



Role of the Endolysosomal System in Parkinson's disease

D.J. Vidyadhara^{1,2}, John E. Lee^{1,2}, Sreeganga S. Chandra^{1,2}

¹Department of Neurology, Yale University School of Medicine, New Haven, CT-06519, USA

²Department of Neuroscience, Yale University School of Medicine, New Haven, CT-06519, USA

Abstract

Parkinson's disease (PD) is one of the most common neurodegenerative disorders, affecting 1–1.5% of the total population. While progress has been made in understanding the neurodegenerative mechanisms that lead to cell death in late stages of PD, mechanisms for early, causal pathogenic events are still elusive. Recent developments in PD genetics increasingly point at endolysosomal (E-L) system dysfunction as the early pathomechanism and key pathway affected in PD. Clathrin-mediated synaptic endocytosis, an integral part of the neuronal E-L system, is probably the main early target as evident in auxilin, RME-8, and synaptojanin-1 mutations that cause PD. Autophagy, another important pathway in the E-L system, is crucial in maintaining proteostasis and a healthy mitochondrial pool, especially in neurons considering their inability to divide and requirement to function an entire life-time. *PINK1* and *Parkin* mutations severely perturb autophagy of dysfunctional mitochondria (mitophagy), both in the cell body and synaptic terminals of dopaminergic neurons, leading to PD. Endolysosomal sorting and trafficking is also crucial, which is complex in multi-compartmentalised neurons. *VPS35* and *VPS13C* mutations noted in PD target these mechanisms. Mutations in *GBA* comprise the most common risk factor for PD and initiate pathology by compromising lysosomal function. This is also the case for *ATP13A2* mutations. Interestingly, α -synuclein and LRRK2, key proteins involved in PD, function in different steps of the endolysosomal pathway and target their components to induce disease pathogenesis. In this review, we discuss these E-L system genes that are linked to PD and how their dysfunction results in PD pathogenesis.

Graphical Abstract

The endolysosomal system is increasingly recognized as the key pathway affected in Parkinson's disease (PD). Clathrin-mediated synaptic endocytosis, an integral part of the neuronal endolysosomal system, is probably the main early target. Autophagy, another important endolysosomal pathway that aids in maintaining proteostasis and a healthy mitochondrial pool is affected. Other crucial pathways such as endolysosomal sorting and trafficking, along with lysosomal degradation are also hampered. Key PD proteins such as α -synuclein and LRRK2 target the endolysosomal components to induce pathology. Here, we comprehensively discuss these mechanisms to better understand the role of endolysosomal system dysfunction in PD pathogenesis.

Corresponding Author: Vidyadhara D. J., Ph.D., Departments of Neurology and Neuroscience, Yale University School of Medicine, New Haven, CT-06519, USA, vidyadhara.dj@yale.edu.

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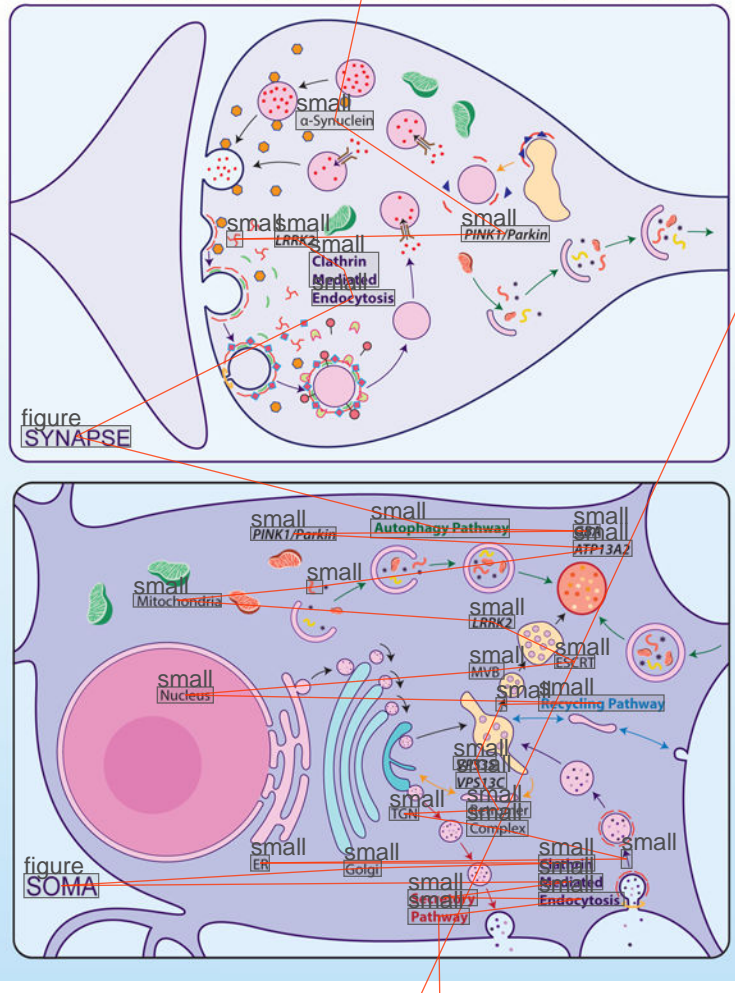
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ENDOLYSOSOMAL SYSTEM TARGETS IN PARKINSON'S DISEASE



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Keywords

Clathrin-mediated endocytosis; Autophagy; Lysosomes; Retromer complex; α -Synuclein; LRRK2

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, clinically identified by motor symptoms, with an increasing recognition of non-motor manifestations (Hoehn & Yahr 1967; Chaudhuri & Schapira 2009). Loss of dopaminergic neurons of the substantia nigra pars compacta (SNpc) (40–50%) and the presence of α -synuclein pathology in surviving neurons are the neuropathological hallmarks of PD. At the onset of motor symptoms, dopamine levels have decreased 60–70% in the dorsal striatum, where most SNpc dopaminergic neurons form synaptic connections (Dickson *et al.* 2009; Ma *et al.* 1997; Kordower *et al.* 2013; Fearnley & Lees 1991). Under physiological conditions, α -synuclein is predominantly present in presynaptic terminals, aiding neurotransmitter release, whereas in PD, α -synuclein tends to aggregate and form Lewy bodies (the defining α -synuclein pathology in PD) (Spillantini *et al.* 1998; Spillantini *et al.* 1997). Aggregation of α -synuclein, perturbed proteostasis, mitochondrial dysfunction, oxidative stress, and impaired calcium homeostasis are the major cell-autonomous mechanisms of PD pathogenesis. Non-

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cell-autonomous mechanisms include cell-to-cell transmission of pathological α -synuclein and neuroinflammation (Poewe *et al.* 2017; Jyothi *et al.* 2015). So far, incomplete knowledge on causal mechanisms has limited treatment strategies to symptomatic relief. Recent studies targeting the endolysosomal (E-L) system reveal that its dysfunction can lead to the mentioned disease mechanisms, suggesting the E-L system as a key pathway in PD pathogenesis. In this review, we discuss important E-L system components, their roles in neuronal survival, and how their dysfunction causes PD.

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1. Endolysosomal system in neurons: Custom-made for neuronal function

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The E-L system comprises of complex, highly dynamic membrane-enclosed tubular-vesicular structures with overlapping properties and functions (Klumperman & Raposo 2014). They facilitate nutritional intake from a cell's microenvironment through endocytosis, neutralize pathogenic materials via phagocytosis, promote cellular proteostasis through autophagy, and maintain overall cellular homeostasis through endosomal sorting and trafficking of various proteins/enzymes across organelles (Klumperman & Raposo 2014; Repnik *et al.* 2013). E-L system functions vary greatly depending on cell type and functional state. Neurons are unique in their highly polarized intracellular compartmentalization of cell body/soma and processes including axons, axon terminals, and dendrites. This organization enables them to form complicated networks that mediate specialized functions (Figure 1). This complexity comes at a price: burdensome demands on the E-L system, which have been increasingly implicated in neurological diseases including PD (Kett & Dauer 2016; Wang *et al.* 2018).

In neurons, synthesis of E-L components occurs primarily in the cell body/soma (Figure 1A). These components are trafficked through the secretory pathway to their subcellular location. A prime example of this is lysosomal hydrolase trafficking. Lysosomal hydrolases are produced in the endoplasmic reticulum, then undergo glycosylation and other post-translational modifications at the Golgi apparatus [e.g. addition of mannose-6-phosphate (M6P) groups] (Nixon & Cataldo 1995). This modification directs hydrolases to lysosomes. More specifically, hydrolases form complexes with transmembrane M6P receptors, which are sequestered into clathrin-coated vesicle (CCVs) budding from the trans-Golgi networks (TGN) to form endosomes/early endosomes (Nixon & Cataldo 1995) (Figure 1A). Inside early endosomes, M6P receptors detach from hydrolases and are recycled back to the TGN for their next round of transport (Saftig & Klumperman 2009), while hydrolases are delivered to lysosomes (discussed below).

