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Review

Crosstalk between presynaptic trafficking and autophagy in Parkinson's disease

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ABSTRACT

Parkinson's disease (PD) is a debilitating neurodegenerative disorder that profoundly affects one's motor functions. The disease is characterized pathologically by denervation of dopaminergic (DAergic) nigrostriatal terminal and degeneration of DAergic neurons in the substantia nigra par compacta (SNpc); however, the precise molecular mechanism underlying disease pathogenesis remains poorly understood. Animal studies in both toxin-induced and genetic PD models suggest that presynaptic impairments may underlie the early stage of DA depletion and neurodegeneration (reviewed in Schirinzi, T., et al. 2016). Supporting this notion, human genetic studies and genomic analysis have identified an increasing number of PD risk variants that are associated with synaptic vesicle (SV) trafficking, regulation of synaptic function and autophagy/lysosomal system (Chang, D., et al. 2017, reviewed in Trinh, J. & Farrer, M. 2013; Singleton, A.B., et al. 2013). Although the precise mechanism for autophagy regulation in neurons is currently unclear, many studies demonstrate that autophagosomes form at the presynaptic terminal (Maday, S. & Holzbaur, E.L. 2014; Vanhauwaert, R., et al. 2017; reviewed in Yue, Z. 2007). Growing evidence has revealed overlapping genes involved in both SV recycling and autophagy, suggesting that the two membrane trafficking processes are inter-connected. Here we will review emergent evidence linking SV endocytic genes and autophagy genes at the presynaptic terminal. We will discuss their potential relevance to PD pathogenesis.

1. Introduction

1.1. Synaptic vesicle recycling

The presynaptic terminal is a highly specialized structure where SVs cluster in the proximity of the electron-dense active zone, and are prepared physically and biochemically for fusion and recycling with the action of a highly conserved group of molecules. Vesicle fusion is mainly triggered by calcium entering through voltage-gated calcium channels upon membrane depolarization. Calcium binds to SV-resident calcium sensors (the synaptotagmin family members) (Brose et al., 1992), and catalyzes the zippering of the four-helix molecular complex, known as the SNAREs, to mediate SV fusion to the plasma membrane. This process occurs in the time scale of several milliseconds. The SNARE complex is quickly dissociated by an ATPase, NSF, and restores the original protein conformation ready for the next fusion event (Sollner et al., 1993). SV protein cargos and lipid components are subjected to recycling, and in some cases, sorting, after the fusion event. Although the mode of SV recycling and the typical time scale are highly debated (reviewed in Granseth et al., 2007; He and Wu, 2007; Smith et al., 2008), it is generally accepted that an adaptor protein (such as AP2) is required for SV cargo detection and clathrin recruitment to initiate the most classic form of endocytosis. Other molecules, such as endophilin, dynamin, synaptojanin1, auxilin, are involved at various stages of the endocytic process to mediate membrane deformation, fission and clathrin uncoating. Majority of the endocytosed SVs are reused after neurotransmitter refilling driven by the pH gradient, while a fraction of them are sorted in the endosome system to become new SVs.

1.2. Autophagy

Autophagy, which refers to “self-eating” from the Greek terms, describes the cellular process in which cytosolic components, including mainly long-lived proteins and organelles are transported to lysosome for degradation (reviewed in Klionsky, 2007; Nixon, 2013). Macroautophagy is the most common form of autophagy subtype and will be simply referred to as “autophagy” in the following discussion unless otherwise noted. Microautophagy is characterized by direct

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invagination of the lysosomal membrane during sequestration of cytoplasmic contents and is the least understood phenomenon in mammalian cells. Chaperone-mediated autophagy, also known as CMA, features the most selective translocation of the cytosolic proteins to the lysosomal lumen via cytosolic chaperones, which are specifically recognized by receptors at the lysosomal membrane (Cuervo, 2010). All three types of autophagy coexist in neurons to maintain cellular homeostasis. They aid in the clearance of misfolded proteins, disease-prone aggregates and damaged organelles and play roles in neurodevelopment and plasticity (Yue, 2007; Wong and Cuervo, 2010). More than 30 autophagy-related (ATG) genes have been identified. Most of them are evolutionarily conserved; they are functionally distinct and involved in the synthesis and trafficking of autophagosomes, fusion between autophagosomes and lysosomes and final degradation in lysosomes. The roles of autophagy genes in neurons and regulation of neuronal autophagy are just beginning to be understood.

1.3. Crosstalk between endocytosis and autophagy

Recent findings have suggested crosstalk between mechanisms in regulating SV endocytosis and autophagy. For example, clathrin, which was known to coat the endocytic membranes during SV recycling, has been reported for its new cooperative roles with $PI(4,5)P_2$ in autophagic lysosome reformation (Rong et al., 2012). Additional evidence also implicated clathrin in the microautophagy pathway (Oku et al., 2017). Besides clathrin, membrane remodeling protein, endophlinA1, and its close binding partner, synaptojanin1, have also been proposed to regulate autophagosome biogenesis as well, which will be detailed in the following sections. Supporting this notion, human genetic studies and genomic analysis have identified an increasing number of PD risk variants that are associated with both SV trafficking and autophagy/lysosomal system (Chang et al., 2017, reviewed in Trinh and Farrer, 2013; Singleton et al., 2013). Although the precise mechanism for autophagy regulation in neurons is currently unclear, many studies demonstrate that autophagosomes form at the presynaptic terminal (Maday and Holzbaur, 2014; Vanhauwaert et al., 2017; reviewed in Yue, 2007), and are transported retrogradely via the assistance of a motor protein, dynein, for further degradation at the cell body (Cheng et al., 2015). Growing evidence has revealed overlapping genes involved in both SV recycling and autophagy, suggesting that the two membrane trafficking processes are inter-connected (Fig. 1).

2. LRRK2 regulates both autophagy and SV trafficking

Dominantly inherited mutations in *LRRK2/PARK8* are linked to both familial and sporadic cases of PD (reviewed in Mata et al., 2006; Paisán-Ruiz et al., 2013; Zimprich et al., 2004). LRRK2 is a large complex protein that contains multiple functional domains, including leucine-rich repeats (LRR), a Ras-of-Complex (Roc) GTPase domain, a C-terminal-of Roc (COR) domain, a tyrosine-kinase-like protein kinase domain and WD40 repeats. Majority of the PD mutations were found within the central Roc-COR tandem and the kinase domain. LRRK2 is considered the most frequent genetic cause for PD, and its role in autophagy and SV trafficking has been extensively studied in the recent years.

2.1. Wildtype LRRK2 in autophagy regulation

Several studies have shown that LRRK2 may regulate autophagy. In an earlier study using human embryonic kidney cell line (HEK293), LRRK2 knockdown was shown to increase autophagic flux (increased Atg8/LC3 lipidation) under starvation conditions (Alegre-Abarrategui et al., 2009), suggesting an inhibitory role of endogenous LRRK2. Using *LRRK2* knockout ($LRRK2^{-/-}$) mice, Jie Shen and colleagues carried out a series of investigations to characterize the physiological function of LRRK2 (Tong, Y., et al. 2010 and 2012; Giaime et al., 2017). Their studies showed that, despite the lack of change in the brain, there is a biphasic change of autophagy in the kidney (Tong et al., 2012): loss of LRRK2 alone leads to an enhanced autophagic flux at 3–4 months; however, in 20-month old $LRRK2^{-/-}$ mice, autophagy flux was reduced (Tong et al., 2010). Interestingly, the DAergic system of the $LRRK2^{-/-}$ mice appeared normal up to 20 months of age (Tong et al., 2010), likely due to compensatory roles of LRRK1, the functional homologue of LRRK2. *LRRK2* and *LRRK1* double deleted ($LRRK^{-/-}$) mice displayed selective loss of DAergic neurons in the SNpc at 15 months, and an accelerated decline of autophagic clearance in the brain compared to WT mice (Giaime et al., 2017). These results suggest a compensatory role of LRRK1 in brain autophagic regulation, which explains the lack of obvious autophagy defects in $LRRK2^{-/-}$ brain. Conditional deletion (or silencing) of LRRK2 at an older age using alternative strategies may be useful in determining the relevance of LRRK2 loss-of-function in brain autophagy and PD pathogenesis.

