Journal of Genetics, Vol. 97, No. 3, July 2018, pp. 715–727
https://doi.org/10.1007/s12041-018-0959-z

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REVIEW ARTICLE

Regulation of membrane dynamics by Parkinson's disease-associated genes

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Received 23 November 2017; revised 15 January 2018; accepted 24 January 2018

Abstract. Parkinson's disease (PD), the second most common neurodegenerative disease after Alzheimer's disease, develops sporadically, and its cause is unknown. However, 5–10% of PD cases are inherited as monogenic diseases, which provides a chance to understand the molecular mechanisms underlying neurodegeneration. Over 20 causative genes have already been identified and are being characterized. These PD-associated genes are broadly classified into two groups: genes involved in mitochondrial functions and genes related to membrane dynamics such as intracellular vesicle transport and the lysosomal pathway. In this review, we summarize the latest findings on the mechanism by which members of the latter group of PD-associated genes regulate membrane dynamics, and we discuss how mutations of these genes lead to dopaminergic neurodegeneration.

Keywords. soluble NSF-attachment protein receptor; phosphoinositide; autophagy; retromer; Rab GTPase; endocytosis; synaptic vesicle.

Introduction

A neurodegenerative disorder, Parkinson's disease (PD) is pathologically characterized by the loss of dopaminergic neurons in the substantia nigra of the midbrain. However, clinically, the autonomic nervous system and olfactory neurons are also affected, suggesting that nerve dysfunction is wider than it was once thought. The recent findings of α-synuclein propagation via neural circuits strengthen this idea. Most PD cases are sporadic, and their aetiology is largely unknown. A small fraction of PD cases are familial, and over 20 causative genes have been identified. In addition, several risk genes and risk loci for sporadic PD have also been reported. These advances have revealed that more than a few PD-associated genes regulate membrane dynamics, which includes endocytosis and exocytosis, vesicular transport and autophagy.

The lipid and protein components of biomembranes vary between organisms and between organelles, and those components determine their properties and identities. These biomembranes provide zones for signal transduction, metabolism, and transport of substances. Extracellular substances and receptors on biomembranes are taken up together with biomembranes by endocytosis and are first transported to the early endosomes (figure 1). From the early endosome, some are transported to the late endosomes and are degraded by the lysosome-mediated degradation system (figure 1). Others are transported to the trans-Golgi network (TGN) by retrograde transport or again transported to the cell membrane via the recycling endosomes (figure 1). Such membrane dynamics are important to maintain cell and organelle activities. During the regulation of membrane dynamics, the composition of the biomembrane changes. Phosphoinositide is one type of phospholipid that is contained in the biomembrane and can become any of seven isomers depending on the phosphorylation mode of the three hydroxyl groups of the inositol ring (figure 2). There is no *in vivo* evidence that the other two hydroxyl groups are subjected to phosphorylation. The combination of phosphorylation in phosphoinositides plays a role in distinguishing the identity of vesicles and organelles, where a variety of proteins that target specific phosphoinositides are involved in regulating membrane dynamics, vesicular sorting, signal transduction and lysosome-dependent degradation.

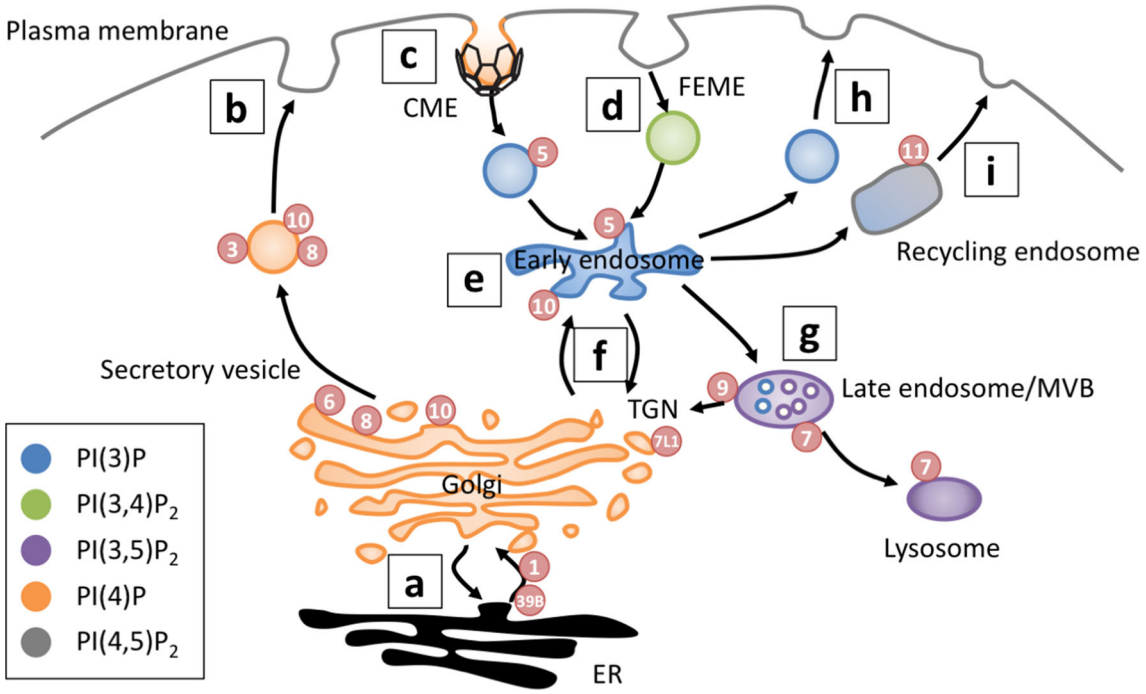

Figure 1. Membrane trafficking in the cell body. (a) ER-to-Golgi trafficking. Newly synthesized proteins, lipids and sugar chains at the ER are transport to the Golgi. (b) Exocytosis from the Golgi. Secretory vesicles including newly generated or recycling molecules depart from the Golgi to the plasma membrane. (c) Clathrin-mediated endocytosis (CME). Clathrin binds to the plasma membrane containing $PI(4,5)P_2$ and regulates budding and fission of endocytosed membrane. (d) Fast Endophilin-mediated endocytosis (FEME). Endophilin-mediated clathrin-independent endocytosis from the plasma membrane. (e) Vesicles retrieved by CME/FEME are transported to the early endosomes. (f) Some endocytosed molecules are transported to the TGN and parts of them are returned to the early endosome. (g) Degradation pathway. Molecules to be broken down are transported to the lysosome via the late endosome and/or multivesicular body. (h) Direct recycling pathway. Membrane budding occurs on the early endosome and recycling vesicles are transported to the plasma membrane. (i) Recycling endosome pathway. Some molecules in the early endosome are transported to the plasma membrane via the recycling endosome. The numbers in the circles correspond to those in the names of the Rab proteins involved in these pathways.