Simultaneously, early endosomes are also derived from the cell surface through the endocytic pathway, an integral part of the E-L system. Many of the nutrients required for neuronal function and surface receptors designated for recycling are internalised through clathrin-mediated endocytosis (CME) at the plasma membrane and delivered to early endosomes (Figure 1A). Early endosomes derived from both the TGN and the plasma membrane mature into late endosomes, where Rab5 and Rab7 play a prominent role (Saftig & Klumperman 2009). This process occurs with the help of the endosomal sorting complex required for transport (ESCRT) (Figure 1A), which facilitates ubiquitin-dependent sorting of cargo/hydrolases from the outer membrane of early endosomes into interluminal vesicles

(ILVs) (Wollert *et al.* 2009). Early endosomes derived from CME, if not sorted, follow a recycling pathway to the plasma membrane (Figure 1A) (Saftig & Klumperman 2009). ILVs are enveloped inside multivesicular bodies (MVBs), which mature into late endosomes. These late endosomes deliver their luminal contents either by forming tubular extensions or by fusing with pre-existing lysosomes to form endolysosomes (Luzio *et al.* 2007). These may be considered the final compartment of the endocytic pathway, where hydrolases degrade endocytic cargo and the membranes of ILVs for reuse (Klumperman & Raposo 2014; Saftig & Klumperman 2009). In addition, there is retrograde transport of receptors (such as M6P, Lamp2a, etc) from endosomes to the TGN for reuse through the retromer complex, comprised mainly of VPS35, VPS26 and VPS29 (Saftig & Klumperman 2009) (Figure 1A). Proteins that are meant for secretion follow a secretory pathway from the TGN (Saftig & Klumperman 2009; Klumperman & Raposo 2014). Selective capture and elimination of a cell's damaged organelles and misfolded proteins happens through a process called macroautophagy (termed hereafter as autophagy) (Ohsumi 2014), an integral part of the E-L system. Double membrane vesicles called autophagosomes encapsulate unwanted/toxic cytosolic constituents including aggregated proteins and deliver them to the lysosome for degradation (Figure 1A) (Kawamata *et al.* 2008; Mizushima 2007). Non-selective autophagy also occurs during starvation, through which cells can recycle proteins and reuse amino acids for the synthesis of basic proteins essential for cell survival (Ohsumi 2014). Therefore, autophagy plays a pivotal role in housekeeping and maintenance of neuronal homeostasis.

Customization of the E-L system for neurons mainly happens in axons (Figure 1B) and synapses (Figure 1C). At presynaptic terminals, vesicle precursors are delivered from the cell body and undergo vesicle cycling to form functional synaptic vesicles (SVs). However, delivery of new vesicle precursors cannot match the number of SVs undergoing exocytosis to achieve neurotransmission at an active synapse (Saheki & De Camilli 2012). This deficit is solved by highly responsive CME, recapturing fused vesicular membranes from the presynaptic membrane to form CCVs, which are in turn converted back to SVs through clathrin uncoating (Figure 1C) (Heuser & Reese 1973; Morgan *et al.* 2000; Saheki & De Camilli 2012). Less understood clathrin-independent bulk endocytosis (Holt *et al.* 2003; Wu & Wu 2007) and the "kiss and run" mechanism (Fesce *et al.* 1994) are also parts of the synaptic endocytic repertoire. In addition, there are dense-core vesicles (DCVs) derived from the TGN that are transported down the axons. Unlike SVs, DCVs do not participate in SV recycling and are primarily used to secrete long-acting neuropeptides (Nurrish 2014). While these processes are mostly confined to the presynaptic compartment, the E-L system's postsynaptic compartment contributes to membrane trafficking pathways primarily aimed at receptor, transporter, and ion-channel recycling (Rosendale *et al.* 2017).

Though this complex E-L system allows a neuron to function far away from its site of protein synthesis, the neuron is beholden to anterograde transport of proteins and membranes synthesized and modified in the soma for maintenance of axons and the synapses (Maday *et al.* 2014). Furthermore, constant synaptic activity, especially in continuously firing neurons like SNpc dopaminergic neurons may result in considerable build-up of damaged organelles, membranes, and misfolded proteins. These need to undergo encapsulation by an autophagosome or endosome (Figure 1C) and retrograde transport to the

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soma for lysosomal degradation (Figure 1B) (Yang *et al.* 2013). Here, long-distance axonal microtubule networks provide significant support to the neuronal E-L system (Yang *et al.* 2013). These spatial challenges to address a unique structural geometry, high metabolic demand, enhanced responsiveness to chemical/electrical stimulation, and the requirement for longevity due to their postmitotic nature, place a tremendous burden on the E-L system. Nigrostriatal dopaminergic neurons have an additional burden in terms of long and highly branched axons, larger surface area, selective transporters for external toxins, large fluctuations in free Ca^{2+} , less buffering capacity, and higher oxidative stress, reducing their threshold for degeneration (Surmeier *et al.* 2017; Vidyadhara *et al.* 2019; Vidyadhara *et al.* 2016; Vidyadhara *et al.* 2017). These vulnerabilities are further exacerbated with aging. Faced with environmental and genetic risk factors, disease pathogenesis abounds.

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2. E-L system dysfunction: the holy grail of PD pathophysiology?

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In 1997, identification of dominant mutations in the α -synuclein (*SNCA*) gene (*PARK1*) which encodes α -synuclein as a cause of familial PD (Polymeropoulos *et al.* 1997), brought a conceptual shift toward the importance of genetics in disease pathogenesis. Subsequent findings revealed various other genetic mutations, with at least 10–15% of PD patients having familial PD of an absolute genetic origin. Leucine-rich repeat kinase 2 (*LRRK2*, *PARK8*) and Parkin (*PRKN*, *PARK2*) mutations are the most common genetic variants of dominant and recessively inherited PD, respectively (Deng *et al.* 2018). Mutations and gene multiplications of *SNCA* (*PARK1* and *PARK4*) (Chartier-Harlin *et al.* 2004; Singleton *et al.* 2003) and Glucosidase Beta Acid (*GBA*) are also highly prevalent, whereas PD pathology was also noted with abnormal forms of Parkinsonism associated with deglycase or DJ1 (*PARK7*), and PTEN induced putative kinase 1 (*PINK1*, *PARK6*) (Deng *et al.* 2018). There is also compelling evidence for a genetic component to sporadic PD. In fact, a rigid distinction between the genetic and environmental basis of PD pathogenesis may not exist considering that cases are phenotypically indistinguishable (except for the onset) (Baba *et al.* 2006), implying genetic and sporadic cases share common underlying mechanisms. Even after considerable studies on these familial mutations and their interaction with environment, the core pathomechanisms of PD are still unclear, limiting treatment strategies. Over the past ten years, advancements in high-throughput sequencing, the application of genome wide association studies (GWAS), and meta-analysis, have identified new genetic mutations (refer to Deng *et al.* 2018 for a list of all PARK genes and risk alleles). Intriguingly, most of these identified genes have links to the E-L system (refer Table 1). Mechanistic studies also suggest that these proteins are the downstream targets of previously identified broad spectrum dysfunction in α -synuclein and LRRK2 mutations. It is now becoming clear that most PD-linked genes (Table 1) and known pathomechanisms are, in one way or another, associated with the E-L system, strongly suggesting this pathway as the key master regulator of PD pathogenesis. Recent studies suggest that neurodegeneration is initiated at the dopaminergic presynaptic terminals of striatum, years before the clinical diagnosis (Imbriani *et al.* 2018). Evidence that most of the E-L system genes associated with PD function at this terminal further establishes their fundamental role in PD pathogenesis. Below, we discuss our understanding of the known functions of these E-L system genes and their dysfunction in PD. We also discuss how other key PD-associated proteins target E-L pathways.

3. Clathrin-mediated synaptic vesicle endocytosis: A prime target?

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SV endocytosis is predominantly mediated through CME (Figure 1C). Following exocytosis, the SV membrane is endocytosed primarily through CME, which involves formation of a clathrin coat, composed of an outer clathrin layer and an inner layer of adaptors (Saheki & De Camilli 2012). The most common adaptors such as AP-2 and AP180 binds to endocytic motifs on the SV membrane, as well as phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂] present in the plasma membrane. Endophilin A1 and α -synuclein drive membrane bending and curvature formation of the developing clathrin coated pit (Westphal & Chandra 2013; Bai *et al.* 2010). Following this, the clathrin coated pit in conjunction with dynamin and other accessory proteins undergoes membrane fission to form a CCV (Saheki & De Camilli 2012). Regeneration of SVs from CCVs and recycling of clathrin and related coat components for further endocytosis requires un-coating of clathrin from CCVs (Figure 1C). The clathrin uncoating step is carried out by the coordinated action of the PI phosphatase synaptojanin-1 and the co-chaperone auxilin with Hsc70. Endophilin A1 and cyclin G Associated Kinase (GAK) also regulate clathrin uncoating to facilitate CME at the presynaptic terminal (Figure 1C) (Saheki & De Camilli 2012). Mutations of these E-L system genes in familial PD envisage synaptic endocytosis dysfunction as a prime trigger for PD pathogenesis.