2.2. LRRK2 PD mutants in autophagy regulation

The G2019S mutation (GS) is the most common LRRK2 variant found in PD patients. Along with the R1441C/G/H (RC/RG/RH) mutations, they represent the most well studied PD mutations in LRRK2. The PD mutations cause enhanced kinase activity. A previous study showed that expression of the RC mutant in HEK293 cells led to impaired autophagic function evidenced by an elevation of large autophagic vacuoles and an increased level of p62 (Alegre-Abarrategui et al., 2009). BAC transgenic mice expressing *human* LRRK2 RG (*h*RG BAC) developed intra-axonal autophagic vacuoles in the nigral DAergic projecting axons, indicating disturbance in the autophagy/lysosomal pathway (Tagliaferro et al., 2015). The GS mutation, on the other hand, was shown to stimulate autophagy in differentiated neuroblastoma cells, and its impact on shortened neurite lengths could be reversed by RNAi-mediated knockdown of Atg8/LC3 or Atg7 (Plowey et al., 2008). However, using a LRRK2 pharmacological inhibitor, IN-1, one study showed that block of LRRK2 kinase activity stimulates autophagy in human neuroglioma cells (Manzoni et al., 2013). With recent evidence on the off-target effect of IN-1 (Luerman et al., 2014), this conclusion remains to be confirmed using more specific LRRK2 kinase inhibitors. In *C. elegans*, expressing either GS or RC mutant of LRRK2 inhibited autophagy (Saha et al., 2013). Despite the existing evidence implying an active role of LRRK2 in autophagy regulation, the results appeared rather conflicting. One study suggests that LRRK2 may activate the CaMKK-β/AMPK pathway and act as a modulator of lysosomal calcium and proton homeostasis to impact autophagy and cell survival (Gómez-Suaga and Hilfiker, 2012). This hypothesis has yet to be verified in other model systems. The exact role and the mechanism for LRRK2 in regulating autophagy thus remain largely unclear.

Clearance of mutant LRRK2 has been thought of as a protective mechanism during protein stress. Besides proteasome degradation (Ko et al., 2009), LRRK2 was also found to be a substrate of CMA and could be degraded in lysosomes (Orenstein et al., 2013). However, unlike typical CMA substrates, which competes for lysosomal association, the lysosomal LAMP-2A binding for LRRK2 exhibit dose-dependent increase upon addition of other CMA substrates. Such atypical interplay between LRRK2 and CMA could compromise the efficient degradation of other CMA substrates, such as α-synuclein (see below). LRRK2 G2019S mutant displayed further impaired degradation by CMA compared to WT proteins, and it also exerts greater inhibition over this pathway (Orenstein, S.J., et al. 2013). It is possible that the impaired CMA leads to an enhanced compensatory macroautophagy function (Plowey et al., 2008), however, whether and how such compensation lead to additional toxic effects in PD pathogenesis remains to be understood.

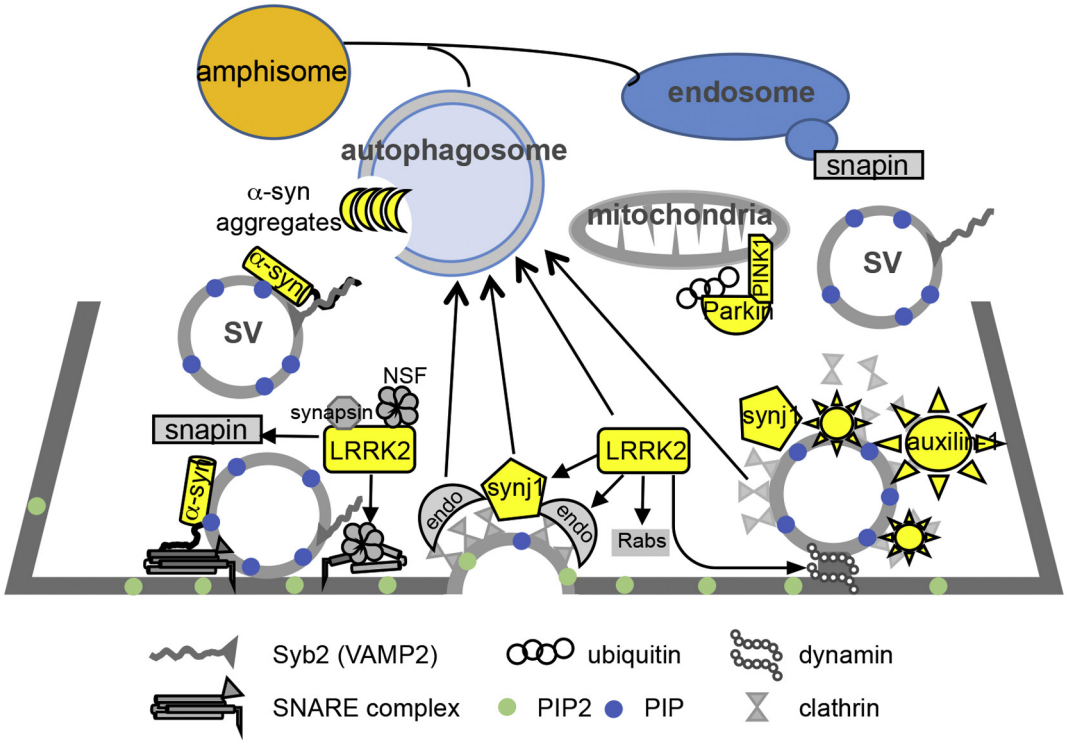

Fig. 1. PD genes in regulating both autophagy and SV trafficking.
A cartoon illustrates several PARK genes (highlighted in yellow) at the presynaptic terminal and their roles in regulating SV exocytosis/endocytosis as well as their proposed connections to autophagy. As a promiscuous kinase, LRRK2 has been shown to regulate SV recycling via phosphorylating several synaptic targets (solid arrow heads), many of which are known to be involved in autophagosome formation (open arrow heads) as well.

2.3. LRRK2 interaction with synaptic proteins

Extensive studies have also suggested a role of LRRK2 in regulating SV recycling. LRRK2 was found to be present in the SV fraction and associate with SV membrane proteins, such as synaptophysin, VAMP2, and synapsin I. It was also shown to interact with cytosolic proteins such as NSF, MUNC18-1, dynamin-1, Rab5b and Rab7L1, and presynaptic membrane proteins, such as syntaxin 1A, which participate in regulating SV fusion and recycling (Piccoli et al., 2014; Cirnaru et al., 2014; Shin et al., 2008; Yun et al., 2015; MacLeod et al., 2013). Besides, LRRK2 coimmunoprecipitates with actin and tubulin, further supporting LRRK2 as part of a complex synaptic protein network involved in neurotransmission and synaptic remodeling (Piccoli et al., 2014; Cirnaru et al., 2014; Plowey et al., 2008).

Most PD-linked mutations (including the G2019S mutation in the LRRK2 kinase domain and the R1441C/G/H and Y1699C mutations in the ROC-GTPase domain) result in enhanced kinase activity (West et al., 2005; Li et al., 2010; Steger et al., 2016; Islam et al., 2016), and therefore the "gain-of-toxic-function" has been proposed as a potential pathogenic mechanism for LRRK2-mediated pathogenesis. The theory has led to identification of numerous targets of the LRRK2 kinase, many of which are synaptic proteins (Fig. 1), such as Snapin (Yun et al., 2013), NSF (Belluzzi et al., 2016), Rab5b (Shin et al., 2008; Yun et al., 2015), endophilin (Matta et al., 2012; Arranz et al., 2015) and synaptojanin1 (Islam et al., 2016; Pan et al., 2017). While many of these potential substrates remain to be validated in vivo, LRRK2 phosphorylation of synaptojanin1 and endophilinA were confirmed in flies expressing human LRRK2 mutants (Matta et al., 2012; Islam et al., 2016). A recent investigation employed a combination of phosphoproteomics, mouse genetics, and pharmacological LRRK2 inhibitors and identified a subset of Rab GTPases, including Rab8, Rab10 and Rab12 as bona-fide LRRK2 substrates (Steger et al., 2016). The pathogenic implication, however, remains to be understood. There are at least 60 Rab genes in the human genome and they function as regulators for a multitude of intracellular membrane trafficking pathways by recruiting effector molecules to their GTP-bound forms (reviewed by Zerial and McBride, 2001). It is likely that phosphorylation of the Rabs results in interference with membrane reorganization during synaptic transmission as well as during autophagosome trafficking and recycling.

