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Presynaptic endocytic factors in autophagy and neurodegeneration

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Neuronal signaling depends on the exocytic fusion and subsequent endocytic retrieval and reformation of neurotransmitter-containing synaptic vesicles at synapses. Recent findings have uncovered surprising roles of presynaptic endocytic proteins in the formation and transport of autophagosomes. These include functions of the membrane remodelling protein endophilin and its downstream effector, the phosphoinositide phosphatase synaptojanin, in autophagosome formation and in Parkinson's disease, the endocytic sorting adaptor CALM in protein degradation via the autophagy/lysosomal pathway in Alzheimer's disease, and the clathrin adaptor complex AP-2 in retrograde transport of signaling autophagosomes to prevent neurodegeneration. These findings reveal unanticipated connections between the machineries for synaptic neurotransmission and neuronal proteostasis and identify presynaptic endocytic proteins as potential targets to treat neurodegenerative diseases.

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Introduction

Neurons in the brain communicate through specialized cell–cell junctions termed synapses, where electrical signals, for example, action potentials, are converted into the exocytic release of chemical neurotransmitters. Neurotransmission is triggered by calcium influx into the presynaptic nerve terminal, which induces the exocytic fusion of neurotransmitter-filled synaptic vesicles (SV) with the presynaptic active zone (AZ) membrane to release their content. SV fusion in addition to soluble NSF attachment protein receptor (SNARE) complex formation requires calcium sensors, most notably the SV membrane protein synaptotagmin, and is orchestrated by multidomain AZ scaffold proteins that link release-ready SVs to sites of calcium influx [1]. To sustain neurotransmission and prevent expansion of the presynaptic plasma membrane, SV fusion is followed by the endocytic recycling and regeneration of SV proteins and lipids [2]. Although the exact mechanisms of SV endocytosis remain debated, endocytic factors such as dynamin, endophilin, as well as components of the clathrin-based endocytic machinery such as AP-2, stonin2/stonedB and AP180 or CALM have been shown to be required for SV recycling and/or reformation *in vivo* [3–5] (Figure 1). Apart from their presynaptic function in the SV cycle, endocytic proteins also play a role in regulating the localization, trafficking and signaling of receptors important for neuronal development. Moreover, presynaptic vesicle cycling must be linked to mechanisms of quality control to ensure the removal of dysfunctional proteins [6,7]. How this is achieved is largely unknown.

Neurons like most other cell types employ several strategies for removing damaged or misfolded proteins that include chaperone-mediated disaggregation, degradation of soluble proteins via the ubiquitin–proteasome system (UPS) and protein turnover via the autophagy–lysosomal pathway. In macroautophagy (simply referred to as autophagy hereafter) a defined cascade of protein interactions that includes the formation of protein conjugates, comprising the ubiquitin-related proteins autophagy-related gene (ATG) 5 and ATG8/LC3/GABARAP, orchestrates the formation of a double membrane pre-autophagosomal structure. This structure, also referred to as the phagophore, matures into a closed autophagosome that delivers its engulfed cytoplasmic material to the lysosome for degradation [8]. The importance of the autophagy system for neuronal health is illustrated by the fact that mice lacking core autophagy proteins suffer from fatal neurodegeneration [9] and the restoration of Atg5 *solely* in the brain is sufficient to rescue from neonatal lethality [10]. Accumulating evidence indicates that upregulation of autophagy can protect against neurodegeneration in several models (see [11] for a recent review), although autophagic activity has also been suggested to contribute to neuronal cell death commonly associated with neurodegenerative diseases [12].

In this review we will focus on recent data indicating that presynaptic endocytic proteins such as endophilin and

AP-2 serve hitherto unknown, mostly endocytosis-independent, roles in the formation and transport of autophagosomes to promote neuronal development and to prevent neurodegeneration (Table 1 and Figure 1) [6,13•].