3.1 The DNAJ family of heat shock proteins in the presynaptic terminal and beyond:

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Auxilin: *DNAJC6* gene encodes auxilin, the major presynaptic endocytic protein linked to PD. Auxilin is a brain specific co-chaperone belonging to the DNAJC family (Table 1), a subclass of heat shock proteins (HSP40). Auxilin plays an important role in clathrin uncoating, recruiting ATP-activated Hsc70 to CCVs, which in turn, disassembles clathrin from vesicles facilitating their recycling (Gorenberg & Chandra 2017; Lemmon 2001) (Figure 1C). Loss-of-function autosomal recessive mutations of auxilin (*PARK19*) have been shown to cause juvenile/early onset PD, first observed in two teenage brothers of a Palestinian family (Edvardson *et al.* 2012). The study noted abnormal mRNA splicing and a decrease in auxilin mRNA levels. Other variants of auxilin mutations were later noted in a Brazilian family with similar mis-splicing of mRNA, whereas mutations affecting the J-domain (p.Q791*, p.Q846* and p.R927G) were observed in a few other consanguineous families (Koroglu *et al.* 2013; Olgiati *et al.* 2016). Along with typical PD symptoms, patients with nonsense mutations have cognitive impairment, one of the cardinal non-motor symptoms of PD. Epilepsy and mental retardation have also been noted for some PD patients with auxilin mutations (Koroglu *et al.* 2013). These human genetic studies suggest widespread neuronal dysfunction besides nigrostriatal dopaminergic neurodegeneration. Hence, future studies on post-mortem brains documenting PD-typical Lewy body pathology and dopaminergic neuronal loss are necessary to concretely categorize the pathology as PD or parkinsonism.

One of the few preclinical studies characterizing *DNAJC6* reveals that auxilin down-regulation leads to progressive dopaminergic neuronal loss and motor disabilities in *Drosophila*. Furthermore, loss of auxilin exacerbates α -synuclein overexpression and paraquat (an environmental toxin)-mediated dopaminergic loss, thus aggravating previously established phenotypes in fly models of PD (Song *et al.* 2017). Interestingly, the brains (deep

RME-8: Receptor mediated endocytosis 8 (RME-8) or DNAJC13 is another DNAJC family co-chaperone and principally functions in regulating retromer and clathrin un-coating dynamics on endosomes (Gorenberg & Chandra 2017) (Figure 1C). *RME-8* mutations (p.N855S) in autosomal-dominant PD (*PARK21*) was noted first in a Dutch-German-Russian family of Mennonite origin, with multiple incidences (Table 1) (Vilarino-Guell *et al.* 2014; Gustavsson *et al.* 2015). Post-mortem brains of PD patients with *RME-8* mutations revealed strong immunoreactivity for Lewy bodies in the substantia nigra, along with brain areas implicated in non-motor symptoms of PD. Nigral Lewy bodies were also found to be co-localized with RME-8 (Vilarino-Guell *et al.* 2014). Unlike other DNAJC proteins

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implicated in PD, *in vitro* studies have revealed that, the *RME-8* mutation confers toxic gain-of-function, leading to endolysosomal cargo trafficking deficits (Norris *et al.* 2017). The *RME-8* mutation is also predicted to affect overall endosomal trafficking, even in the soma and postsynaptic compartment (Vilarino-Guell *et al.* 2014), unlike the synapse-specific dysfunction predicted for auxilin. Recently, the link between *RME-8* and PD has been muddled by an independent analysis of the same Mennonite family which did not find *RME-8* mutations. Instead, all the affected individuals were identified to carry mutations in *TMEM230* (Deng *et al.* 2016), which also functions in E-L system, perhaps at synapses. Hence, further studies are warranted to confirm the genetic link between *RME-8* and PD. Still, evaluating the RME-8 mediated E-L system dysfunction might provide valuable insights in understanding PD.

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3.2: Synaptojanin-1 and endophilin A: Partners in crime?

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Two other major synaptic endocytic proteins besides the DNAJ family that are implicated in PD are synaptojanin-1 (SYNJ1) and endophilin A family. SYNJ1 is a Sac domain-containing protein with two different phosphatase domains; 5-phosphatase and Sac1 which target different phosphoinositide phosphates (PIPs) (McPherson *et al.* 1996). 5-phosphatase regulates SV endocytosis by dephosphorylating PI(4,5)P₂ to facilitate uncoating of CCVs in co-operation with Hsc70 and auxilin (Figure 1C), whereas Sac1 domain has gained importance due to its link to PD (*PARK20*, Table 1) (Mani *et al.* 2007; Cremona *et al.* 1999; Saheki & De Camilli 2012). The p.R258Q missense mutations in the Sac domain were identified independently in two consanguineous families of Italian and Iranian origin. Homozygous missense mutations of *SYNJ1* Sac domain are noted to cause autosomal recessive PD with extrapyramidal features identical to patients with auxilin mutations (Krebs *et al.* 2013; Quadri *et al.* 2013; Olgiati *et al.* 2014). Interestingly, they responded poorly to L-dopa treatment, indicating the need for preclinical studies to understand the exact pathomechanisms. Recent observations by Cao *et al.*, suggest that the Sac domain plays a previously unappreciated role in clathrin uncoating. Mice with *SynJ1* Sac mutations (knock-in) developed PD-like characteristics and featured synaptic endocytosis defects, including accumulating of CCVs. Furthermore, elevated auxilin and parkin levels were noted in these mice, suggesting the profound effect of Sac mutations on other proteins linked to PD (Cao *et al.* 2017). Studies on fibroblasts from patients with *SYNJ1* mutations suggested defective endocytic trafficking (Quadri *et al.* 2013). Other studies have shed light on the involvement of SynJ1 in regulating synaptic autophagy. Mutations in the Sac1 domain of *SynJ1* in both *Drosophila* neurons and *PARK20* patient-derived iPSCs severely affected synaptic autophagosome formation but not endocytosis (Vanhouwaert *et al.* 2017). This discrepancy and the precise relationship between SV endocytosis and synaptic autophagy remains to be clarified. However, the *Drosophila* model revealed loss of dopaminergic neurons, providing evidence for the contribution of synaptic autophagy defects in pathogenesis of PD (Vanhouwaert *et al.* 2017). Considering its dual enzymatic activity, SynJ1 is also believed to play a role in fast endophilin-mediated, clathrin-independent endocytosis which is an alternative to CME (Watanabe *et al.* 2018) and several other processes. Further studies are warranted to understand if SYNJ1 exert its effects on PD pathogenesis through any of the alternative endocytic pathways.

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SYNJ1 recruitment to endocytic membrane is facilitated by endophilin A (Figure 1C), a protein drawing attention in PD pathogenesis for a while, despite its locus variation being just identified as a risk factor (Table 1) (Chang *et al.* 2017). This is because of endophilin A's close association with SYNJ1 (Song & Zinsmaier 2003; Cao *et al.* 2017) and its link to most important proteins involved in PD. One of our earlier studies indicated that overexpression of α -synuclein decreases endophilin A levels, while deletion of synucleins had the opposite effect (Westphal & Chandra 2013). As noted in *SynJ1* knock-ins, endophilin KO also leads to synaptic vesicle endocytosis defects, accumulation of CCVs, elevation of parkin levels, and other parkinsonian features (Cao *et al.* 2014; Milosevic *et al.* 2011). Gain-of-function mutations in LRRK2 hyper-phosphorylate endophilin A, the fly homolog, leading to synaptic endocytosis dysfunction (Matta *et al.* 2012). LRRK2-mediated phosphorylation of endophilin A is also noted to regulate synaptic autophagy (Soukup *et al.* 2016). Owing to the relatively higher frequency of LRRK2 mutations and its involvement in sporadic PD, endophilin A may prove to be another crucial protein involved in PD.

Headline heading

4. Autophagy in PD; cause and effect, hand in hand:

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Neurons need to constantly maintain homeostasis by preserving healthy organelles and functional proteins, while selectively capturing and eliminating damaged organelles and misfolded proteins. Autophagy, an integral component of the E-L system, is an intracellular proteostasis pathway that performs this house cleaning function (Nixon 2013; Suresh *et al.* 2018b; Mizushima *et al.* 1998) (Figure 1A, C). It was initially identified in cell lines to function in maintaining energy homeostasis during critical periods of development and in response to nutrient depletion (Mizushima 2007). Intriguingly, nutrient depletion does not induce autophagy in neurons, but a complete lack of autophagy can cause lethal neurodegeneration, emphasizing its important role in survival (Hara *et al.* 2006; Komatsu *et al.* 2006). Since neurons are postmitotic and long-lived, they are particularly vulnerable to accumulation of toxic proteins (Nixon 2013). In addition, greater dependency of neurons on mitochondria for energy instead of glycolytic pathways demands constant mitochondrial turnover (Kann & Kovacs 2007), leading to many malfunctioning mitochondria. Both these factors play major roles in the pathogenesis of PD.