2.4. LRRK2 regulation in SV trafficking

In parallel to the biochemical information on the altered protein network at the presynaptic terminal, LRRK2 has been shown in various experimental settings to modulate synaptic transmission and SV recycling.

2.4.1. LRRK2 and SV endocytosis

An early study showed that in cultured hippocampal neurons, expressing either wildtype (WT), GS or RC mutants of LRRK2 slowed SV endocytosis, while knocking down *LRRK2* by RNAi led to a more significant impairment in SV endocytosis (Shin et al., 2008). This study, however, contradicts with a recent study using the same optical probe (pHluorin) that failed to observe changes in SV endocytosis in cultured hippocampal neurons from *LRRK2*$^{-/-}$ or *LRRK1* and *LRRK2* double deleted mice (Maas et al., 2017). Interestingly, *LRRK2*$^{-/-}$ striatal neurons displayed impaired SV endocytosis (Arranz et al., 2015). Although it is unclear whether such alteration occurs in vivo or is associated with the role of LRRK2 in PD pathogenesis, it suggests that LRRK2 may regulate SV recycling in a cell type specific manner. Supporting this notion, our recent study showed that hyperactivity of the LRRK2 GS mutation specifically slowed SV endocytosis in cultured midbrain neurons (containing DAergic neurons), but not cortical neurons (Pan et al., 2017). In our study, we have also observed a modest slowing of SV endocytosis in *LRRK2*$^{-/-}$ cortical neurons while sparing the midbrain neurons. In *drosophila* neuromuscular junction (NMJ),

either deleting the *LRRK* gene or expressing human *LRRK2 GS* resulted in the slowing of SV endocytosis (Matta et al., 2012). The authors proposed that such bi-directional regulation of SV endocytosis by LRRK2 might be due to the unique biophysical role endophilinA (endoA) as a LRRK2 substrate (Ambroso et al., 2014). It is possible that both endoA and its close binding partner, synaptojanin1, are compromised by LRRK2 phosphorylation (Pan et al., 2017; Islam et al., 2016), leading to a failed protein network for supporting efficient SV recycling. Nevertheless, the differential regulation of this network in distinct cell types awaits further investigation.

2.4.2. LRRK2 and SV exocytosis

Removing endogenous LRRK2 has not been reported to alter SV exocytosis during continuous synaptic activity. However, in primary cortical neurons prepared from *h*LRRK2 GS BAC mice, SV exocytosis was substantially enhanced, which can be reversed by a LRRK2 kinase inhibitor, GSK2578215A (Belluzzi et al., 2016). Similar kinase activity-dependent facilitation of SV exocytosis was also observed for cortical neurons derived from *m*LRRK2 GS BAC mice (Pan et al., 2017). In contrast, exocytosis of midbrain neurons was not affected by the GS mutation. The enhanced exocytosis in GS expressing cortical neurons could be due to phosphorylation of NSF, which results in enhanced dissociation of the SNARE complex (Belluzzi et al., 2016). In the brief time scale when exocytosis was examined in the above studies, calcium homeostasis is believed to play an essential role in modulating SV fusion. Recent evidence for a direct regulatory role of LRRK2 on CaV2.1 channel (Bedford et al., 2016) calls for a more detailed analysis in this arena. As calcium was shown to regulate autophagy (reviewed in Bootman et al., 2017), it is unclear if the change of calcium levels in response to action potential also alters autophagy pathway at axon terminal.

2.4.3. LRRK2 and synaptic transmission

In rat cortical neurons, Plowey et al. showed that LRRK2 GS mutant enhances glutamatergic synapse activity by increasing the number of contacts (Plowey et al., 2014). A recent in vivo study using *h*LRRK2 GS KI mice, however, found no difference in the number of glutamatergic synapse in the striatum, despite the overly active glutamatergic transmission observed at postnatal day 21 (Matikainen-Ankney et al., 2016). The authors also found that the effect is kinase activity dependent and originates from the presynaptic site, consistent with the GS mutation-induced enhancement in exocytosis in cultured cortical neurons (Belluzzi et al., 2016; Pan et al., 2017). In aged (8–12 months) *m*LRRK2 GS BAC mice, the enhanced basal level of excitatory synaptic transmission was found in the hippocampal Schaffer collateral–CA1 synapse. However, the change did not appear to be from the presynaptic terminal (Sweet et al., 2015). Although there are apparent differences in the age and the brain region examined, it is certainly possible that LRRK2 modulates synaptic transmission from both pre- and postsynaptic sites.

DAergic transmission has been independently evaluated by cyclic voltammetry as conventional electrical recording cannot access the G-protein coupled DA receptors. Using a complement of LRRK2 kinase inhibitors, DA release was not altered at various stimulations patterns (Qin et al., 2017). In 12-month old *m*LRRK2 GS BAC mice, we previously reported decreased release of DA in response to single as well as prolonged stimulations (Li et al., 2010). Similar impairment was found in two other different *h*LRRK2 GS transgenic lines (Liu et al., 2015; Chou et al., 2014). However, in *h*LRRK2 RC BAC mice, evoked DA release was not altered at 6–8 weeks of age (Sanchez et al., 2014), despite their abnormal DA-dependent motor functions starting at 6 months (Li et al., 2009). In a recent study in *h*LRRK2 WT, GS and RC BAC transgenic rats, evoked DA release was also found reduced when rats aged to 18–22 months (Sloan et al., 2016). These results from multiple groups and the examination of different animal models consistently suggest an age-dependent inhibitory role of LRRK2 PD mutants in DAergic transmission. The mechanism of reduced DAergic transmission remains unknown. Whether it is due partially to altered SV exocytosis and endocytosis is yet to be demonstrated in the in vivo model. Other impairments, such as a decreased capability for neurotransmitter refilling in aged DA neurons (Liu et al., 2015), may also account for the LRRK2 mutation-induced age-dependent impairment in DAergic transmission.

2.5. The role of LRRK2 in autophagy/SV trafficking crosstalk

Despite substantial evidence demonstrating separate roles of LRRK2 in SV trafficking and in autophagy regulation, it remains unclear whether these are two independent pathways of LRRK2 are interconnected. The challenge lies in the lack of knowledge and strategy to study autophagy in neurons from the central nervous system where SV trafficking is well characterized. Several elegant works reported autophagosome formation at the presynaptic terminal (Maday and Holzbaur, 2014; Vanhauwaert et al., 2017), however, most of these works are based on mammalian periphery neurons (dorsal root ganglion/DRG neurons) or non-mammalian synapses (*Drosophila* neuromuscular junction/NMJ). Autophagosome formation in nerve terminals of central neurons has not been reported or consistently observed using conventional autophagy induction strategies, such as starvation or inhibition of the mTOR pathway. It is likely that autophagy is not a robust and frequent cellular event at central synapses and cannot be easily captured; or, perhaps autophagosome formation in central synapses require unconventional induction strategies, which are yet to be identified. Nevertheless, it remains possible that LRRK2 uses similar molecular signaling complexes in mediating both SV trafficking and autophagosome formation at nerve terminals. Careful analyses in DRG neurons or *Drosophila* NMJ, similar to what has been demonstrated for endophilinA (detailed below), may help elucidate this potential crosstalk.

3. Presynaptic protein α-synuclein interacts with autophagy and SV membrane

A-Synuclein (α-syn), encoded by the *SNCA/PARK1, 4* genes, is the key protein component of Lewy bodies present in the parkinsonian brain. It is a presynaptic terminal-enriched small (14 kDa) protein containing three functional domains: the highly conserved N-terminal domain, which can form amphipathic α helices for lipid interaction (Davidson et al., 1998), the central hydrophobic NAC domain, which is involved in formation of β-sheet rich amyloid-like fibrils (Giasson et al., 2001), and an unfolded C-terminal tail, which is known to bind VAMP2/synaptobrevin-2. All PD-linked disease mutations, A30P, E46K, H50Q, G51D and A53T (Polymeropoulos et al., 1997; Kruger et al., 1998; Zarranz et al., 2004; Proukakis et al., 2013; Appel-Cresswell et al., 2013; Lesage et al., 2013), lie in the amphipathic region, suggesting a role of α-syn/lipid interaction in PD pathogenesis. Besides genetic variants, α-syn overexpression (gene duplication or triplication) also leads to familial early-onset severe forms of PD (Singleton et al., 2003; Chartier-Harlin et al., 2004).