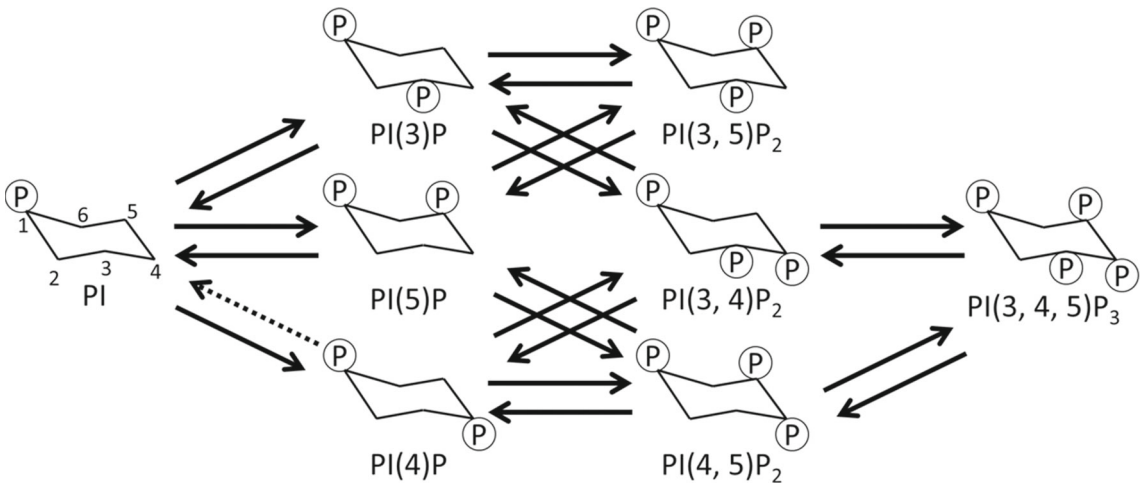

Figure 2. Metabolism of phosphoinositides. P, phosphate group; PI, phosphatidylinositol; PI(3)P, phosphatidylinositol 3-phosphate; PI(4)P, phosphatidylinositol 4-phosphate; PI(5)P, phosphatidylinositol 5-phosphate; $PI(3,4)P_2$, phosphatidylinositol 3,4-bisphosphate; $PI(3,5)P_2$, phosphatidylinositol 3,5-bisphosphate; $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; $PI(3,4,5)P_2$, phosphatidylinositol 3,4,5-triphosphate. Solid arrows, synthesis pathways are confirmed *in vitro* and/or *in vivo*; dashed arrow, synthesis pathway is unknown.

Axonal transport and synaptic activity are distinctive features of neurons. Upon the change in the electrical potential of the membrane and the subsequent inflow of Ca^{2+}, neurotransmitter release occurs by the integration of synaptic vesicle (SV) membrane into presynaptic membrane. The membranes of SVs after neurotransmitter release are retrieved via recycling mechanisms and are regenerated as SVs after refilling with neurotransmitters (figure 3). The SV recycling mechanism ensures sustained synaptic activity, which is particularly important for dopaminergic neurons with the pacemaking property (Guzman *et al.* 2010).

Advances in studies to understand the roles of PD-associated proteins in neurons have suggested that *α-synuclein*, *LRRK2*, *Vps35*, *Auxilin*/*DNAJC6*, *Synaptojanin 1*, *RME-8*/*DNAJC13*, *Vps13C* and *ATP13A2* are involved in membrane dynamics, including SV dynamics (figure 4; table 1). We introduce the latest findings, trying to propose intersecting pathogenic pathways where multiple PD genes are involved.

SV regulation by α-synuclein

Accumulation and aggregation of disease-specific abnormal proteins are common features of neurodegenerative diseases, which suggests that 'protein management' is dysregulated in affected neurons. In PD, the neuronal inclusions of aggregation-prone presynaptic protein α-synuclein, which are named Lewy bodies, are believed to be a key factor for dopaminergic neuron death. α-Synuclein is an abundant neuronal protein that accounts for 0.1% of total brain protein (Iwai *et al.* 1995). Mutations and SNPs of the *α-synuclein* gene are highly correlated with PD risk (Polymeropoulos *et al.* 1997; Satake *et al.* 2009; Simon-Sanchez *et al.* 2009; Nalls *et al.* 2014). The finding that the gene triplication of *α-synuclein* is responsible for the *PARK4* locus strongly indicates that increased intracellular concentration of α-synuclein becomes a risk for PD (Singleton *et al.* 2003; Farrer *et al.* 2004). The physiological roles of α-synuclein had not been fully understood because there are three synuclein homologues, α-synuclein, β-synuclein and γ-synuclein, in vertebrate genomes and because single knockout of the *α-synuclein* gene in mice does not produce apparent neuronal phenotypes. However, the studies on a triple knockout of all three *synuclein* genes and the finding that a genetic interaction of α-synuclein with a soluble NSF-attachment protein receptor (SNARE)-complex assembly chaperone cysteine-string protein-α (CSP α) suggest that α-synuclein regulates assembly of the SNARE complex (Chandra *et al.* 2005; Burre *et al.* 2010; Greten-Harrison *et al.* 2010) (figure 5). During the release and retrieval cycle of SVs, α-synuclein is thought to repeatedly bind and dissociate from the acidic phospholipid surface of SVs via its N-terminal, seven repeated motifs of 11 residues with the consensus sequence

Table 1. PD genes related to membrane dynamics.

	Gene symbol	Gene name	Possible functions	Hereditary form	Age of onset (early or late)	Lewy body pathology
PD causative genes	*PARK1*/*PARK4*	α-Synuclein (SNCA)	Regulation of synapse vesicle trafficking	AD	Early	+
	PARK8	LRRK2	Protein kinase	AD	Late	+/–
	PARK9	ATP13A2	Lysosomal ATPase	AR	Early	?
	PARK17	Vps35	Retromer component	AD	Late	–
	PARK19	Auxilin	Endocytosis	AR	Early	+
	PARK20	Synaptojanin 1	Polyphosphoinositide phosphatase	AR	Early	+
	PARK21	RME-8/TMEM230*	Endosomal transport	AD	Late	+
	PARK23	Vps13c	Endosomal transport	AR	Early	+
Risk loci	*RAB7L1*/*Rab29*	Rab7L1	Rab family protein			
	GAK	GAK	Cochaperone with a kinase domain			
	Rab39B	Rab39B	Rab family protein			
	INPP5F/*Sac2*	INPP5F	Phosphoinositide 4-phosphatase			

*Mutations of *TMEM230*, which is proposed to regulate SV trafficking, have been reported in the same pedigree in which *RME-8* has been assigned as the gene linked to PD (Deng *et al.* 2016). However, there are few studies from different populations to support the role of TMEM230 in PD. More evidence is still needed to clarify this question. ?, Brain pathology is not available from any patient diagnosed with Kufor–Rakeb disease.