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4.1 PINK1 and Parkin:

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Role in Mitophagy and Beyond. Under physiological conditions, autophagy plays a crucial role in clearing unwanted proteins, as well as damaged mitochondria through a targeted mechanism called mitophagy (Nguyen *et al.* 2016). Loss-of-function mutations in *PINK1* and *Parkin* (*PARK2* and *PARK6*), result in recessively-inherited early-onset PD and significantly, both genes were discovered to be essential for mitophagy (Kitada *et al.* 1998; Valente *et al.* 2004; Mori *et al.* 1998). *PINK1* is a serine/threonine protein kinase that acts as a surveillance molecule for mitochondrial health and accumulates on the outer membrane of abnormal mitochondria (Nguyen *et al.* 2016). *PINK1* attracts recruitment of *Parkin* to damaged mitochondria, which through its E3 ubiquitin ligase activity (Kane *et al.* 2014; Narendra *et al.* 2010), coats the mitochondria with ubiquitin triggering autophagosomes to engulf the mitochondria (Narendra *et al.* 2008), culminating in lysosomal degradation (Figure 1A, C). Discoveries of *PINK1* and *Parkin* mutations were the first genes to shed

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light on the direct involvement of autophagy and E-L system in PD pathogenesis (Pickrell & Youle 2015). These observations also helped in validating previous neurotoxic studies (Przedborski *et al.* 2004) which identified mitochondrial dysfunction as one of the primary causes of neurodegeneration. Though the symptoms are indistinguishable from sporadic cases, several studies on brains of PD patients with *Parkin* mutations do not show α -synuclein aggregation and Lewy body pathology (Doherty & Hardy 2013). Only one report suggests PINK1-linked parkinsonism to be associated with Lewy pathology (Samaranch *et al.* 2010). This prompts us to speculate if there are alternative neuropathological cascades in PD that are α -synuclein independent but centered around the E-L system. In addition to their well-defined roles in mitophagy, Parkin was identified to modulate E-L system through Rab7 ubiquitination (Song *et al.* 2016), whereas PINK1 phosphorylates some Rab proteins (Lai *et al.* 2015). It is also worth noting that several of these rab proteins (for example Rab7) regulate mitochondrial dynamics and quality control independent of Parkin/PINK1 (Wang *et al.*, 2018). *Parkin* mutations are also known to directly affect retromer recruitment (Williams *et al.* 2018) and interact strongly with VPS35 by ubiquitinating it (Martinez *et al.* 2017; Malik *et al.* 2015). Parkin binds to endophilin A and functions in its ubiquitination (Cao *et al.* 2014). Parkin also ubiquitinates EPS15 to regulate epidermal growth factor receptors and phosphoinositide 3-kinase (PI(3)K)-Akt signalling that plays a role in neuronal survival (Fallon *et al.* 2006). These developments suggest mitophagy-independent routes these proteins may take towards PD. Further investigations of Parkin and PINK1 in this direction might provide crucial insights in understanding neurodegenerative mechanisms that are Lewy-body independent.

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4.2. Synaptic autophagy and axonal transport: a road less travelled in PD research

α -Synuclein aggregates initially in presynaptic termini, where the protein is enriched (Kramer & Schulz-Schaeffer 2007). α -Synuclein aggregates are a major target for autophagy-mediated degradation (Xilouri *et al.* 2008). In both genetic and sporadic PD, aggregated α -synuclein is likely to enhance autophagosome formation to meet the higher demand for its degradation (Wong & Holzbaur 2015). Once threshold reaches beyond compensatory limits, mutant/overexpressed α -synuclein starts to accumulate, turning detrimental to cellular homeostasis. In fact, A53T and A30P α -synuclein mutants blocked their own uptake and other substrates by lysosomes for degradation through the chaperone-mediated autophagy pathway *in vitro* (Cuervo *et al.* 2004). Dopamine-modified α -synuclein is identified to further exacerbate this process, which may explain the selective vulnerability of dopaminergic neurons in PD (Martinez-Vicente *et al.* 2008). A recent study on mammalian cells and transgenic mice shows that α -synuclein overexpression mediated autophagy defects are exerted through Rab1a inhibition (Winslow *et al.* 2010). Further understanding of the mechanisms for α -synuclein-autophagy crosstalk in PD might have crucial therapeutic applications. Backing this notion, both *in vitro* and *in vivo* studies show that enhancing autophagy and autophagosome-lysosome fusion could ameliorate PD-like features by clearing aggregated α -synuclein (Suresh *et al.* 2017; Suresh *et al.* 2018a).

Several other PD causing genes associated with the E-L system exert their pathological effects through impairing synaptic autophagy. The R258Q SynJ1 mutation directly affects autophagosome biogenesis and maturation at synaptic terminals of flies and neurites of

human iPSC derived neurons. The observation of dopaminergic neuronal loss in these flies suggests autophagosome generation defects as one of the possible mechanisms through which SynJ1 mutations lead to dopaminergic neurodegeneration and PD (Vanhauwaert *et al.* 2017). Presynaptic endocytic membrane bending protein endophilin A also has functions in providing docking sites for autophagy proteins and facilitating autophagosome expansion. This is achieved by phosphorylation of endophilin A-BAR domain by LRRK2, as noted in a fly model (Soukup & Verstreken 2017). Congruent to these observations, mice lacking endophilin A revealed deficiency in autophagosome formation and developed a Parkinsonian phenotype (Murdoch *et al.* 2016). These studies, along with recent finding of clathrin involvement in autophagy regulation (Rong *et al.* 2012), suggest a cooperative crosstalk between SV endocytosis and autophagy.

Macromolecules and organelles in the presynaptic terminal travel a long distance from the soma, making them old and vulnerable. Rapid turnover of proteins, membranes, receptors, and vesicles in the pre-synapse assigns autophagy a major role in maintaining synaptic homeostasis. This tasks synaptic autophagy with quality control responsibilities. Though there is compelling evidence that compromised autophagy contributes to PD, these studies primarily looked at the soma from a proteinopathy or mitophagy perspective (Lynch-Day *et al.* 2012). Selective inactivation of autophagy by conditional knockout of autophagy protein atg7 in dopaminergic neurons leads to enhanced dopamine release and rapid presynaptic recovery with abnormal axonal profiles (Hernandez *et al.* 2012). Conversely, autophagy enhancement through mTOR inhibition slows dopamine release, reduces the number of synaptic vesicles, and increases autophagosome density in the axons (Hernandez *et al.* 2012). These results emphasize the importance of synaptic autophagy in normal dopamine transmission and further evaluation is warranted to understand if additional synaptic autophagy defects exist, which may lead to PD pathogenesis.

Another unique and stressful situation for neurons is that autophagosomes loaded with unwanted materials from an active presynaptic terminal, need to be transported a long distance to the soma for lysosomal degradation (Figure 1B). Microtubule and scaffolding proteins aid in this retrograde axonal transport of autophagosomes and are crucial for the normal functioning of E-L system (Birdsall & Waites 2019). During their transport, autophagosomes mature into autolysosomes by fusing with late-endosomes/lysosomes towards the proximal end of axon (Nikoletopoulou & Tavernarakis 2018). There are recent studies suggesting lysosomal degradation at the distal end, especially of mitophagosome mediated by Parkin-PINK1. This is predicted to provide rapid neuroprotection against oxidative stress by damaged mitochondria, without requirement for retrograde transport (Ashrafi *et al.* 2014; Ashrafi & Schwarz 2015). Axonal transport aids this function through anterograde transport of E-L system components required for degradation from soma towards distal end. Any defect in this to-and-fro transport machinery can completely clog the E-L system and other processes. Considering its functional significance in neuronal survival, axonal transport might hold clues for understanding pathological mechanisms. Abnormal axonal swelling with lysosomal accumulation has been noted in Alzheimer's disease, blocking the retrograde transport of autophagosomes (Gowrishankar *et al.* 2015; Nixon *et al.* 2005). Similar understanding on the health of axonal transport machinery and

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its dynamics in PD is crucial towards finding commonalities amongst neurodegenerative diseases.