3.1. α-synuclein and autophagy

Three degradation pathways, including CMA, autophagy and Ub-proteasome were shown to regulate the turnover of α-syn. A-syn contains a pentapeptide motif (KFERQ), which can be recognized by the chaperone protein HSC70, essential for CMA-mediated degradation (Cuervo et al., 2004). HSC-70 bound α-syn can then be transported into the lysosome via interaction with LAMP-2A. Mutant forms of α-syn (A30P, A53T) can bind much more strongly to LAMP-2A, and therefore failed to be transported across the membrane (Martinez-Vicente et al., 2008). Similar to LRRK2 and mutant LRRK2, which were discussed above, such interference with normal function of CMA translocation complex reduces degradation of other CMA substrates, including damaged and misfolded proteins, resulting in cytotoxicity. A-syn can be

modified by oxidized dopamine, creating DA-α-syn, which displays a phenotype closely resembling that of the mutant α-syn. In various experimental conditions, CMA defect was observed when expressing DA-α-syn (Martinez-Vicente et al., 2008). Whether such α-*syn*-mediated toxicity underlies the pathogenic mechanism of PD remains to be tested particularly in vivo.

Besides CMA, current evidence also demonstrated a role of α-syn in interfering with macroautophagy or autophagy. Although mutant A30P and A53T α-syn blocks CMA (Cuervo et al., 2004), other studies found that expressing *h*A53T mutant α-syn enhanced autophagosome formation, likely due to a higher demand to remove α-syn proteins (Stefanis et al., 2001; Yu et al., 2009). In contrast, in mammalian cell lines, expressing *h*E46K mutant impairs autophagy induction. In cultured cells or neurons treated with preformed α-syn fibrils (PFFs), α-syn inclusions/aggregates, once formed, were shown to be refractory to degradation by autophagy due to reduced autophagosome clearance (Tanik et al., 2013). The exact mechanism for α-*syn*-mediated disruption of autophagy, however, remains controversial. Results obtained from mammalian cells and in transgenic mice suggest that overexpression of α-syn could compromise autophagy via Rab1a inhibition, accompanied by mislocalization of the autophagy protein, Atg9 (Winslow et al., 2010); and that overexpression of the E46K mutant could inhibit autophagy via deregulate the JNK1-bcl-2 pathway, but not the mTOR pathway (Yan et al., 2014). Whether the same regulation applies to other α-syn mutations is yet to be elucidated. As lipid interaction with α-syn might be essential for revealing its pathogenic role, more investigation of the impact of lipid metabolism on autophagy regulation is needed. Earlier studies suggested that α-syn inhibits phospholipase D (PLD) (Jenco et al., 1998; Ahn et al., 2002), a pivotal enzyme that regulates a multitude of phospholipid species and membrane trafficking pathways (reviewed in Cazzolli et al., 2006). Whether such alteration affects autophagosome formation remains unknown and awaits further investigation.

3.2. α-synuclein in SV trafficking

In the presynaptic terminal, it is believed that α-syn exists in the equilibrium of two forms: a native unfolded monomeric form and a SV membrane bound α-helical form. Evidence has shown that the membrane bound form is physiologically relevant and may form higher-order multimers to promote SNARE complex formation during SV exocytosis via interaction with VAMP2 (Burre et al., 2010; Diao et al., 2013; Burre et al., 2013) (Fig. 1). Surprisingly, deletion of α-syn, or even both α- and β-syn, had led to only minor impairments on glutamatergic transmission, which were observed during prolonged stimulation (Murphy et al., 2000; Cabin et al., 2002; Chandra et al., 2004). The nigral DAergic system, however, appears to be affected, which is represented by increased release of DA with paired or continuous stimuli in α-syn null mice (Abeliovich et al., 2000; Yavich et al., 2004; Senior et al., 2008). The enhanced release was shown to be a result of increased rate of SV refilling due to loss of α-syn (Yavich et al., 2004). However, it is somewhat at odds with the findings in hippocampal neurons, where the mobilization of the reserve pool of SVs is affected (Murphy et al., 2000). Introducing WT or mutant forms of α-syn has resulted in conflicting results. Transgenic mice expressing *h*WT α-syn impairs SV exocytosis in hippocampal neurons (Yavich et al., 2004), which is consistent with the findings in rat midbrain DAergic neurons (Nemani et al., 2010; Gaugler et al., 2012) or neuroendocrine cells (Larsen et al., 2006) overexpressing α-syn. Interestingly, while expressing A53T or E46K mutant of α-syn results in a similar inhibitory effect in SV exocytosis, A30P mutation failed to affect the rate of exocytosis (Nemani et al., 2010). Although it may seem plausible considering that only the A30P (not A53T or E46K) mutant protein exhibits significantly reduced membrane binding (Jo et al., 2002; Bussell Jr. and Eliezer, 2004; Bodner et al., 2010), it suggests an intriguing possibility that different mutations of α-syn work through distinct cellular pathways in PD pathogenesis. More studies are required to understand how α-syn and its mutants interact with SV membranes in various neuronal types and modulate their dynamics in response to synaptic activities. These studies may ultimately lead to a better understanding of how α-syn/lipid interaction intersects SV recycling and autophagosome biogenesis.

4. EndophilinA: a junction of SV endocytosis and autophagy

Mammalian endophilinA (endoA) contains a family of three homologous genes - endoA1 (encoded by *SH3GL2*), endoA2 (encoded by the *SH3GL1*) and endoA3 (encoded by *SH3GL3*), among which, endoA1 is the only brain-specific isoform. EndoA is a member of the BAR protein family involved in membrane deformation, and its SH3 domain in the carboxyl terminal is capable of recruiting dynamin and synaptojanin1 (Fig. 1). The role of endoA1 in neuronal SV recycling was described 20 years ago (Ringstad et al., 1997). Following studies have demonstrated the impaired SV endocytosis in multiple animal models when two or more endoA isoforms are disrupted (Guichet et al., 2002; Verstreken et al., 2002; Schuske et al., 2003; Milosevic et al., 2011). It was not until recently that endoA was shown as a direct substrate of LRRK2 kinase at the NMJ of *Drosophila* (Matta et al., 2012). The authors have also found that the slowed SV endocytosis in *LRRK* mutant flies could be reversed by heterozygous mutation of the *Drosophila* endoA gene (Arranz et al., 2015). Although the in vivo evidence from a mammalian model is still lacking, this work represents the first evidence that endoA might be involved in PD pathogenesis via interaction with *LRRK2*. More interestingly, *SH3GL2* is now revealed in a meta-analysis as a significant risk gene for sporadic PD patients (Chang et al., 2017). The current research progress has brought a growing interest in understanding the role of endoA in DAergic neuron degeneration.

EndoA triple knockout (TKO) mice are lethal shortly after birth. Mice with endoA deficiency exhibit age-dependent motor impairments and progressive ataxia (Murdoch et al., 2016) reminiscent of neurodegenerative phenotypes in endoA1 and endoA2 double deleted (DKO) mice (Milosevic et al., 2011). Transcriptional analysis for the DKO and TKO hippocampus revealed the deregulation of autophagy and the Ub-proteasome system (Murdoch et al., 2016). Consistent with this idea, Soukup et al. showed that *Drosophila* endoA facilitates autophagosome formation at the presynaptic terminal by formation of highly curved membranes upon phosphorylation by LRRK. The curved membranes act as docking stations for recruitment of additional Atg proteins necessary for autophagosome maturation (Soukup S.F., et al. 2016). Although endoA-assisted membrane deformation is also required for SV endocytosis (Bai et al., 2010), and LRRK phosphorylation of endoA also modulates SV recycling (Matta et al., 2012), the role of endoA in autophagy regulation may be independent of its action in SV endocytosis (Soukup S.F., et al. 2016). The most compelling evidence is that LRRK phosphomimetic and phosphodead mutant of endoA exhibited opposite roles in autophagosome formation, but similar inhibitory roles in SV endocytosis.