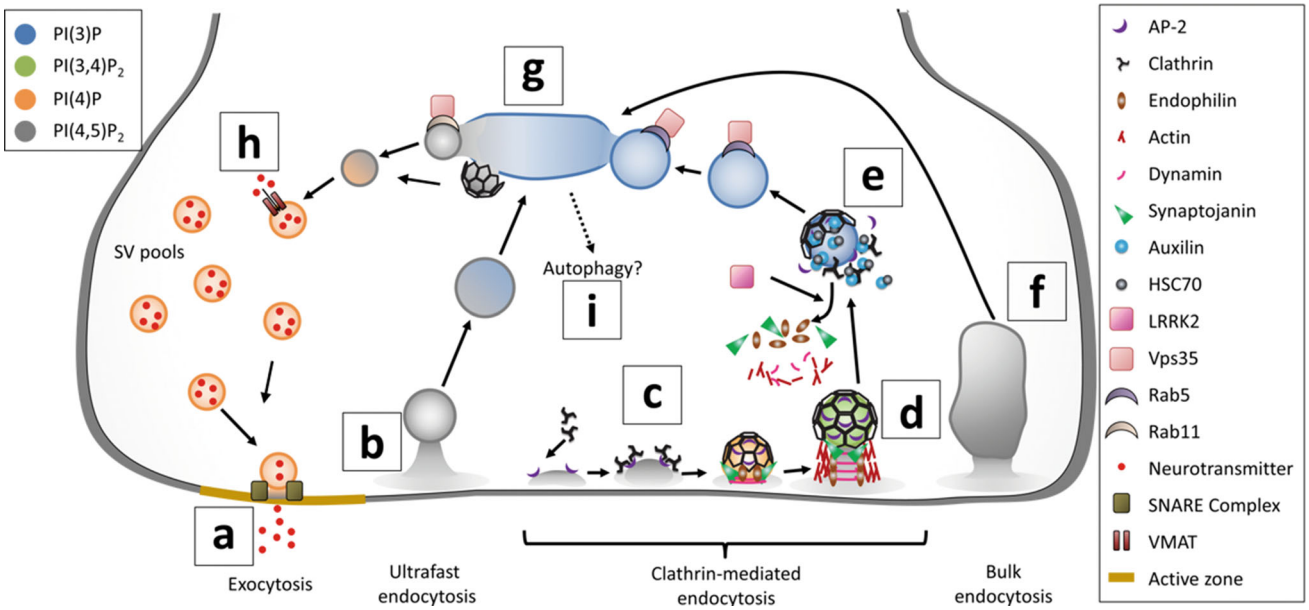

Figure 3. Membrane dynamics in the neuronal presynapse. (a) SVs are docked at the active zone of the synaptic membrane. Neurotransmitters are released by the activity of the SNARE complex upon neuronal excitation. (b) Ultrafast endocytosis. Clathrin-independent endocytosis mediates direct recycling of SVs very rapidly. (c) Clathrin-mediated endocytosis. AP-2 binds to the external membrane sites of the active zone and induces clathrin coating. (d) Endophilin, Synaptojanin and Dynamin regulate the fission of clathrin-coated vesicles in conjunction with actin as a pulling force. (e) Clathrin uncoating. Auxilin and Hsc70 remove clathrin from endocytosed vesicles. LRRK2-mediated phosphorylation of Endophilin A and Synaptojanin 1 promotes the dissociation of the Endophilin A-Synaptojanin 1 complex from endocytosed vesicles. Vps35 and Rab5 transport vesicles to the synaptic endosome. (f) Bulk endocytosis. Strong neuronal activity induces bulk endocytosis to recycle large amounts of membrane. (g) Synaptic endosome. Endocytosed vesicles are fused to the synaptic endosome. Budding vesicles from the synaptic endosome regenerate SVs, where Vps35 and Rab11- and/or clathrin-mediated budding is involved. (h) SV maturation. SVs are filled with neurotransmitters through transporters such as vesicular monoamine transporter (VMAT). (i) Autophagy. Starvation and/or neuronal activity induces (macro)autophagy at the synapses.

XKTKEGVXXXX. SVs are tethered to the presynaptic membrane via the SNARE complex, which is composed of the t-SNARE proteins syntaxin-1 and SNAP-25, and the v-SNARE protein synaptobrevin-2/VAMP2 (figure 5). α-Synuclein facilitates the assembly of the SNARE complex by forming a multimer to produce chaperone activity on the surface of SVs but does not cause a significant change in synaptic strength (Burre *et al.* 2014) (figure 5). When α-synuclein is released from SVs, α-synuclein presents as an unfolded monomeric form. These findings imply that the altered composition of membrane lipids and the dysregulation of SV cycling are the elements of α-synuclein aggregation.

The property of α-synuclein for membrane binding and deformation activities also appears to modulate slower exocytosis of large dense core vesicles, which include monoamines and peptides (Logan *et al.* 2017). The increased expression of both wild-type and disease-associated mutant forms of α-synuclein reduces the number of exocytotic events, while wild-type α-synuclein, but not disease-associated mutants, accelerates the kinetics of exocytosis at physiological levels, promoting fusion pore dilation (Logan *et al.* 2017). This finding suggests that exocytotic inhibition by α-synuclein is more relevant to the pathogenesis of PD. Currently, providing integrative modeling on the roles of α-synuclein in chaperone activity for the SNARE complex and exocytotic inhibition is difficult. Further studies on the physiological and pathological roles of α-synuclein in synaptic activity are required.