heading

5. Sometimes, there are traffic issues: E-L system trafficking in PD

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The multi-compartmentalised nature of neurons puts tremendous pressure on the endosomal sorting and trafficking machinery, which is also true in case of nigrostriatal neurons. The vacuolar protein sorting (VPS) group of proteins that form retromer complex comprising of VPS35, VPS26, and VPS29 are crucial in retrograde trafficking and sorting of protein cargos from endosomes to the TGN, facilitating their recycle and reuse (Figure 1A) (Haft *et al.* 2000; Seaman *et al.* 1998). The retromer complex also functions in sorting proteins/cargo to regulate endosomal and lysosomal maturation (Vagnozzi & Pratico 2018). Pedigrees with single allelic mutation in *VPS35* revealed autosomal dominant, late-onset, L-dopa-responsive PD, with striking similarities to idiopathic PD (Vilarino-Guell *et al.* 2011; Wider *et al.* 2008). Broadly, it is proposed that the mutant *VPS35* fails to associate with other components of the retromer complex, resulting in impaired retrograde trafficking (Williams *et al.* 2017). Induced loss-of-function mutations of *VPS35 in vitro* resulted in enlargement of late-endosomes and aggregation of α -synuclein oligomers (Follett *et al.* 2016). Another study on flies revealed ceramide accumulation due to VPS35 deficiency, suggesting lysosomal dysfunction (Lin *et al.* 2018). α -synuclein accumulation in nigral dopaminergic neurons, loss of dopaminergic neurons, reduced dopamine transporter levels in the striatum, and locomotory deficits were noted in VPS35-deficient mice (Ishizu *et al.* 2016; Tang *et al.* 2015a). Further *in vitro* studies of *VPS35* (D620N) mutant cells revealed dysfunction in endosome-to-Golgi retrieval of lysosomal membrane proteins (LAMP2a) and accelerated lysosomal degradation of LAMP2a (Tang *et al.* 2015a). LAMP2a is considered important in directing misfolded α -synuclein to lysosomes, suggesting a mechanism for α -synuclein accumulation in VPS35 deficiency (Tang *et al.* 2015a). *VPS35* mutations also affect autophagy and dendritic AMPA (α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor trafficking (Munsie *et al.* 2015; Zavodszky *et al.* 2014). *VPS35* mutations bring severe abnormality in mitochondrial dynamics, as noted in cultured dopaminergic neurons, mice SNpc neurons, and in PD patient derived fibroblasts. (Tang *et al.* 2015b). Oxidative stress implicated in sporadic PD might mediate pathogenesis through VPS35-driven mitochondrial defects (Wang *et al.* 2016). VPS35 also has a role in biogenesis of mitochondria-derived vesicles, further suggesting that its dysfunction cause mitochondrial pathology and neurodegeneration (Sugiura *et al.* 2014). VPS35 is functionally linked to LRRK2, boosting its kinase activity (Mir *et al.* 2018). In fact, decreased VPS35 levels were noted in the SNpc of PD patients with *LRRK2* mutations (Zhao *et al.* 2018). Interestingly, overexpression of VPS35 ameliorated locomotor deficits and enhanced lifespan in *LRRK2* mutant models (Linhart *et al.* 2014; MacLeod *et al.* 2013), which was also seen in *Parkin* mutant models (Malik *et al.* 2015). Neuroprotection was also noted against MPP⁺ induced toxicity in dopaminergic neurons (Bi *et al.* 2013). Along with its E-L sorting functions, these links between VPS35 and α -synuclein, LRRK2, Parkin, lysosomal dysfunction, autophagy, mitochondrial perturbations, and oxidative stress strongly suggest VPS35 as a good therapeutic target.

Content

Another VPS family member associated with PD is VPS13C. Five allelic variations in *VPS13C* gene can lead to autosomal recessive, early-onset PD, primarily due to premature termination of VPS13C (Lesage *et al.* 2016; Schormair *et al.* 2018). Patients were noted with cardinal PD symptoms and extrapyramidal signs. Post-mortem studies revealed α -synuclein/ubiquitin positive-Lewy bodies in the brain stem, limbic system, hippocampus, and cortical associative areas (Lesage *et al.* 2016). A few studies reveal that VPS13 functions in the TGN-endosomal cycle (De *et al.* 2017), whereas recent discoveries have also located it on the outer mitochondrial membrane (Lesage *et al.* 2016). In fact, siRNA-mediated silencing of VPS13C resulted in lower mitochondrial membrane potential, mitochondrial fragmentation, increased respiratory rates, exacerbated Parkin-dependent mitophagy, and upregulation of Parkin in response to mitochondrial damage (Lesage *et al.* 2016). A new study also revealed VPS13C's role in forming membrane contact sites and facilitating lipid transfer between the ER and mitochondria (Kumar *et al.* 2018). This is noteworthy, considering lipid transfer's increasing recognition in disease pathogenesis.

Headline
heading**6. Lysosomes: PD may also begin at the end of the tunnel**

Content

Since lysosomes are the destination for most E-L system vesicles carrying a variety of cargos for degradation and recycling (Figure 1A), dysfunction in any of its components results in lysosomal storage disorders, severely perturbing cellular homeostasis. A growing body of evidence suggests Lysosomal Storage Disorders increase risk for PD pathogenesis (Robak *et al.* 2017). Among them, Gaucher disease (GD) confers the greatest risk, where 5–15% of GD patients or carriers develop PD later in life, to an extent where causal *GBA* mutations are the most common genetic risk factor for PD (Table 1) (Aharon-Peretz *et al.* 2004; Bultron *et al.* 2010; Simon-Sanchez *et al.* 2009). *GBA* encodes glucocerebrosidase 1 (Gcase1), which facilitates breakdown of glucosylceramide (GlcCer) into glucose and ceramide. In its absence, GlcCer accumulates and undergoes partial enzymatic deacylation to glucosylsphingosine and other more complex glycosphingolipids, which mediate disease pathogenesis. *GBA* is a relatively well studied E-L system gene with regards to PD, both in terms of post-mortem brains, clinical investigations, and basic research. Reduced Gcase1 activity in substantia nigra, putamen, and other brain areas of PD patients with *GBA* mutations have been noted along with GlcCer and glycosphingolipids accumulation (Alcalay *et al.* 2015; Gegg *et al.* 2012). Interestingly, similar observations were made even in sporadic PD patients (Alcalay *et al.* 2015; Rocha *et al.* 2015), suggesting an even broader role for *GBA* than just mutations and potentially involve epigenetic and environmental modifiers of *GBA*. Gcase1 levels decrease with aging, the major risk factor for PD (Hallett *et al.* 2018; Rocha *et al.* 2015), indicating *GBA*'s possible role in lowering the nigral neurons' threshold to develop PD. Cognitive areas such as hippocampus, frontal cortex, and amygdala also showed relative decrease in GCase1 activity, along with a significant elevation of hippocampal glucosylsphingosine in sporadic cases (Gegg *et al.* 2012). This correlates with a higher Lewy body burden identified in the hippocampus and medial temporal regions, as well as a diffused cortical Lewy body pathology in *GBA*-linked PD (Hallett *et al.* 2018; Watson & Leverenz 2010). These pathological observations strengthen clinical findings that cognitive deterioration is severe in PD patients with *GBA* mutations compared to that of sporadic cases (Liu *et al.* 2016; Mata *et al.* 2016). These features indicate mouse models of

PD with *GBA* mutations are important in studying lipid dyshomeostasis and understand mechanisms of cognitive dysfunction in PD.

A recent study by Taguchi et al., provides evidence for an association between loss of Gcase1 activity, glycosphingolipids accumulation, and α -synuclein aggregation, both *in vitro* and *in vivo* models of *Gba* mutation (Taguchi *et al.* 2017). Glucosylsphingosine was shown to promote pathological α -synuclein aggregates, capable for further self-templating, was shown to accumulate in the brains of young homozygous *Gba* mutant and KO mice, implicating this glycosphingolipid in PD pathogenesis. Even in heterozygous *Gba* mutations, the Gcase1 activity decreased with age to an extent where mice reached threshold for haploinsufficiency, mimicking the human condition. Furthermore, the *Gba* mutant mice, when crossed with well characterised α -synuclein transgenic PD mice, revealed accelerated PD progression including α -synuclein aggregation and motor deficits (Taguchi *et al.* 2017). α -Synuclein aggregation has also been seen in human iPSCs derived neurons from GD and PD patients that are exposed to Gcase1 inhibitors, and in those carrying *GBA* mutations (Zunke *et al.* 2018). Mutations in *GBA* also cause misprocessing of Gcase1 in the ER, leading to ER retention of Gcase1. This produces ER stress and auto-lysosomal perturbations causing an abnormal lipid profile and increased α -synuclein accumulation and secretion (Fernandes *et al.* 2016; Ron & Horowitz 2005). This study along with a few other observations suggest dopaminergic dysfunction due to gain-of-function mutations in *Gba* (Cullen et al. 2011; Fernandes et al. 2016; Sidransky & Lopez 2012). However, therapeutic strategies based on loss-of-function observations have been successful, arguing against a gain of function model. Activation of residual Gcase1 by small molecules and chaperones and ameliorating glycosphingolipid accumulation by inhibiting GlcCer synthase lowered α -synuclein pathology and reinstated lysosomal health (Richter *et al.* 2014; Charvin *et al.* 2018; Sardi *et al.* 2017). These strategies are already being tested in clinics (Richter *et al.* 2014).

ATPase cation transporting 13A2 (ATP13A2), another lysosomal protein which localises on its membrane (Figure 1A) with unknown function, has been implicated in PD (PARK9, Table 1). Autosomal recessive, early-onset parkinsonism due to *ATP13A2* mutations were first noted in Kufor-Rakeb Syndrome, followed by recent studies in other forms of parkinsonism (Di Fonzo *et al.* 2007; Eiberg *et al.* 2012; Park *et al.* 2011; Ramirez *et al.* 2006). Fibroblasts from these patients showed impaired lysosomal acidification and proteostasis, leading to inefficiency in substrate degradation and autophagosome clearance. These observations were replicated in *ATP13A2* knockdown dopaminergic cell lines, where its restoration re-established lysosomal homeostasis and ameliorated cell death (Dehay *et al.* 2012). Downregulation of ATP13A2 was also seen in nigral neurons of sporadic PD patients, along with traces in Lewy bodies (Dehay *et al.* 2012). A loss-of-function *ATP13A2* mutant mouse model revealed age related lysosomal dysfunction, protein aggregation, and gliosis leading to motor abnormalities. Interestingly, the levels of total and oligomeric α -synuclein was normal, despite ubiquitinated protein aggregation, cathepsin-D dysfunction, and autophagy defects (Kett *et al.* 2015). These observations suggest that *ATP13A2* mutations may lead to PD through an α -synuclein independent mechanism.