The results presented in the series of work by Verstreken and colleagues are extremely exciting. It is also the first time that autophagy and SV endocytosis, the two presumably independent membrane trafficking processes, were examined at the presynaptic terminal as a potential cooperation via a common signaling pathway involving endoA. There are, however, several questions remain to be addressed. For example, how does LRRK phosphorylated endoA differentiate its designated role in SV endocytosis versus autophagy? Does endoA regulate SV trafficking and autophagy in a comparable way as in mammalian synapses? A major discrepancy between the fly NMJ and the mammalian central neuron synapse is that starvation (amino acid deprivation) does not induce autophagosome formation in cultured mouse hippocampal neurons (Maday and Holzbaur, 2016) as does for fly NMJ (Vanhauwaert et al., 2017; Soukup S.F., et al. 2016; Murdoch et al., 2016). In addition, there has been no report to date showing synaptic

activity-induced autophagosome formation in central neurons as does for fly NMJ, despite the observation that autophagosomes preferentially form at the presynaptic terminals of cultured dorsal root ganglion (DRG) and hippocampal neurons at basal conditions (Maday and Holzbaur, 2016).

5. Synaptojanin1 and auxilin in SV endocytosis and autophagy

Mutations in *SYNJ1* and *DNAJC6* were identified in the recent years in families with juvenile atypical Parkinsonism and are known as *PARK20* and *PARK19*, respectively (Krebs et al., 2013; Quadri et al., 2013; Olgiati et al., 2014; Kirola et al., 2016; Taghavi et al., 2017; Edvardson et al., 2012; Köroğlu et al., 2013). Patients carrying these mutations show similar early-onset symptoms and are often accompanied by seizure and other pyramidal dysfunctions. Synaptojanin1 (synj1) and auxilin are known to be close partners involved in clathrin coat removal after SV endocytosis (Fig. 1). Mice with synj1 or auxilin deletion die shortly after birth, and their synapses exhibit accumulation of clathrin-coated vesicles (CCVs) (Cremona et al., 1999; Yim et al., 2010).

Synj1 is an essential inositol phosphatase enriched in the presynapticterminal and auxilin is less characterized compared to synj1. Synj1 is a 145-kDa protein containing three functional domains: the SAC1-like domain, which hydrolyzes inositol monophosphates, such as PI3P and PI4P; the 5′-phosphatase domain, which hydrolyzes PI(4,5)P_2 at the 5′ position to produce PI4P; and the highly variable C-terminal proline-rich domain, which can be recruited by endoA during SV endocytosis. All three domains are involved in regulating SV recycling at various stages of synaptic activity (Mani et al., 2007). Interestingly, the disease related mutations are only present in the two phosphatase domains, suggesting an involvement of lipid deregulation in *SYNJ1*-mediated pathogenesis. Transgenic mice expressing disease mutation R258Q in the SAC1-like domain (*RQ KI* mice) recapitulated human symptoms and displayed dystrophic changes in the nigral DAergic terminals (Cao et al., 2017). In addition to an increased number of CCVs and reduced SV density in the dorsal striatum, onion-like membrane structures were observed in nigral DAergic terminals and striatal neurons, reminiscent of a burdened autophagy system in the absence of the essential autophagy genes (Komatsu et al., 2007; Nishiyama et al., 2007). In line with this observation, a recent study showed that the SAC1-like domain of synj1 is required for autophagosome maturation at presynaptic terminals of fly NMJ (Vanhauwaert et al., 2017). The missense mutation in the SAC1-like domain (Krebs et al., 2013) impairs the recruitment of Atg18a/WIPI2 to further interrupt Atg8/LC3 lipidation. The evidence of synj1-mediated autophagy in mammalian central synapses is yet to be demonstrated. Interestingly, the role for clathrin and PI(4,5)P_2 in regulating autophagic lysosome reformation (ALR) was reported (Rong et al., 2012), and whether synj1 is involved in ALR in neurons by regulating PI(4,5)P_2 remains unclear.

Similar to fly endoA, which is regulated by LRRK in NMJ, mammalian synj1 was shown to be phosphorylated by LRRK2 in the proline-rich domain and the phosphorylation may comprise in part synj1 endocytic function (Pan et al., 2017; Islam et al., 2016). *SYNJ1* haploinsufficiency leads to slowed SV endocytosis in midbrain neurons, reminiscent of those from *m*LRRK2 GS BAC mice. Combining *mLRRK2 GS* and *SYNJ1*$^{+/-}$ mutations did not further impair SV endocytosis but reduced exocytosis during continuous stimulation, indicating a complex genetic interaction between *SYNJ1* and *LRRK2* in regulating SV trafficking. Given the recent advances in identifying the interplay among LRRK2 (LRRK), endoA and synj1, it is possible that the three molecules form a tripartite signaling complex in regulating synaptic membrane trafficking and autophagy, both of which may go awry in the pathogenesis of PD.

6. Autophagy proteins regulate SV recycling

Autophagy is a highly conserved cellular process across species. Deletion of essential autophagy gene *ATG5* or *ATG7* in brain leads to autophagy failure, accumulation of ubiquitinated proteins, neurodegeneration and mortality soon after birth (Kuma, et al., and Komatsu et al., 2006), suggesting that autophagy is neuroprotective by constant removal of cellular waste. Conditional deletion of *ATG7* in DAergic neurons results in enlarged, dystrophic DAergic terminals, age-dependent accumulation of α-syn, progressive loss of DAergic neurons and motor function deficits in mice (Friedman et al., 2012). Interestingly, a separate study showed that loss of Atg7 in DAergic neurons also leads to enhanced DA release as well as faster recovery after SV depletion (Torres and Sulzer, 2012). In contrast, inhibition of mTOR by rapamycin, which induces formation of autophagosomes in the presynaptic terminals of DAergic neurons, leads to decreased dopamine release (Hernandez et al., 2012). Whether such modulation in DA transmission is mediated by Atg7 and occurs in other parts of the brain is yet to be demonstrated in greater detail.

There is additional evidence suggesting that interference with the autophagy pathway could also modulate synaptic transmission. For example, Snapin is a SNAP-associated protein that was initially identified as a SNAP25 binding protein (Chheda et al., 2001). Its roles in mediating late endosomal trafficking (Cai et al., 2010; Kim et al., 2012), lysosomal acidification and autophagosome maturation (Shi et al., 2017) have recently been characterized. Snapin may form complex with dynein upon autophagosome fusion with late endosomes in distal axons, and thereby facilitate the retrograde transport of autophagic clearance at the soma (Cheng et al., 2015). Disruption of snapin or snapin-dynein interaction results in accumulation of autophagosomes in distal axons and synaptic terminals, indicating its involvement in autophagy clearance. Interestingly, *SNAPIN*$^{-/-}$ cortical neurons also displayed reduced glutamatergic transmission (Pan et al., 2009) and impaired SV exocytosis (Di Giovanni and Sheng, 2015). In addition, snapin may regulate the SV pool size by interacting with dynein and facilitating the SV trafficking to the endosomal pathway (Di Giovanni and Sheng, 2015). Such regulation may represent an additional mechanism for crosstalk between SV trafficking and autophagy. SNAP29 is known as one of the three SNARE proteins besides syntaxin17 and VAMP8, in mediating fusion between autophagosomes and lysosomes in autophagy maturation step (Itakura et al., 2012; Takáts et al., 2013). Our early studies have found that SNAP29 could also act as a clamp for NSF-mediated SNARE complex dissociation at the presynaptic terminal and thereby inhibit continuous exocytosis at low frequencies (Su et al., 2001; Pan et al., 2005). The potential interplay between the two SNAP-29 regulated events remains unclear. An emergent school of thoughts propose that autophagy proteins are present at presynaptic terminals for degradation and turnover of presynaptic components, such as active zone proteins and SVs (Lüningschrör et al., 2017). The small GTPase, Rab26, has been implicated in this mechanism as it colocalizes with Atg16L1, LC3B and Rab33B (Binotti et al., 2015) and specifically directs synaptic vesicles to preautophagosomal structures (Lüningschrör et al., 2017). Although the current evidence of cross talk between autophagy and SV trafficking is rather limited, it has already begun to emerge as an exciting field of research for further elucidation of the interaction of the two membrane trafficking processes at presynaptic terminals.

7. Conclusion and outlook

The emerging evidence suggests extensive interaction between SV trafficking and autophagy pathways. Importantly several PD associated genes were found to play roles in regulating both SV trafficking and autophagy (Fig. 1). In support of above notion, other PD related genes such as *SYT11* (Pihlstrøm et al., 2013; Wang et al., 2016; Bento et al., 2016; Wang et al., 2018), *TMEM230* (Deng et al., 2016; Kim et al.,

2017) and VPS35 (Vilariño-Güell et al., 2011; Zimprich et al., 2011; Inoshita et al., 2017) are also implicated in these two pathways. While the existing evidence shows that SV trafficking and autophagy are regulated by an overlapping set of genes, how exactly these overlapping genes switch for regulation of different pathways has yet to be elucidated. The challenge facing the field is lack of a reliable readout system for autophagy in the presynaptic terminals and the complexity in autophagy regulation at the mammalian central nerve terminals. Further studies are required to address these important and exciting questions. Gaining insight into the mechanism for the crosstalk between SV trafficking and autophagy may assist to reveal novel therapeutic targets for PD.