Pathological roles of α-synuclein in membrane trafficking

Vesicular trafficking in the cell body transports cargos packed with proteins from the endoplasmic reticulum (ER) to the cell membrane via the Golgi apparatus (figure 1). At the TGN, budded vesicles containing the cargos are sorted, according to their contents, to the plasma membrane, endosomes, or lysosomes, or are recycled to the ER. Studies using yeast, nematode and *Drosophila* models of PD showed that α-synuclein disrupts the transport between the ER and the Golgi, leading to ER stress (Cooper *et al.* 2006; Gitler *et al.* 2008). In these models, overexpression of small GTPases Rab1, Rab3 and Rab8 suppresses the toxicity of α-synuclein. Because Rab1 and Rab8 regulate ER-Golgi and post-Golgi trafficking, respectively, and because Rab3 is involved in SV tethering and docking at the

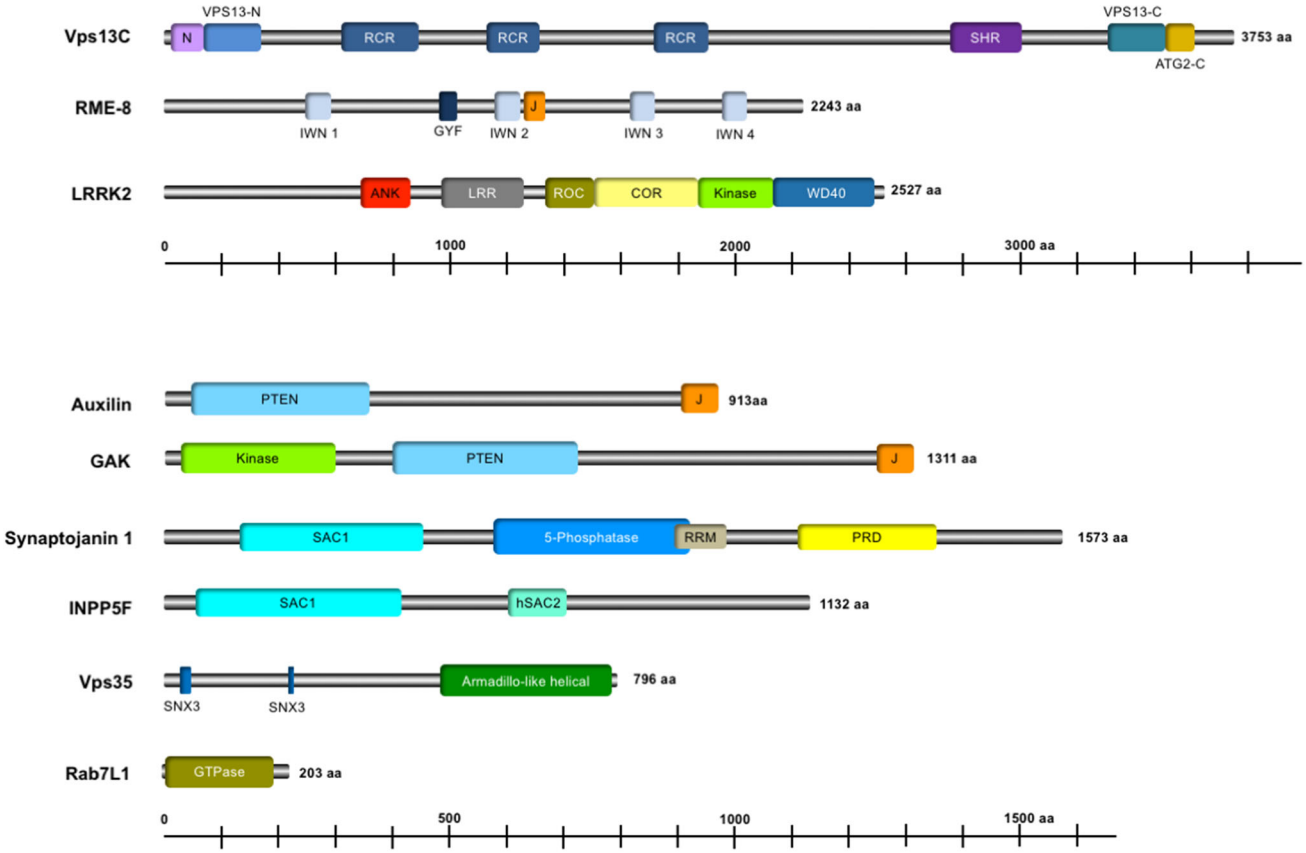

Figure 4. Domain structures of PD gene products related to membrane dynamics. *ATP13A2* encodes an inorganic ion transporter that contains a core of multiple transmembrane domains, but it is not shown here (Holemans *et al.* 2015). *INPP5F* encodes an inositol 4-phosphatase that functions in the early endocytic pathway (Hsu *et al.* 2015; Nakatsu *et al.* 2015). N, N-terminal region of Chorein; Vps13-N, N-terminal conserved region in Vps13 family proteins; Vps13-C, C-terminal conserved region in Vps13 family proteins; RCR, Repeated coiled region; SHR, SHR (SHORT-ROOT transcription factor)-binding domain; ATG2-C, Region homologous to the C-terminal domain of ATG2A (1723–1829 aa), which is required for ATG2A localization to both the autophagic membrane and lipid droplets. IWN, IWN repeat with an approximately 90-amino acid stretch containing seven invariant amino acids; GYF, glycine-tyrosine-phenylalanine domain; J, J domain; ANK, Ankyrin repeat; LRR, Leucine-rich repeats; ROC, Ras of complex proteins domain; COR, C-terminal of Roc GTPase domain; Kinase, Serine/threonine protein kinase domain; WD40, WD40 repeat domain; PTEN, PTEN-like domain that is composed of a phosphatase domain and a following C2 domain; SAC1, SAC1-homology domain with PI4P phosphatase activity; 5-Phosphatase, PI5P phosphatase domain; RRM, RNA recognition motif; PRD, Proline-rich domain; hSAC2, SAC2 homology domain; SNX3, A region to interact with SNX3; GTPase, Small GTPase domain.

presynapse, α-synuclein may affect several vesicular trafficking pathways (figure 1).

Mutations in Rab39B, which are primarily responsible for X-linked mental retardation associated with autism and epilepsy, also cause intellectual disability i.e. comorbid with early-onset PD with Lewy body pathology (Giannandrea *et al.* 2010; Wilson *et al.* 2014). Hence, loss-of-function mutations in Rab39B may be a potential risk factor for α-synuclein accumulation and subsequent development of PD. Rab39B is abundantly expressed in the brain and is localized to the Golgi compartment. Although the physiological role of Rab39B is largely unknown, a recent study indicates that Rab39B regulates ER-Golgi trafficking of GluA2, a subunit of AMPA-type glutamate receptors, thereby controlling the surface expression of GluA2 and excitatory synaptic transmission (Mignogna *et al.* 2015).