7. E-L system; a key target of key PD-associated proteins?

7.1. α -synuclein:

Misfolded α -synuclein can trigger PD pathogenesis in the presynaptic terminals of nigrostriatal neurons (Imbriani *et al.* 2018; Kramer & Schulz-Schaeffer 2007; Spinelli *et al.* 2014). This may as well be one of the earliest pathogenic events in PD, where α -synuclein aggregates target SV endocytosis, SV trafficking, and autophagy components of E-L system to initiate or exacerbate dopaminergic neuronal dysfunction.

α -Synuclein and other family members function in presynaptic terminals, where they modulate SV distribution and neurotransmission (Burre 2015; Greten-Harrison *et al.* 2010; Vargas *et al.* 2017) (Figure 1C). The biophysical properties of synucleins reveal their ability to sense and modify membrane curvature (Shen *et al.* 2012; Westphal & Chandra 2013; Middleton & Rhoades 2010). These functions suggest that synucleins regulate SV exocytosis and endocytosis (Vargas *et al.* 2014; Greten-Harrison *et al.* 2010). Studies in KO mice in fact show that synucleins normally function post SV exocytosis, in fusion pore expansion and/or control the kinetics of SV endocytosis by facilitating membrane curvature (Lautenschlager *et al.* 2017; Logan *et al.* 2017; Vargas *et al.* 2014). α -Synuclein also appears to control SV availability for release by regulating SV clustering (Diao *et al.* 2013; Vargas *et al.* 2017).

On a pathological level, several studies show that overexpression of α -synuclein inhibits neurotransmission. The locus of this consistent effect is not clear. Studies have shown that elevated α -synuclein levels and oligomeric α -synuclein inhibit SV exocytosis in a SNARE-dependent manner or affecting SV clustering (Huang *et al.* 2019; Nemani *et al.* 2010). On the other hand, considerable evidence suggests that aggregated α -synuclein primarily impacts SV endocytosis, which is of relevance to E-L system genes linked to PD. In lamprey giant synapses, injection of aggregated α -synuclein is identified to inhibit clathrin uncoating as evidenced by the accumulation of CCVs when SV recycling was evoked by high-frequency stimulation (Busch *et al.* 2014). A recent study also suggests that extra synuclein might sequester Hsc70 thus effect clathrin uncoating, subsequently causing SV recycling defects (Banks *et al.* 2019). Similar observation was made in mammalian Calyx of Held synapse, where acute elevation of monomeric and mutant α -synuclein levels decreased SV endocytosis with frequency dependent effect on SV exocytosis. This was also seen upon transgenic expression of α -synuclein (Eguchi *et al.* 2017; Xu *et al.* 2016). . Overexpression of mutant α -synuclein also affects dopamine transporter trafficking on the presynaptic membrane, leading to dopamine dyshomeostasis (Kisos *et al.* 2014). The observations that overexpression and deletion of synucleins have similar effects on SV exo- and endocytosis (Lautenschlager *et al.* 2017) suggest that SV cycling is exquisitely sensitive to α -synuclein levels. Further understanding of the precise mechanisms for interactions of α -synuclein and synaptic E-L system proteins involved in PD will provide crucial insights on early causal pathogenic events in PD.

At the soma, aggregated α -synuclein mainly targets E-L system trafficking, especially the retromer pathway. Recent studies propose that aggregated α -synuclein disrupts the retromer complex by interacting through VPS26 or VPS29, and thus preventing the recruitment of

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adaptors like Snx3 and SNX-BAR dimers (Chung *et al.* 2017; Patel & Witt 2018).. Deficiency of VPS35 in dopaminergic neurons impairs endosome-to-Golgi retrieval of receptors like Lamp2a, accelerating its lysosomal degradation. Lamp2a is a critical receptor for chaperone-mediated autophagy of misfolded α -synuclein, so its deficiency leads to a vicious cycle of α -synuclein accumulation (Tang *et al.* 2015a), which might be the case in patients with VPS35 mutations. Overexpression of α -synuclein also directly compromises macroautophagy through inhibition of Rab1a (Winslow *et al.* 2010). It is also pertinent to note that aggregated α -synuclein in the soma is a key target of treatment strategies in development through induction of macroautophagy (Suresh *et al.* 2017; Suresh *et al.* 2018a).

Recent developments suggest that α -synuclein pathology spreads in a prion-like manner through synaptically connected regions in a manner consistent with Braak staging (Braak *et al.* 2003; Luk *et al.* 2012). The E-L system plays a prominent role in cell-to-cell transmission of preformed α -synuclein filaments, leading to the spread of phospho- α -synuclein pathology. Internalization of fibrillar α -synuclein occurs via endocytosis (as well as tunnelling nanotubules) and follows the neuronal endosomal pathway to lysosomal degradation (Rodriguez *et al.* 2018). Heparan sulfate proteoglycans, lymphocyte-activation gene 3, $\alpha 3$ isoform of Na^+/K^+ ATPase, and a few endocytic vesicle receptors are predicted to act as receptors for α -synuclein preformed fibrils (Holmes *et al.* 2013; Ihse *et al.* 2017; Mao *et al.* 2016; Shrivastava *et al.* 2015). *In vitro* studies suggest that internalized α -synuclein fibrils are localized in both early endosomes and late endolysosomes (Karpowicz *et al.* 2017; Lee *et al.* 2008b). The lipid bilayer of endosomes restricts cytoplasmic spillage, so most endocytosed α -synuclein is degraded by lysosomes. How a fraction of α -synuclein preformed fibrils escapes this barrier and goes on to initiate seeding is still unclear (Bieri *et al.* 2018). Studies of α -synuclein axonal transport predict that retrograde transport of α -synuclein preformed fibrils is mediated by endocytic vesicles and is twice as fast and more efficient than anterograde transport (Brahic *et al.* 2016). This strengthens the notion that PD pathology spreads retrogradely from the synaptic terminal to soma. The E-L system is also involved in exocytosis of fibrillized α -synuclein, facilitating its prion-like spread. An ER-bound enzyme with chaperone activity USP19 (Ubiquitin carboxyl-terminal hydrolase 19) has been shown to deliver misfolded α -synuclein to late endosomes, which then fuses with the plasma membrane through a unique process called misfolding-associated protein secretion (Lee *et al.* 2016). Another recent study suggests that the DNAJ/Hsc70 complex could facilitate α -synuclein exocytosis (Fontaine *et al.* 2016). Overexpression of ATP13A2 and GBA is also noted to increase exocytosis of α -synuclein (Fernandes *et al.* 2016; Tsunemi *et al.* 2014). Together, these observations identify the E-L system as the primary conduit for the spread of α -synuclein-mediated pathology in PD.

heading
7.2. LRRK2:

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Leucine-Rich Repeat Kinase 2 (LRRK2) is a multidomain large protein with both GTPase and kinase activity along with other domains for protein-protein interaction (Kett & Dauer 2012). Epidemiological, histopathological, and clinical investigations have all established *LRRK2* gene mutations as the most common genetic cause of PD (*PARK8*, Table 1) (Paisan-Ruiz *et al.* 2004; Zimprich *et al.* 2004), with a considerable contribution to sporadic cases (Kett & Dauer 2012). Over 8 identified *LRRK2* mutations cause PD. The mutation at

G2019S is the most common and is known to increase LRRK2's activity (Kett & Dauer 2012; Smith *et al.* 2006; West *et al.* 2005). LRRK2 functions have been explored through PD-related studies, in which increased G2019S kinase activity reveals its close association with the E-L system. LRRK2's role in vesicular trafficking, lysosomal homeostasis, and autophagy are well accepted. Phosphorylation by LRRK2 is essential for a subset of RAB GTPases' function of endolysosomal vesicular sorting and trafficking (Steger *et al.* 2017; Steger *et al.* 2016). In an overexpression system, RAB7L1 was noted to interact strongly with LRRK2 at the switch-II site (Steger *et al.* 2017), unlike as seen in *in vitro* kinase assays (Steger *et al.* 2016). Several *LRRK2* mutations significantly affect RAB pathways, where variations in Rab7L1's interactions with LRRK2 have been identified to increase the risk of sporadic PD. In fact, recent GWAS identify *RAB29* encoding RAB7L1 (*PARK16*, Table 1) as an independent risk gene for PD (Satake *et al.* 2009). Rab7L1 upregulation could attenuate LRRK2-induced toxicity in dopaminergic neurons (MacLeod *et al.* 2013). This is predicted to be functioning through VPS35 and VPS26 (Linhart *et al.* 2014). VPS35 also cooperates with LRRK2 to regulate SV recycling and dopaminergic synaptic release (Inoshita *et al.* 2017), thus suggesting a prominent role for the retromer complex in LRRK2-mediated PD pathogenesis.