References

Abeliovich, A., et al., 2000. Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. Neuron 25, 239–252.
Ahn, B.H., et al., 2002. Alpha-Synuclein interacts with phospholipase D isozymes and inhibits pervanadate-induced phospholipase D activation in human embryonic kidney-293 cells. J. Biol. Chem. 277, 12334–12342.
Alegre-Abarrategui, J., et al., 2009. LRRK2 regulates autophagic activity and localizes to specific membrane microdomains in a novel human genomic reporter cellular model. Hum. Mol. Genet. 18, 4022–4034.
Ambroso, M.R., et al., 2014. Endophilin A1 induces different membrane shapes using a conformational switch that is regulated by phosphorylation. Proc. Natl. Acad. Sci. U. S. A. 111, 6982–6987.
Appel-Cresswell, S., et al., 2013. Alpha-synuclein p.H50Q, a novel pathogenic mutation for Parkinson's disease. Mov. Disord. 28, 811–813.
Arranz, A.M., et al., 2015. LRRK2 functions in synaptic vesicle endocytosis through a kinase-dependent mechanism. J. Cell Sci. 128, 541–552.
Bai, J., et al., 2010. Endophilin functions as a membrane-bending molecule and is delivered to endocytic zones by exocytosis. Cell 143, 430–441.
Bedford, C., et al., 2016. LRRK2 regulates voltage-gated calcium channel function. Front. Mol. Neurosci. 9, 35.
Belluzzi, E., et al., 2016. LRRK2 phosphorylates pre-synaptic N-ethylmaleimide sensitive fusion (NSF) protein enhancing its ATPase activity and SNARE complex disassembling rate. Mol. Neurodegener. 11, 1.
Bento, C.F., et al., 2016. The Parkinson's disease-associated genes ATP13A2 and SYT11 regulate autophagy via a common pathway. Nat. Commun. 7, 11803.
Binotti, B., et al., 2015. The GTPase Rab26 links synaptic vesicles to the autophagy pathway. elife 4, e05597.
Bodner, C.R., et al., 2010. Differential phospholipid binding of alpha-synuclein variants implicated in Parkinson's disease revealed by solution NMR spectroscopy. Biochemistry 49, 862–871.
Bootman, M.D., et al., 2017. The regulation of autophagy by calcium signals: do we have a consensus? Cell Calcium pii: S0143-4160 (17), 30123–30129.
Brose, N., et al., 1992. Synaptotagmin: a calcium sensor on the synaptic vesicle surface. Science 256, 1021–1025.
Burre, J., et al., 2010. Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. Science 329, 1663–1667.
Burre, J., et al., 2013. Properties of native brain α-synuclein. Nature 498, E4–6.
Bussell Jr., R., Eliezer, D., 2004. Effects of Parkinson's disease-linked mutations on the structure of lipid-associated alpha-synuclein. Biochemistry 43, 4810–4818.
Cabin, D.E., et al., 2002. Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged repetitive stimulation in mice lacking alpha-synuclein. J. Neurosci. 22, 8797–8807.
Cai, Q., et al., 2010. Snapin-regulated late endosomal transport is critical for efficient autophagy-lysosomal function in neurons. Neuron 68, 73–86.
Cao, M., et al., 2017. Parkinson sac domain mutation in synaptojanin 1 impairs clathrin uncoating at synapses and triggers dystrophic changes in dopaminergic axons. Neuron. 93, 882–896 (e5).
Cazzolli, R., et al., 2006. Phospholipid signalling through phospholipase D and phosphatidic acid. IUBMB Life 58, 457–461.
Chandra, S., et al., 2004. Double-knockout mice for alpha- and beta-synucleins: effect on synaptic functions. Proc. Natl. Acad. Sci. U. S. A. 101, 14966–14971.
Chang, D., et al., 2017. A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. Nat. Genet. 49, 1511–1516.
Chartier-Harlin, M.C., et al., 2004. Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet 364, 1167–1169.
Cheng, X.T., et al., 2015. Axonal autophagosomes recruit dynein for retrograde transport through fusion with late endosomes. J. Cell Biol. 209, 377–386.
Chheda, M.G., et al., 2001. Phosphorylation of Snapin by PKA modulates its interaction with the SNARE complex. Nat. Cell Biol. 3, 331–338.
Chou, J.S., et al., 2014. (G2019S) LRRK2 causes early-phase dysfunction of SNpc dopaminergic neurons and impairment of corticostriatal long-term depression in the PD transgenic mouse. Neurobiol. Dis. 68, 190–199.
Cirnaru, M.D., et al., 2014. LRRK2 kinase activity regulates synaptic vesicle trafficking and neurotransmitter release through modulation of LRRK2 macro-molecular complex. Front. Mol. Neurosci. 27, 7–49.
Cremona, O., et al., 1999. Essential role of phosphoinositide metabolism in synaptic vesicle recycling. Cell 99, 179–188.
Cuervo, A.M., 2010. Chaperone-mediated autophagy: selectivity pays off. Trends Endocrinol. Metab. 21, 142–150.
Cuervo, A.M., et al., 2004. Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. Science 305, 1292–1295.
Davidson, W.S., et al., 1998. Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. J. Biol. Chem. 273, 9443–9449.
Deng, H.X., et al., 2016. Identification of TMEM230 mutations in familial Parkinson's disease. Nat. Genet. 48, 733–739.
Di Giovanni, J., Sheng, Z.H., 2015. Regulation of synaptic activity by snapin-mediated endolysosomal transport and sorting. EMBO J. 34, 2059–2077.
Diao, J., et al., 2013. Complexin-1 enhances the on-rate of vesicle docking via simultaneous SNARE and membrane interactions. J. Am. Chem. Soc. 135, 15274–15277.
Edvardson, S., et al., 2012. A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. PLoS One 7, e36458.
Friedman, L.G., et al., 2012. Disrupted autophagy leads to dopaminergic axon and dendrite degeneration and promotes presynaptic accumulation of alpha-synuclein and LRRK2 in the brain. J. Neurosci. 32, 7585–7593.
Gaugler, M.N., et al., 2012. Nigrostriatal overabundance of α-synuclein leads to decreased vesicle density and deficits in dopamine release that correlate with reduced motor activity. Acta Neuropathol. 123, 653–669.
Giaime, E., et al., 2017. Age-dependent dopaminergic neurodegeneration and impairment of the autophagy-lysosomal pathway in LRRK-deficient mice. Neuron 96, 796–807.
Giasson, B.I., et al., 2001. A hydrophobic stretch of 12 amino acid residues in the middle of alpha-synuclein is essential for filament assembly. J. Biol. Chem. 276, 2380–2386.
Gómez-Suaga, P., Hilfiker, S., 2012. LRRK2 as a modulator of lysosomal calcium homeostasis with downstream effects on autophagy. Autophagy 8, 692–693.
Granseth, B., et al., 2007. Clathrin-mediated endocytosis: the physiological mechanism of vesicle retrieval at hippocampal synapses. J. Physiol. 585, 681–686.
Guichet, A., et al., 2002. Essential role of endophilin A in synaptic vesicle budding at the drosophila neuromuscular junction. EMBO J. 21, 1661–1672.
He, L., Wu, L.G., 2007. The debate on the kiss-and-run fusion at synapses. Trends Neurosci. 30, 447–455.
Hernandez, D., et al., 2012. Regulation of presynaptic neurotransmission by macroautophagy. Neuron 74, 277–284.
Inoshita, T., et al., 2017. Vps35 in cooperation with LRRK2 regulates synaptic vesicle endocytosis through the endosomal pathway in drosophila. Hum. Mol. Genet. 26, 2933–2948.
Islam, S., et al., 2016. Human R1441C LRRK2 regulates the synaptic vesicle proteome and phosphoproteome in a drosophila model of Parkinson's disease. Hum. Mol. Genet. 25, 5365–5382.
Itakura, E., et al., 2012. The hairpin-type tail-anchored SNARE syntaxin 17 targets to autophagosomes for fusion with endosomes/lysosomes. Cell 151, 1256–1269.
Jenco, J.M., et al., 1998. Regulation of phospholipase D2: selective inhibition of mammalian phospholipase D isoenzymes by alpha- and beta-synucleins. Biochemistry 37, 4901–4909.
Jo, E., et al., 2002. Defective membrane interactions of familial Parkinson's disease mutant A30P alpha-synuclein. J. Mol. Biol. 315, 799–807.
Kim, H.J., et al., 2012. Beclin-1-interacting autophagy protein Atg14L targets the SNARE-associated protein Snapin to coordinate endocytic trafficking. J. Cell Sci. 125, 4740–4750.
Kim, M.J., et al., 2017. The Parkinson's disease-linked protein TMEM230 is required for Rab8a-mediated secretory vesicle trafficking and retromer trafficking. Hum. Mol. Genet. 26, 729–741.
Kirola, L., et al., 2016. Identification of a novel homozygous mutation Arg459Pro in SYNJ1 gene of an Indian family with autosomal recessive juvenile Parkinsonism. Parkinsonism Relat. Disord. 31, 124–128.
Klionsky, D.J., 2007. Autophagy: from phenomenology to molecular understanding in less than a decade. Nat. Rev. Mol. Cell Biol. 8, 931–937.
Ko, H.S., et al., 2009. CHIP regulates leucine-rich repeat kinase-2 ubiquitination, degradation, and toxicity. Proc. Natl. Acad. Sci. U. S. A. 106, 2897–2902.
Komatsu, M., et al., 2006. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature 441, 880–884.
Komatsu, M., et al., 2007. Essential role for autophagy protein Atg7 in the maintenance of axonal homeostasis and the prevention of axonal degeneration. Proc. Natl. Acad. Sci. U. S. A. 104, 14489–14494.
Köroğlu, Ç., et al., 2013. DNAJC6 is responsible for juvenile parkinsonism with phenotypic variability. Parkinsonism Relat. Disord. 19, 320–324.
Krebs, C.E., et al., 2013. The Sac1 domain of SYNJ1 identified mutated in a family with early-onset progressive parkinsonism with generalized seizures. Hum. Mutat. 34, 1200–1207.
Kruger, R., et al., 1998. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nat. Genet. 18, 106–108.
Larsen, K.E., et al., 2006. Alpha-synuclein overexpression in PC12 and chromaffin cells impairs catecholamine release by interfering with a late step in exocytosis. J. Neurosci. 26, 11915–11922.
Lesage, S., et al., 2013. G51D α-synuclein mutation causes a novel parkinsonian-pyramidal syndrome. Ann. Neurol. 73, 459–471.
Li, Y., et al., 2009. Mutant LRRK2(R1441G) BAC transgenic mice recapitulate cardinal features of Parkinson's disease. Nat. Neurosci. 12, 826–828.
Li, X., et al., 2010. Enhanced striatal dopamine transmission and motor performance with LRRK2 overexpression in mice is eliminated by familial Parkinson's disease mutation G2019S. J. Neurosci. 30, 1788–1797.
Liu, G., et al., 2015. Selective expression of Parkinson's disease-related leucine-rich repeat kinase 2 G2019S missense mutation in midbrain dopaminergic neurons impairs dopamine release and dopaminergic gene expression. Hum. Mol. Genet. 24, 5299–5312.
Luerman, G.C., et al., 2014. Phosphoproteomic evaluation of pharmacological inhibition of leucine-rich repeat kinase 2 reveals significant off-target effects of LRRK-2-IN-1. J. Neurochem. 128, 561–576.
Lüningschrör, P., et al., 2017. Plekhg5-regulated autophagy of synaptic vesicles reveals a pathogenic mechanism in motoneuron disease. Nat. Commun. 8, 678.
Maas, J.W., et al., 2017. Endogenous leucine-rich repeat kinase 2 slows synaptic vesicle recycling in striatal neurons. Front. Synaptic Neurosci. 23, 5–9.
MacLeod, D.A., et al., 2013. RAB7L1 interacts with LRRK2 to modify intraneuronal