Mutations in the glucocerebrosidase (GCase) gene (*GBA1*), which encodes a lysosomal enzyme that catalyses the hydrolysis of glycolipid glucocerebroside to ceramide and glucose, is a strong risk for PD. Gaucher disease (GD) is the most frequent lysosomal storage disorder caused by homozygous mutations in *GBA1*, and the formation of α-synuclein-positive Lewy bodies is observed in GD patients. Impaired GCase activity causes α-synuclein accumulation, which in turn leads to the inhibition of intracellular trafficking of GCase, falling in a vicious cycle of pathogenic progression (Mazzulli *et al.* 2011). In a similar context, lysosomal integral membrane protein type-2 (LIMP-2) regulates the trafficking of GCase, and loss of LIMP-2 causes the accumulation of α-synuclein in mice by impairing GCase activity and lysosomal function (Rothaug *et al.* 2014). Inhibition of the lysosomal enzyme trafficking by the accumulation of α-synuclein is rescued

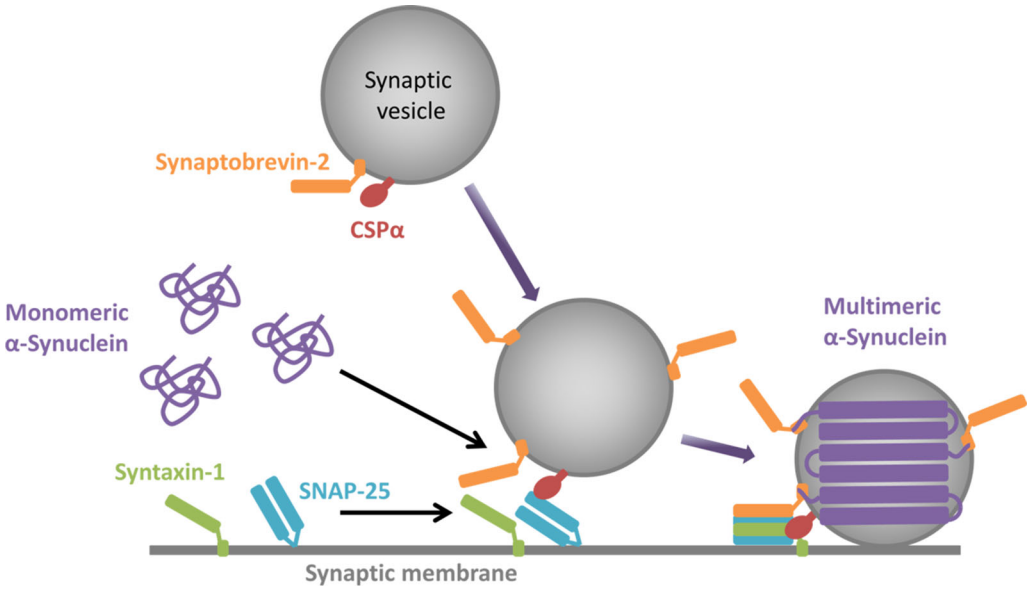

Figure 5. Physiological roles of α-Synuclein at presynapses. α-Synuclein has an N-terminal helical domain for binding to acidic phospholipid surfaces and a less conserved acidic C-terminus for binding to Synaptobrevin-2. Cysteine-string protein-α (CSPα) is a presynaptic Hsp40-like co-chaperone that forms a chaperone complex with Hsc70 and the small glutamine-rich tetratricopeptide-repeat protein SGT, facilitating the folding of synaptic SNARE proteins including SNAP-25, Syntaxin-1 and Synaptobrevin-2. α-Synuclein assists in this step through binding to Synaptobrevin-2. Soluble α-Synuclein is natively unfolded and monomeric. Upon binding to SVs during docking and priming of the vesicles, α-synuclein folds into an amphipathic α-helix and multimerizes, which promotes SNARE complex assembly.

by Rab1a overexpression, alleviating α-synuclein toxicity (Mazzulli *et al.* 2016). Thus, the inhibition of trafficking of lysosomal enzymes from the ER to lysosomes by the accumulation of α-synuclein or mutations in the enzyme receptors appears to be a critical point in PD pathogenesis.

Two PD-associated genes in clathrin-mediated vesicle trafficking

Clathrin-mediated endocytosis is thought to be one of the major mechanisms of endocytosis (figure 1). *Auxilin* (Edvardson *et al.* 2012; Koroglu *et al.* 2013; Olgiati *et al.* 2016) and *Synaptojanin1* (Krebs *et al.* 2013; Quadri *et al.* 2013; Olgiati *et al.* 2014; Kirola *et al.* 2016), the two genes responsible for autosomal recessive forms of PD with early onset, are specifically expressed in neurons and are thought to be involved in neuronal clathrin-mediated endocytosis (figure 6). Clathrin forms a triskelion trimer as a unit of a heavy chain and a light chain, and covers vesicles by polymerization of multiple clathrin molecules. Receptor endocytosis begins with the recruitment of clathrin to the receptor-integrated membrane area, forming a clathrin-coated pit. The binding of the clathrin adapter protein AP-2 to phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) on the plasma membrane recruits and polymerizes clathrin, which stimulates membrane invagination and subsequent recruitment of membrane squeezing and scission molecules such as Endophilin, Dynamin and Synaptojanin family proteins (Rohde *et al.* 2002; Ferguson *et al.* 2009; Milosevic *et al.* 2011). Endophilin localizes at the neck of the clathrin-coated pit and drives membrane curvature by its N-terminal BAR domain and binds to the Dynamin GTPase via its C-terminal SH3 domain, which induces GTP-mediated scission of the neck in cooperation with local tension of F-actin as a pulling force on the invaginated membrane (Sundborger *et al.* 2011). Synaptojanin, which is a neuronal phosphoinositide phosphatase that hydrolyzes $PI(4,5)P_2$, is also recruited by Endophilin, and dephosphorylation of $PI(4,5)P_2$ by Synaptojanin at the neck stimulates Dynamin to promote membrane scission (Cestra *et al.* 1999; Schuske *et al.* 2003; Verstreken *et al.* 2003; Chang-Ileto *et al.* 2011). After the fission and subsequent vesicle generation, clathrin rapidly dissociates from clathrin-coated vesicles. Auxilin and Hsc70 are involved in clathrin uncoating (Fotin *et al.* 2004). Auxilin binds to phosphatidylinositol phosphates and to Hsc70 through its PTEN-like domain and J domain, respectively (Guan *et al.* 2010; Kalli *et al.* 2013) (figure 4). Animals with loss of function of Endophilin or Synaptojanin exhibit a similar phenotype in which clathrin-coated vesicles, but not clathrin-coated pits, are accumulated in the synaptic terminals (Milosevic *et al.* 2011). These studies suggest that Endophilin and Synaptojanin are dispensable for fission of clathrin-coated vesicles from synaptic membranes, but rather important for clathrin uncoating (Milosevic *et al.* 2011).