LRRK2 regulates lysosomal protein trafficking and morphology (Eguchi *et al.* 2018; Kuwahara *et al.* 2016). In *LRRK2* mutants, perinuclear lysosomal clustering was noted, mediated by increased phosphorylation of RAB7 (Hockey *et al.* 2015). Two-pore channels are the calcium channels present on acidic vesicles of the E-L system and help in regulating their trafficking and fusion of lysosomes with autophagosomes/endosomes (Calcraft *et al.* 2009). Abnormal lysosomal morphology and density seen in fibroblasts from patients with LRRK2 mutations could be reversed by inhibiting these two-pore channels (Hockey *et al.* 2015). Thus, these channels might be one potential mediator of E-L system dysfunction in LRRK2-associated PD. Lysosome-related proteins involved in PD such as Gcase1, Lamp2A and ATP13A2 are also downregulated in PD patients with *LRRK2* mutations (Zhao *et al.* 2018), suggesting lysosomes are one of LRRK2's prime targets.

Mechanisms for LRRK2-mediated autophagy defects are still elusive. The ability of LRRK2 kinase inhibitors to reverse autophagy dysfunction suggest that kinase activity may be significant (Manzoni *et al.* 2013; Saez-Atienzar *et al.* 2014). Mutant *LRRK2* is also a substrate for chaperone-mediated autophagy, impairment of which could exacerbate pathological implications (Orenstein *et al.* 2013). Accumulation of MVBs and malformed autophagosomes were noted in *LRRK2*R1441C mutations (Alegre-Abarrategui *et al.* 2009). Intra-axonal autophagic vacuole defects in nigral dopaminergic projections were seen in mice expressing human LRRK2 (Li *et al.* 2009; Tagliaferro *et al.* 2015). It also regulates synaptic autophagy through phosphorylation of endophilin A (Soukup *et al.* 2016). G2019S mutations are known to induce autophagy through MAPK/ERK kinases (Bravo-San Pedro *et al.* 2012; Bravo-San Pedro *et al.* 2013), and some studies suggest LRRK2-mediated autophagy regulation is largely independent of mTOR (Manzoni *et al.* 2013). Since RAB-mediated trafficking is crucial for autophagy, which is also affected in *LRRK2* mutations, it is important to evaluate if autophagy defects are the consequences of RAB dysregulation or a direct effect of *LRRK2* mutations.

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LRRK2 regulates the SV cycle by interacting with E-L system proteins that are also implicated in PD: auxilin, SYNJ1, and endophilin A. LRRK2 overactivity phosphorylates auxilin in its clathrin binding domain, making their interaction weaker. This leads to disruption of SV endocytosis (Nguyen & Krainc 2018). This was noted in patient iPSC-derived dopaminergic neurons, along with accumulation of oxidized dopamine, increased α -synuclein levels, and decreased Gcase1 activity (Nguyen & Krainc 2018).

Hyperphosphorylation of endophilin A and synJ1 by LRRK2 significantly altered synaptic endocytosis (Islam *et al.* 2016; Matta *et al.* 2012; Pan *et al.* 2017). A recent study showed that LRRK2 can directly bind to clathrin-light chains through its GTPase domain, affecting Rac1-mediated endosomal dynamics (Schreij *et al.* 2015). LRRK2 also perturbs synaptic exocytosis through phosphorylation of N-ethylmaleimide-Sensitive Factor which upregulates SNARE disassembly (Belluzzi *et al.* 2016). Furthermore, LRRK2 is highly expressed in dorsal striatal medium spiny neurons (MSNs), the postsynaptic compartment of nigrostriatal pathway. G2019S mutation of *LRRK2* lead to abnormally high activity of MSNs and morphological perturbations during development (Matikainen-Ankney *et al.* 2016). Abnormal synaptogenesis and dopamine receptor activation in the developing MSNs mediated by dysfunctional protein kinase A was seen in *LRRK2*R1441C mutation (Parisiadou *et al.* 2014). These defects can permanently modify the circuit structure and function, predisposing the individuals to both motor and non-motor dysfunctions of PD. *LRRK2* mutation-mediated endocytosis defects were noted predominantly in these MSNs, along with nigral dopaminergic neurons and not in cortical or hippocampal neurons (Pan *et al.* 2017; Maas *et al.* 2017). This suggests a heightened vulnerability in the E-L system of nigrostriatal circuit for *LRRK2* mutations.

Increased LRRK2 kinase activity mediated elevation of exocytosis may also be relevant for cell-to-cell transfer of misfolded α -synuclein (Bae *et al.* 2018; Schapansky *et al.* 2018; Lin *et al.* 2009). LRRK2 can selectively recruit to overloaded lysosomes and divert undegraded materials out of the kidney cells, along with secretion of α -synuclein from endosomes (Eguchi *et al.* 2018). Studies also demonstrate heightened uptake of α -synuclein fibrils through endocytosis in the presence of pathogenic LRRK2 (Bae *et al.* 2018). However, LRRK2's role in prion-like spreading of α -synuclein remains speculative, until confirmation that similar mechanisms exist in neurons.

8. Future Directions:

Clinical description and diagnosis of PD has changed little since James Parkinson's characterization of the disease 200 years ago (Parkinson 2002). In the 1960s, identification of dopamine deficiency as the cause of motor abnormalities led to symptomatic relief for patients (Carlsson *et al.* 1957). Though some success has been noted with deep brain stimulation (Limousin *et al.* 1998), we are still relying on symptomatic relief. The last three decades of basic research have enriched our understanding of PD's cellular and molecular mechanisms, bringing tangible hope for disease modifying treatment strategies. Identification of risk genes and conceptual shifts in our understanding through genetic models point to the E-L system dysfunction as the key mediator of PD pathogenesis. Further strengthening this concept, PD pathogenesis appears to begin at synaptic terminals where endo and exocytotic machinery is most active and where α -synuclein and LRRK2 exert their

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effects through the E-L system and vice-versa. Studies so far suggest that altered flux through the E-L system in nigral dopaminergic neurons that have lesser threshold for degeneration is the key trigger for neurodegeneration. Moving forward, critical elucidation of the mechanisms behind sorting, trafficking, and recycling defects demands detailed studies of auxilin and other DNAJ family proteins, along with SYNJ1, endophilin A, and the retromer complex. Studying these genes will also provide crucial insights into less explored roles of synaptic autophagy, axonal transport, and clathrin-independent endocytosis in PD. Recent surveys of PD patients note preservation of cognitive functioning as a major unmet need. Further investigation of GBA mutations implicated in PD is a promising avenue considering GBA's known involvement in cognitive disorders like Dementia with Lewy Bodies. Along with GBA, studying ATP13A2 and other lysosomal genes will enrich our understanding of lysosomal dysfunction, potentially bringing us closer to repurposing drugs used in Lysosomal Storage Disorders for PD. Affirming ongoing clinical trials targeting autophagy, α -synuclein, and LRRK2, an enhanced understanding of E-L system-mediated pathogenesis will prove crucial in developing novel treatment strategies and diagnostic tools for PD.

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heading

Abbreviations:

other

PD

Parkinson's disease

other

SNpc

Substantia Nigra pars compacta

other

E-L

other

Endolysosomal

other

M6P

other

Mannose-6-phosphate

other

TGN

other

Trans-Golgi Network

other

CME

other

Clathrin-Mediated Endocytosis

other

ESCRT

other

Endosomal Sorting Complex Required for Transport

other

ILVs

other

Interluminal Vesicles

other

MVBs

other

Multivesicular Bodies

other

CCVs

other

Clathrin Coated Vesicles

other

GAK

other

Cyclin G Associated Kinase

other

GBA

other

Glucosidase Beta Acid

other

DCVs

other

Dense Core Vesicles

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heading
LRRK2

other

Leucine-rich repeat kinase 2

other

PARK

Parkinson's disease gene

PRKN

Parkin

other

SNCA

Alpha-synuclein gene

other

GWAS

Genome Wide Association Study

other

SVs

Synaptic Vesicles

other

PI(4,5)P₂

Phosphatidylinositol 4,5-bisphosphate

other

L-dopa

Levodopa

other

KO

Knockout

other

RME-8

Receptor Mediated Endocytosis 8

other

SYNJ1/SynJ1

Synaptojanin-1

other

PIPs

Phosphoinositide Phosphates

other

ATP13A2

ATPase cation transporting 13A2

other

PINK1

PTEN Induced Putative Kinase 1

other

VPS

Vacuolar Protein Sorting

other

LAMP2a

Lysosome-Associated Membrane Glycoprotein 2

other

AMPA

α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

other

GD

Gaucher Disease

other

Gcase1

Glucocerebrosidase 1

other

GlcCer

Glucosylceramide

other

SNARE

Soluble N-ethylmaleimide-sensitive factor Attachment protein Receptors

other

HSPGs

Heparan Sulfate Proteoglycans

other

USP19

Ubiquitin Carboxyl-Terminal Hydrolase 19

other

MAPK/ERK

Mitogen-Activated Protein Kinase/ Extracellular Signal-Regulated Kinase

other

GTPase

Guanosine Triphosphatase

other

PI(4,5)P₂

Phosphatidylinositol 4,5-bisphosphate

other

Hsc70

Heat Shock Cognate 70

other

siRNA

small interfering Ribonucleic acid

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other iPSCs	induced Pluripotent Stem Cells
other HSP40	Heat Shock Protein 40
other MSNs	Medium Spiny Neurons

