill specialized functions. In fact, knockdown of key autophagy genes can elicit a different phenotype in different populations of neurons. For example, the loss of ATG5 in neural progenitors or Purkinje cells results in rapid onset of neurodegeneration [6,8]. In the study by Overhoff *et al*., however, a comparable duration of ATG5 loss in inhibitory neurons does not elicit neurodegeneration in the hippocampus, striatum, or entorhinal cortex [2]. Moreover, the complexity of roles for autophagy is not limited to neurons. Recent work demonstrates that subpopulations of astrocytes in the aging hippocampus are also differentially vulnerable to a loss of autophagy [9].

In addition to cell-type adaptations, what are the stimulus-specific adaptations for autophagy at the synapse? Overhoff *et al*. propose a model where the indirect regulation of AMPAR trafficking by autophagy may be important in homeostatic synaptic scaling and combating excitotoxicity. An important consideration in this model is how autophagy may regulate AMPAR trafficking both indirectly through PKA signaling and directly by targeting GluR1 containing AMPARs for autophagic degradation. Of note, the latter function seems to be elicited during long-term depression (LTD), but not during baseline neurotransmission [1,10]. Models of LTD stimulate autophagy, which in turn promotes LTD via degradation of AMPARs [1,10]. Proteomic profiling of purified autophagic organelles revealed an increase in AMPAR subunits and components of the PSD during LTD [10]. Thus, the nature of the cargo degraded by autophagy can be regulated by synaptic plasticity. In fact, autophagy has been also implicated in synaptic signaling by controlling synaptic vesicle release and long-term memory formation [1]. How is autophagy able to decode slight variations in synaptic signaling events to accomplish these diverse and highly localized functions? Future work that

identifies context-specific (e.g., long-term potentiation vs. LTD) and compartment-specific (e.g., pre- vs. postsynaptic) signals upstream of autophagy will help elucidate the adaptability of this pathway. An emerging theme in the field is that the complexity of roles for autophagy in the brain depends on neuronal subtype, neuronal compartment, contributions from neighboring glia, onset and duration of autophagy deficiency, and activity or stress stimulus [1].

The work by Overhoff *et al*. illuminates how neuronal populations can adapt autophagy to control the synaptic proteome and modulate the excitability of the synapse. Such work is critical for expanding our understanding of the contributions of autophagy to conditions such as autism spectrum disorders and epilepsy, which are characterized by disruptions in the balance between excitatory and inhibitory activity.

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Declaration of interests

The authors declare no competing interests.

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