Endophilin-mediated clathrin/AP-2-independent endocytosis of some classes of receptors, including dopaminergic D3 and D4 receptors, has also been characterized as fast endophilin-mediated endocytosis (FEME), in

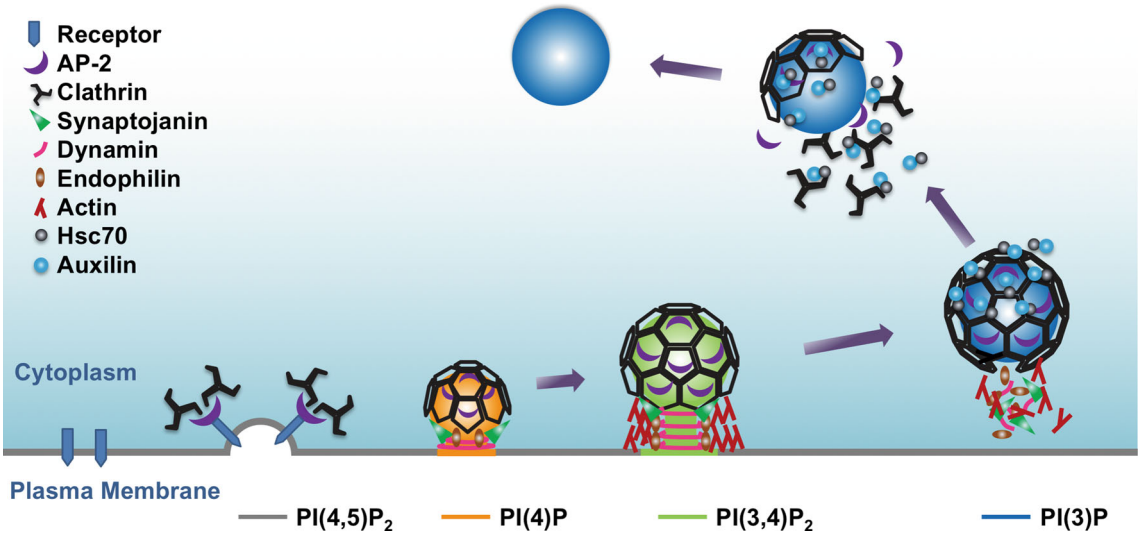

Figure 6. Clathrin-coated vesicle fission and uncoating at synapses. AP-2 is involved in the assembly of endocytic clathrin-coated pits, followed by the recruitment of Endophilin and Synaptojanin along with Dynamin to the neck of late-stage pits, leading to scission of clathrin-coated vesicles. Then, Auxilin and Hsc70 remove clathrin from endocytosed vesicles.

which at least Dynamin and the $PI(3,4)P_2$-binding protein lamellipodin are involved (Boucrot *et al.* 2015; Renard *et al.* 2015) (figure 1).

The functional relationship in neuronal synaptic endocytosis between Auxilin and Synaptojanin 1 is strongly suggested by a study describing a phenotypic similarity in *Auxilin*-deficient and *Synaptojanin 1*-deficient neurons, in which clathrin and AP-2 but not Synaptobrevin are abnormally accumulated (Yim *et al.* 2010). The defect of clathrin uncoating activity in Auxilin loss is partly rescued by GAK, a homolog of Auxilin with an additional N-terminal kinase domain (figure 4). GAK is ubiquitously expressed, and SNPs in the *GAK* gene locus have also been reported as a risk for PD (Pankratz *et al.* 2009).

Clathrin-mediated endocytosis along with AP-2 has been well characterized in SV endocytosis at presynapses as well as receptor endocytosis at the plasma membrane (figures 3 and 6). Upon release of neurotransmitters, SVs fused with plasma membrane are retrieved by endocytosis from regions adjacent to the active zones for SV secretion and are regenerated as SVs. A recent study using electron microscopy with a technique for high spatial and temporal resolution has proposed that actin-dependent and Dynamin-dependent ultrafast endocytosis mediates very fast SV recycling, which occurs within 100 ms at the external sites of the active zone in a clathrin-independent manner (figure 3). On the other hand, clathrin function is required to regenerate SVs from the synaptic endosomes after ultrafast endocytosis. Similar to the SV budding from the synaptic endosomes, clathrin also functions in vesicle budding from the TGN to endosome. Supporting that observation, Auxilin functions in and is enriched in the ER and Golgi trafficking pathways (Zhou *et al.* 2011; Ding *et al.* 2016). Although knockdown of Auxilin in dopaminergic neurons of *Drosophila* affects locomotor activity and the survival of dopaminergic neurons, potentiating α-synuclein toxicity, which clathrin pathway is affected in PD by Auxilin deficiency remains an issue for future research (Song *et al.* 2017).

In humans, there are two Synaptojanin proteins, Synaptojanin 1 and Synaptojanin 2, and the PD-associated Synaptojanin 1 is almost exclusively expressed in brain and is enriched at synapses (Nemoto *et al.* 1997; Verstreken *et al.* 2003). Synaptojanin 1 contains an N-terminal SAC1 phosphatase domain, a central 5-phosphatase domain and C-terminal proline-rich domain for the interaction with SH3 domain-containing endocytic proteins (Krebs *et al.* 2013) (figure 4). The PD-associated mutations of Synaptojanin 1 have been found in the SAC1 domain that hydrolyzes PI(3)P, PI(4)P, and $PI(3,5)P_2$ (Guo *et al.* 1999; Zhong *et al.* 2012). Knock-in of the PD mutation R258Q (RQ) of Synaptojanin 1 in mice, in which neurons show a massive accumulation of Auxilin and clathrin and impaired SV endocytosis, exhibits defects in motor functions and a shorter lifespan (Cao *et al.* 2017). Moreover, axons of dopaminergic neurons in the dorsal striatum of the knock-in mice contain accumulated dopamine transporter and large multilayered membrane structures, which might be associated with an early stage of PD pathology.