protein sorting and Parkinson's disease risk. Neuron 77, 425–439.
Maday, S., Holzbaur, E.L., 2014. Autophagosome biogenesis in primary neurons follows an ordered and spatially regulated pathway. Dev. Cell 30, 71–85.
Maday, S., Holzbaur, E.L., 2016. Compartment-specific regulation of autophagy in primary neurons. J. Neurosci. 36, 5933–5945.
Mani, M., et al., 2007. The dual phosphatase activity of synaptojanin1 is required for both efficient synaptic vesicle endocytosis and reavailability at nerve terminals. Neuron 56, 1004–1018.
Manzoni, C., et al., 2013. Inhibition of LRRK2 kinase activity stimulates macroautophagy. Biochim. Biophys. Acta 1833, 2900–2910.
Martinez-Vicente, M., et al., 2008. Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. J. Clin. Invest. 118, 777–788.
Mata, I.F., et al., 2006. LRRK2 in Parkinson's disease: protein domains and functional insights. Trends Neurosci. 29, 286–293.
Matikainen-Ankney, B.A., et al., 2016. Altered development of synapse structure and function in striatum caused by Parkinson's disease-linked LRRK2-G2019S mutation. J. Neurosci. 36, 7128–7141.
Matta, S., et al., 2012. LRRK2 controls an EndoA phosphorylation cycle in synaptic endocytosis. Neuron 75, 1008–1021.
Milosevic, I., et al., 2011. Recruitment of endophilin to clathrin-coated pit necks is required for efficient vesicle uncoating after fission. Neuron 72, 587–601.
Murdoch, J.D., et al., 2016. Endophilin-A deficiency induces the Foxo3a-Fbxo32 network in the brain and causes dysregulation of autophagy and the ubiquitin-proteasome system. Cell Rep. 17 (4), 1071–1086.
Murphy, D.D., et al., 2000. Synucleins are developmentally expressed, and alpha-synuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons. J. Neurosci. 20, 3214–3220.
Nemani, V.M., et al., 2010. Increased expression of alpha-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis. Neuron 65, 66–79.
Nishiyama, J., et al., 2007. Aberrant membranes and double-membrane structures accumulate in the axons of Atg5-null Purkinje cells before neuronal death. Autophagy 3, 591–596.
Nixon, R.A., 2013. The role of autophagy in neurodegenerative disease. Nat. Med. 19, 983–997.
Oku, M., et al., 2017. Evidence for ESCRT- and clathrin-dependent microautophagy. J. Cell Biol. 216, 3263–3274.
Olgiati, S., et al., 2014. PARK20 caused by SYNJ1 homozygous Arg258Gln mutation in a new Italian family. Neurogenetics 15, 183–188.
Orenstein, S.J., et al., 2013. Interplay of LRRK2 with chaperone-mediated autophagy.Interplay of LRRK2 with chaperone-mediated autophagy. Nat. Neurosci. 16, 394–406.
Paisán-Ruiz, C., et al., 2013. LRRK2: cause, risk, and mechanism. J. Parkinsons Dis. 3, 85–103.
Pan, P.Y., et al., 2005. SNAP-29-mediated modulation of synaptic transmission in cultured hippocampal neurons. J. Biol. Chem. 280, 25769–25779.
Pan, P.Y., et al., 2009. Snapin facilitates the synchronization of synaptic vesicle fusion. Neuron 61, 412–424.
Pan, P.Y., et al., 2017. Parkinson's disease-associated LRRK2 hyperactive kinase mutant disrupts synaptic vesicle trafficking in ventral midbrain neurons. J. Neurosci. 37, 11366–11376.
Piccoli, G., et al., 2014. Leucine-rich repeat kinase 2 binds to neuronal vesicles through protein interactions mediated by its C-terminal WD40 domain. Mol. Cell. Biol. 34, 2147–2161.
Pihlstrøm, L., et al., 2013. Supportive evidence for 11 loci from genome-wide association studies in Parkinson's disease. Neurobiol. Aging 34, 1708 (e7-13).
Plowey, E.D., et al., 2008. Role of autophagy in G2019S-LRRK2-associated neurite shortening in differentiated SH-SY5Y cells. J. Neurochem. 105, 1048–1056.
Plowey, E.D., et al., 2014. Mutant LRRK2 enhances glutamatergic synapse activity and evokes excitotoxic dendrite degeneration. Biochim. Biophys. Acta 1842, 1596–1603.
Polymeropoulos, M.H., et al., 1997. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276, 2045–2047.
Proukakis, C., et al., 2013. A novel α-synuclein missense mutation in Parkinson disease. Neurology 80, 1062–1064.
Qin, Q., et al., 2017. Effects of LRRK2 inhibitors on nigrostriatal dopaminergic neurotransmission. CNS Neurosci. Ther. 23, 162–173.
Quadri, M., et al., 2013. Mutation in the SYNJ1 gene associated with autosomal recessive, early-onset parkinsonism. Hum. Mutat. 34, 1208–1215.
Ringstad, N., et al., 1997. The SH3p4/Sh3p8/SH3p13 protein family: binding partners for synaptojanin and dynamin via a Grb2-like Src homology 3 domain. Proc. Natl. Acad. Sci. U. S. A. 94, 8569–8574.
Rong, Y., et al., 2012. Clathrin and phosphatidylinositol-4,5-bisphosphate regulate autophagic lysosome reformation. Nat. Cell Biol. 14, 924–934.
Saha, S., et al., 2013. Regulation of autophagy by LRRK2 in *Caenorhabditis elegans*. Neurodegener. Dis. 13, 110–113.
Sanchez, G., et al., 2014. Unaltered striatal dopamine release levels in young Parkin knockout, Pink1 knockout, DJ-1 knockout and LRRK2 R1441G transgenic mice. PLoS One 9, e94826.
Schirinzi, T., et al., 2016. Early synaptic dysfunction in Parkinson's disease: insights from animal models. Mov. Disord. 31, 802–813.
Schuske, K.R., et al., 2003. Endophilin is required for synaptic vesicle endocytosis by localizing synaptojanin. Neuron 40, 749–762.
Senior, S.L., et al., 2008. Increased striatal dopamine release and hyperdopaminergic-like behaviour in mice lacking both alpha-synuclein and gamma-synuclein. Eur. J. Neurosci. 27, 947–957.