Endocytic membrane remodelling and lipid metabolizing enzymes in autophagosome biogenesis and synaptic proteostasis

In neurons, autophagosomes largely form in distal axons as well as at synapses and are transported to the soma where they fuse with lysosomes [14]. Although not much is known about the exact pathway of autophagy initiation in neurons it is thought to require the assembly of a protein scaffold that aids the formation of a curved membrane template. Membrane remodelling often involves members of the bin-amphiphysin-rvs (BAR) domain protein family. Prominent members of the BAR protein family are endophilins A1–3, proteins involved in clathrin-mediated [15] and clathrin-independent endocytosis [16]. Endophilins were identified based on their ability to associate with endocytic proteins such as the fissioning GTPase dynamin and the phosphoinositide phosphatase synaptojanin 1, an enzyme required for removal of endocytic clathrin coats during SV recycling [17,18]. Consistent with this, genetic studies in flies, worms and mice have shown that loss of endophilins results in defects in neurotransmission, defective SV recycling, and an accumulation of clathrin-coated SVs, in particular at inhibitory synapses. However, endophilin double and triple knockout (KO) mice, in addition to these phenotypes, suffer from severe neurodegeneration that limits their lifespan [15]. Recent work from two groups shows that endophilin plays an additional unexpected role in synaptic autophagosome biogenesis (Table 1) [19••,20••]. Endophilin was shown to colocalize with autophagosomes and its loss in flies [20••] or mice [19••] interfered with the stimulation-induced formation of autophagosomes. The authors propose a mechanism whereby endophilin via its BAR domain creates areas of high membrane curvature that can serve as docking sites for autophagic proteins that drive subsequent steps of autophagosome formation [21]. Interestingly, endophilin is functionally connected to LRRK2 (PARK8) and the E3 ubiquitin ligase Parkin (PARK2), proteins genetically linked to Parkinson's disease (PD) and its expression is elevated in brains from PD as well as Alzheimer's disease (AD) patients [22,23]. LRRK2 is a large multidomain protein containing functional GTPase and kinase domains. Mutations in LRRK2 are linked to autosomal dominant forms of PD and are the most common genetic cause of both familial and sporadic PD in humans. Mice deficient in LRRK2 and its functional homolog LKKR1 suffer from early mortality and age-dependent neurodegeneration [24]. Disease-causing mutations are clustered within its GTPase and kinase domains, and either impair its GTPase activity or enhance its kinase activity. LRRK2 has been shown to regulate SV endocytosis through phosphorylation of the BAR domain of endophilin [25,26]. Interestingly, endophilin BAR domain phosphorylation is also necessary for the induction of autophagy at synapses and LRRK2 kinase-inactive mutants fail to support autophagosome formation [20••]. Endophilin also associates with the E3 ubiquitin ligases Parkin, an enzyme linked to autosomal recessive juvenile-onset PD by promoting mitophagy [27], and FBXO32 [19••] (Figure 1). The latter colocalizes with endophilin on membrane vesicles and tubules and upregulation of FBXO32 expression in endophilin KO mice has been hypothesized to cause apoptotic death of neurons. Interestingly, endophilin and FBXO32 appear to be part of a larger gene regulatory network that coordinates neuronal protein homeostasis by coordinating autophagy/lysosome-mediated protein turnover with the UPS [19••].

An important evolutionary conserved function of endophilins is their ability to bind and recruit dynamin and the phosphoinositide phosphatase synaptojanin [18], a protein overexpressed in Down syndrome [28]. Synaptojanin is a neuronally enriched lipid phosphatase that contains two phosphatase domains: a central 5-phosphatase domain that converts $PI(4,5)P_2$ to phosphatidylinositol 4-phosphate [PI(4)P] [17] and an N-terminal Sac1 domain with less defined substrate specificity

Table 1

Endocytic proteins in autophagy and neurodegeneration.

Endocytic protein	Proposed role in endocytosis	Proposed role in autophagy	Neurological disorders
Endophilin	Endocytic adaptor for synaptojanin and dynamin	Generates membrane curvature and docking sites for autophagic proteins	Parkinson's disease Alzheimer's disease
LRRK2	Regulates SV cycle by phosphorylating endophilin BAR domain	Stimulates autophagosome formation by phosphorylating endophilin BAR domain	Parkinson's disease Alzheimer's disease
Synaptojanin	Phosphatase required for clathrin uncoating	Regulates WIPI2 uncoating at autophagosomal membranes	Parkinson's disease
CALM	Mediates sorting of VAMP2	Autophagic cargo receptor targeting APP to autophagosomes	Alzheimer's disease Autism spectrum disorders
AP-2	Major adaptor protein for clathrin	Retrograde transport of autophagosomes	?