Synaptic endocytosis regulated by PD genes

The *LRRK2* gene encodes a Roco family protein containing Roc and COR domains accompanied by a kinase domain and a WD40 domain, and has been identified

as a causative gene for autosomal dominant late-onset PD (Paisan-Ruiz *et al.* 2004; Zimprich *et al.* 2004) (figure 4). Further, its SNPs have also been detected as PD risk in multiple GWAS analyses (Satake *et al.* 2009; Simon-Sanchez *et al.* 2009). Many reports suggest that PD-associated mutations cause increased kinase activity, thus implying dysregulation of the phosphorylation substrate(s) of LRRK2 in PD (West *et al.* 2005; Gloeckner *et al.* 2006; Smith *et al.* 2006; Imai *et al.* 2008; Steger *et al.* 2016). LRRK2 is localized in Rab5-positive early and Rab7-positive late endosomes and is suggested to regulate endosomal trafficking as well as the autophagy–lysosomal pathway either directly or indirectly (Dodson *et al.* 2012, 2014; Gomez-Suaga *et al.* 2012, 2014; Orenstein *et al.* 2013; Imai *et al.* 2015). Vps35, mutations of which cause an autosomal dominant form of PD, forms the retromer complex with Vps26 and Vps29 and regulates vesicular trafficking in the endosome-to-Golgi pathway and the endosome-to-cell surface pathway (Vilarino-Guell *et al.* 2011; Zimprich *et al.* 2011).

The GTPase Rab5 is involved in an early step of presynaptic endocytosis. Expression of dominant-negative forms of Rab5 produced enlarged endocytic intermediates at synapses in *Drosophila* and shows reduced synaptic transmission, suggesting that SV recycling is impaired (Shimizu *et al.* 2003; Wucherpfennig *et al.* 2003). Similar synaptic phenotypes were also observed in a *Drosophila* close homologue of LRRK2 (*dLRRK*), *Vps35* and *Synaptojanin* mutant flies, which also suggests that LRRK2 and Vps35, along with Synaptojanin 1, function in SV endocytosis (Verstreken *et al.* 2003; Matta *et al.* 2012; Inoshita *et al.* 2017).

In *Drosophila*, dLRRK phosphorylates Endophilin A, one of the Endophilin family proteins, promoting the detachment of Endophilin A from endocytosed SVs upon scission; clathrin machinery does not appear to be involved in this context (Matta *et al.* 2012). A similar molecular mechanism has been demonstrated using *LRRK2* mutant mice (Arranz *et al.* 2015). In contrast, a study using cortical neuron cultures revealed that SV motility and recycling are enhanced by the reduction of LRRK2 activity (Piccoli *et al.* 2011). Another study reported that hyperphosphorylation of Synaptojanin 1 in the proline-rich domain by the PD-associated LRRK2 G2019S mutant promotes the dissociation of Synaptojanin 1 from Endophilin A, which results in slowed endocytosis at the synapse (Pan *et al.* 2017).

Loss of Vps35 and a PD-associated Vps35 mutant cause reduced neurotransmission and appearance of enlarged vesicles at presynapses in *Drosophila* (Inoshita *et al.* 2017). Manipulation of the activity of dLRRK, Endophilin A, Rab5 or Rab11 rescues the synaptic defects and impaired motor behaviours. These observations suggest that defects in SV endocytosis and recycling at presynapses are among the major causes of PD aetiology. The retromer containing Vps35 is also localized at dendritic spines of neurons and regulates the recycling of glutamate receptors (Choy *et al.* 2014; Munsie *et al.* 2015). Thus, postsynaptic dysregulation in neurotransmission could also contribute to dopaminergic neurodegeneration.

PD genes involved in retrograde transport and the autophagy–lysosomal pathway

In the cell body, vesicular transport from the early endosome has multiple pathways: retrograde trafficking to the TGN, the recycling pathway to the plasma membrane and lysosomal degradation pathways (figure 1). A genetic modifier screen for endosomal trafficking identified *Synaptojanin*, *Vps35* and *Vps13* in yeast, which implies that corresponding PD genes could act in the vesicular transport of the cell body (Luo and Chang 1997). Transport from the early endosome to the TGN and the recycling pathway is mediated by the Vps35-containing retromer complex. While PD-associated Vps35 mutations do not inhibit the formation of the retromer complex with Vps29 and Vps26, these mutations impair the binding of Vps35 to the FAM21-containing WASH complex, which nucleates branched F-actin networks on the surface of endosomes (McGough *et al.* 2014; Zavodszky *et al.* 2014; Follett *et al.* 2016). The retromer complex together with sorting nexin 27 (SNX27), which has a PX domain for PI(3)P binding, and the WASH complex cooperate in endosome-to-cell surface recycling of proteins, whereas the PX domain-containing protein SNX3 and Rab7 appear to recruit the retromer to the late endosome (Temkin *et al.* 2011; Zech *et al.* 2011; Harrison *et al.* 2014). PD mutations of Vps35 could affect both the recycling and late endosome-lysosomal pathways in conjunction with the trafficking of the autophagy protein ATG9 to autophagosomes (Zavodszky *et al.* 2014). The *RME-8* gene, which has been isolated from the *PARK21* locus, is also involved in retromer-mediated protein sorting (Vilarino-Guell *et al.* 2014). RME-8 interacts with the WASH complex subunit FAM21 along with the PX domain-containing protein SNX1 and regulates endosomal tubulation and the retrograde sorting pathway (Popoff *et al.* 2009; Freeman *et al.* 2014). Identification of RME-8, Vps35, Vps13 and Auxilin in a functional screen for endocytosis using *Drosophila* S2 cells strongly suggests that the alteration of vesicle endocytosis is a key element of PD aetiology (Korolchuk *et al.* 2007). Characterization of LRRK2, Auxilin and RME-8 as regulators of the Notch/Delta signalling pathway in *Drosophila* supports the idea that these PD genes are involved in endocytosis and endosomal recycling pathways (Hagedorn *et al.* 2006; Gomez-Lamarca *et al.* 2015; Imai *et al.* 2015).

Among four members of Vps13 in human genomes, Vps13C has been identified as associated with an autosomal recessive early-onset form of PD (Lesage *et al.* 2016). In yeast, there is a single gene for Vps13, which binds

to a variety of phosphoinositides, including PI(4)P and PI(4,5)P_2, in addition to phosphatidic acid (PA) and is required for the TGN-late endosome transport (De *et al.* 2017). Yeast genetic studies suggest roles for Vps13 in organelle contact sites including the ER-mitochondrial junction (Lang *et al.* 2015; Park *et al.* 2016). In mammalian cells, Vps13C is reported to be localized on the outer mitochondrial membrane, and knockdown of Vps13C induced mitochondrial fragmentation and promoted PINK1/Parkin-mediated mitophagy, the lack of which is believed to be a cause of PD pathogenesis (Lesage *et al.* 2016). In contrast, another mammalian study reported that Vps13C is a lysosome-resident protein that regulates the stability of a β-galactoside-binding animal lectin, galectin-12, for adipocyte differentiation. Thus, further studies will be required to understand the critical roles of Vps13C in dopaminergic neurons.