Shi, B., et al., 2017. SNAPIN is critical for lysosomal acidification and autophagosome maturation in macrophages. Autophagy 13 (2), 285–301.
Shin, N., et al., 2008. LRRK2 regulates synaptic vesicle endocytosis. Exp. Cell Res. 314, 2055–2065.
Singleton, A.B., et al., 2003. Alpha-synuclein locus triplication causes Parkinson's disease. Science 302, 841.
Singleton, A.B., et al., 2013. The genetics of Parkinson's disease: progress and therapeutic implications. Mov. Disord. 28, 14–23.
Sloan, M., et al., 2016. LRRK2 BAC transgenic rats develop progressive, L-DOPA-responsive motor impairment, and deficits in dopamine circuit function. Hum. Mol. Genet. 25, 951–963.
Smith, S.M., et al., 2008. Synaptic vesicle endocytosis: fast and slow modes of membrane retrieval. Trends Neurosci. 31, 559–568.
Sollner, T., et al., 1993. A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell 75, 409–418.
Soukup, S.F., et al., 2016. A LRRK2-dependent EndophilinA phosphoswitch is critical for macroautophagy at presynaptic terminals. Neuron. 92, 829–844.
Stefanis, L., et al., 2001. Expression of A53T mutant but not wild-type alpha-synuclein in PC12 cells induces alterations of the ubiquitin-dependent degradation system, loss of dopamine release, and autophagic cell death. J. Neurosci. 21, 9549–9560.
Steger, M., et al., 2016. Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a subset of Rab GTPases. elife 29, 5.
Su, Q., et al., 2001. SNAP-29: a general SNARE protein that inhibits SNARE disassembly and is implicated in synaptic transmission. Proc. Natl. Acad. Sci. U. S. A. 98, 14038–14043.
Sweet, E.S., et al., 2015. The Parkinson's disease-associated mutation LRRK2-G2019S impairs synaptic plasticity in mouse hippocampus. J. Neurosci. 35, 11190–11195.
Taghavi, S., et al., 2017. A clinical and molecular genetic study of 50 families with autosomal recessive parkinsonism revealed known and novel gene mutations. Mol. Neurobiol. http://dx.doi.org/10.1007/s12035-017-0535-1.
Tagliaferro, P., et al., 2015. An early axonopathy in a hLRRK2(R1441G) transgenic model of Parkinson disease. Neurobiol. Dis. 82, 359–371.
Takáts, S., et al., 2013. Autophagosomal Syntaxin17-dependent lysosomal degradation maintains neuronal function in drosophila. J. Cell Biol. 201, 531–539.
Tanik, S.A., et al., 2013. Lewy body-like α-synuclein aggregates resist degradation and impair macroautophagy. J. Biol. Chem. 88, 15194–15210.
Tong, Y., et al., 2010. Loss of leucine-rich repeat kinase 2 causes impairment of protein degradation pathways, accumulation of alpha-synuclein, and apoptotic cell death in aged mice. Proc. Natl. Acad. Sci. 107, 9879–9884.
Tong, Y., et al., 2012. Loss of leucine-rich repeat kinase 2 causes age-dependent bi-phasic alterations of the autophagy pathway. Mol. Neurodegener. 9, 2–7.
Torres, C.A., Sulzer, D., 2012. Macroautophagy can press a brake on presynaptic neurotransmission. Autophagy 8, 1540–1541.
Trinh, J., Farrer, M., 2013. Advances in the genetics of Parkinson disease. Nat. Rev. Neurol. 9, 445–454.
Vanhauwaert, R., et al., 2017. The SAC1 domain in synaptojanin is required for autophagosome maturation at presynaptic terminals. EMBO J. 36, 1392–1411.
Verstreken, P., et al., 2002. Endophilin mutations block clathrin-mediated endocytosis but not neurotransmitter release. Cell 109, 101–112.
Vilariño-Güell, C., et al., 2011. VPS35 mutations in Parkinson disease. Am. J. Hum. Genet. 89, 162–167.
Wang, C., et al., 2016. Synaptotagmin-11 inhibits clathrin-mediated and bulk endocytosis. EMBO Rep. 17, 47–63.
Wang, C., et al., 2018. Synaptotagmin-11 is a critical mediator of parkin-linked neurotoxicity and Parkinson's disease-like pathology. Nat. Commun. 9, 81.
West, A.B., et al., 2005. Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. Proc. Natl. Acad. Sci. 102, 16842–16847.
Winslow, A.R., et al., 2010. α-synuclein impairs macroautophagy: implications for Parkinson's disease. J. Cell Biol. 190, 1023–1037.
Wong, E., Cuervo, A.M., 2010. Autophagy gone awry in neurodegenerative diseases. Nat. Neurosci. 13, 805–811.
Yan, J.Q., et al., 2014. Overexpression of human E46K mutant α-synuclein impairs macroautophagy via inactivation of JNK1-Bcl-2 pathway. Mol. Neurobiol. 50, 685–701.
Yavich, L., et al., 2004. Role of alpha-synuclein in presynaptic dopamine recruitment. J. Neurosci. 24, 11165–11170.
Yim, Y.I., et al., 2010. Endocytosis and clathrin-uncoating defects at synapses of auxilin knockout mice. Proc. Natl. Acad. Sci. U. S. A. 107, 4412–4417.
Yu, W.H., et al., 2009. Metabolic activity determines efficacy of macroautophagic clearance of pathological oligomeric alpha-synuclein. Am. J. Pathol. 175, 736–747.
Yue, Z., 2007. Regulation of neuronal autophagy in axon: implication of autophagy in axonal function and dysfunction/degeneration. Autophagy 3, 139–141.
Yun, H.J., et al., 2013. LRRK2 phosphorylates Snapin and inhibits interaction of Snapin with SNAP-25. Exp. Mol. Med. 16, 34–45.
Yun, H.J., et al., 2015. An early endosome regulator, Rab5b, is an LRRK2 kinase substrate. J. Biochem. 157, 485–495.
Zarranz, J.J., et al., 2004. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann. Neurol. 55, 164–173.
Zerial, M., McBride, H., 2001. Rab proteins as membrane organizers. Nat. Rev. Mol. Cell Biol. 2, 107–117.
Zimprich, A., et al., 2004. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 44, 601–607.
Zimprich, A., et al., 2011. A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease. Am. J. Hum. Genet. 89, 168–175.