Roles for PD-associated genes in the lysosomal degradation pathways have also been reported. Loss of Vps35 results in enlarged autophagosomes and lysosomes in *Drosophila*, which is caused by the block of retromer-mediated trafficking of lysosomal enzymes such as cathepsin L from the TGN to the lysosomes via cargoes, leading to lysosomal dysfunction (Maruzs *et al.* 2015). A similar observation has been reported in Vps35 heterozygous mice, in which the dopaminergic neurons of Lamp1-positive late endosomes/early endosomes were found to be enlarged (Tang *et al.* 2015). In contrast, Lamp2-positive lysosomes are reduced in size, a finding caused by the impairment of Lamp2a endosome-to-Golgi retrieval by Vps35 that leads to the accumulation of α-synuclein likely through defects in Lamp2-mediated chaperone-mediated autophagy (Tang *et al.* 2015). A well characterized cargo of the retromer containing Vps35 is the cation-independent mannose 6-phosphate receptor that binds to newly synthesized lysosomal enzymes in the TGN and that delivers them to lysosomal compartments (Seaman 2004). The iron transporter divalent metal transporter 1 (DMT1) has also been shown as a cargo regulated by the retromer (Tabuchi *et al.* 2010). An alternative splicing isoform of DMT1-II is involved in iron uptake in cooperation with the transferrin receptor, which is recycled from endosomes to the plasma membrane by the retromer. The alteration of iron metabolism may lead to mitochondrial dysfunction and iron deposition in the affected regions in PD.

LRRK2-deficient rodents exhibit degeneration in kidney's proximal tubule epithelial cells, where the accumulation of secondary lysosomes as well as the autophagy-related protein p62/SQSTM1 is observed (Tong *et al.* 2010; Herzig *et al.* 2011; Hinkle *et al.* 2012; Ness *et al.* 2013). However, there is no evidence that the nigrostriatal pathway is affected in these rodent models. However, double knockout of *LRRK2* and its homologue *LRRK1* in mice produces lysosomal dysfunction and mild neuronal death in dopaminergic neurons (Giaime *et al.* 2017).

Rab7L1, which encodes a Rab GTPase, is a risk gene for sporadic PD within the *PARK16* locus. Rab7L1 is involved in vesicular sorting at the Golgi (Satake *et al.* 2009; Simon-Sanchez *et al.* 2009; MacLeod *et al.* 2013). A genetic interaction between the clathrin adapter *AP-3*, *LRRK2* and *Rab7L1* has been shown in the axon terminals of *C. elegans* neurons (Kuwahara *et al.* 2016). Importantly, *Rab7L1*-deficient mice exhibit lysosomal pathology in proximal tubule epithelial cells of the kidney, a phenotype highly similar to that of *LRRK2*-deficient mice (Kuwahara *et al.* 2016). Double knockout of *Rab7L1* and *LRRK2* does not exacerbate the renal phenotype in mice and the axonal phenotype in *C. elegans*, suggesting that *Rab7L1* is an upstream regulator of LRRK2.

The gene responsible for early-onset PD with pyramidal degeneration and dementia (Kufor–Rakeb syndrome) encodes ATP13A2, a lysosomal P-type ATPase, which is proposed to act as a Mn^{2+} and/or Zn^{2+} transporter (Ramirez *et al.* 2006; Gitler *et al.* 2009; Tsunemi and Krainc 2014). The N-terminal hydrophobic extension of ATP13A2, which is required for its late endosomal/lysosomal localization, binds to PI(3,5)P_2 and PA and regulates its ATPase activity, conferring protective effects against mitochondrial stress (Holemans *et al.* 2015). Loss of *ATP13A2* results in α-synuclein accumulation (Gitler *et al.* 2009; Tsunemi and Krainc 2014), where the impairment of the autophagy-dependent or autophagy-independent lysosomal degradation pathway appears to be involved (Bento *et al.* 2016; Demirsoy *et al.* 2017). A study reporting genomewide screening and network analysis of genes modifying α-synuclein toxicity in yeast has uncovered that *ATP13A2* and *Vps35*, in addition to *Synaptojanin* and *Rab7L1*, are indeed involved in the turnover of α-synuclein (Khurana *et al.* 2017).

Involvement of PD-associated genes in autophagy at presynaptic terminals has also been proposed, which could occur in a synaptic activity-dependent manner. LRRK2-mediated phosphorylation of Endophilin A causes shallow membrane curvature, which in turn recruits ATG3, an ATG8-activating enzyme, to PI(3)P-containing membranes (Sakoh-Nakatogawa *et al.* 2013; Soukup *et al.* 2016). In *Drosophila* Synaptojanin RQ knock-in models, the roles of Synaptojanin in synaptic autophagy have been shown. The PI(3)P- and PI(3,5)P_2-binding proteins of the ATG18 family, which are essential for autophagosome formation, has lost its mobility at autophagosomes probably due to impaired hydrolysis of phosphoinositides by Synaptojanin RQ, blocking autophagosome maturation at synapses (Obara *et al.* 2008; Vanhauwaert *et al.* 2017).

Conclusions

Overall, these studies on the functions of a subset of PD genes indicate that the impairment of vesicular trafficking

to the lysosomes, including autophagy as well as lysosomal maintenance, initiates neurotoxic α-synuclein aggregation, which is a critical factor for PD pathogenesis. However, why dopaminergic neurons are especially affected in PD remains an open question. This issue might be explained by the properties of dopaminergic neurons. A recent report has demonstrated that dopamine induces α-synuclein oligomerization, which is thought to be a seed for α-synuclein propagation and Lewy body formation (Luk *et al.* 2009; Masuda-Suzukake *et al.* 2013; Mor *et al.* 2017). Another study has noted that dopamine oxidation inactivates GCase, leading to lysosomal dysfunction and α-synuclein accumulation (Burbulla *et al.* 2017). In this context, mitochondrial oxidative stress caused by mutations of PD genes that regulates mitochondrial functions could also be involved. Another question arises from the fact that several PD genes have been characterized as endocytosis-associated molecules. Considering that Synaptojanin 1 functions primarily at presynapses, alteration of the SV regeneration pathway must be a risk of α-synuclein aggregation, along with propagation at synapses (Verstreken *et al.* 2003; Marza *et al.* 2008; Chen *et al.* 2014). Further studies to address this issue are warranted.

Acknowledgements

This study was supported by Grants-in-Aid for Scientific Research (16K09679 to TI, 17H04049 to YI, 15H04842 to NH) from JSPS in Japan and was partly supported by a grant from Otsuka Pharmaceutical (NH and YI).

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