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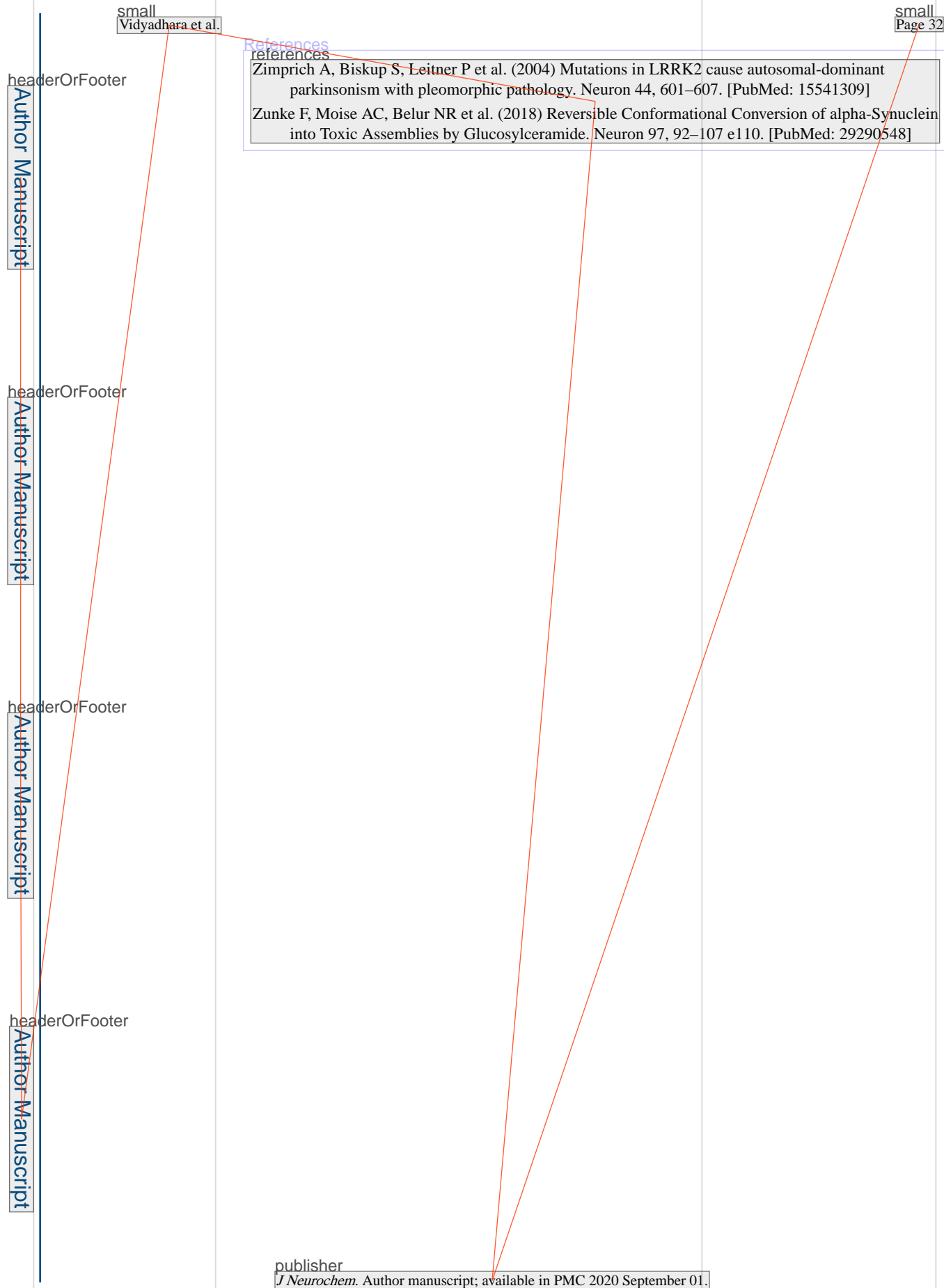
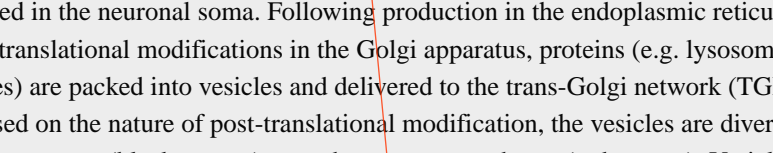




Image	Description
	<p>A. Soma: E-L system proteins that form structural and functional components are synthesized in the neuronal soma. Following production in the endoplasmic reticulum (ER) and post-translational modifications in the Golgi apparatus, proteins (e.g. lysosomal hydrolases) are packed into vesicles and delivered to the trans-Golgi network (TGN). In the TGN, based on the nature of post-translational modification, the vesicles are diverted either towards lysosomes (black arrows) or to the secretory pathway (red arrows). Vesicles that are destined for lysosomes bud off from the TGN to form early endosomes. Simultaneously, early endosomes carrying nutrients and surface receptors for recycling are also derived from the plasma membrane through clathrin mediated endocytosis (CME, purple arrows). These early endosomes, with the help of the endosomal sorting complex required for transport (ESCRT), pass through highly dynamic tubulovesicular structures like multivesicular bodies (MVB) and/or late endosomes before fusing with lysosomes. Leucine-rich repeat kinase 2</p>

Content
ImageDescription

(LRRK2) plays a significant role in this sorting, whereas Glucocerebrosidase 1 (GBA) and ATPase cation transporting 13A2 (ATP13A2) are crucial for normal lysosomal function. Early endosomes from the cell surface that are not sorted follow the recycling pathway back to the plasma membrane (blue arrows). Retrograde transport of receptors that are used for sorting from early endosomes to TGN occurs through the retromer complex where Vacuolar Protein Sorting 35 (VPS35) and VPS13C take part (yellow arrows). An expanding membrane sac called a phagophore sequesters malfunctioning organelles (e.g. sick mitochondria) and misfolded proteins to form a double-membraned autophagosomes. These later fuses with lysosomes and releases its contents for degradation and recycling of components; the complete process is called autophagy (green arrows). PTEN Induced Putative Kinase 1 (PINK1) and Parkin are important in identifying sick mitochondria and diverting them to a specialized form of autophagy called mitophagy.

B. Axon: Axons form a crucial bridge for retrograde transport of endosomes/ autophagosomes from presynaptic terminals to soma for lysosomal degradation. A gradual development of phagophore to full-fledged autophagosome happens within the axon (green arrows) as the encapsulated cargo arrives towards proximal end.

C. Synapse: Neurotransmitter release in the presynaptic terminal is regulated principally by the synaptic vesicle cycle, an integral part of the neuronal E-L system. Initially, endosomes derived from the soma forms synaptic vesicles (SV) in the nerve terminal where RME-8 plays a role in association with clathrin (yellow arrow). Neurotransmitters are loaded into these SVs through specialized transporters. Upon arrival of an action potential in the synaptic terminal, these SVs fuse with the plasma membrane at active sites and release neurotransmitters via exocytosis (black arrows). Empty SVs are then recycled through CME (Purple arrows). α -Synuclein and endophilin A play roles here in membrane bending and SV curvature formation. Clathrin forms a layer on these invaginations with the help of adaptors, and in conjunction with dynamin leads to membrane fission forming clathrin coated vesicles (CCV). Clathrin uncoating is a prerequisite for SV recycling, which is performed by coordinated action of auxilin, Hsc70, endothelin A1, and synaptojanin 1. Phosphorylation by LRRK2 is necessary for activation of these proteins. Synaptic autophagy and mitophagy are also crucial in maintaining the health of the presynaptic terminal (green arrows), where PINK1/Parkin are crucial.

Content

table
Table 1:

callout

headerOrFooter PD genes linked to E-L system with their PARK nomenclature, proteins they encode and their primary function

Image

PD Genes that are linked to E-L system				
PARK	Gene	Protein	Function	Reference
PARK1	SNCA	α -Synuclein	Synaptic vesicle cycling	Polymeropoulos et al. 1997
PARK2	PRKN	Parkin	E3 Ubiquitin Ligase	Mori et al. 1998 Kitada et al., 1998
PARK4	SNCA	α -Synuclein	Synaptic vesicle cycling	Singleton et al., 2003 Chartier-Harlin et al., 2004
PARK6	PINK1	PINK1	Serine/Threonine Kinase	Valente et al., 2004
PARK8	LRRK2	LRRK2	Kinase/GTPase	Paisán-Ruiz et al., 2004 Zimprich et al., 2004
PARK9	ATP13A2	ATPase type 13A2	ATPase	Ramirez et al., 2006 Di Fonzo et al., 2007
PARK16	RAB29	Ras-Related Protein Rab-7L1	Rab GTPase	Satake et al. 2009
PARK17	VPS35	VPS35	Retromer	Wider et al., 2008, Vilarinho-Güell et al., 2011
PARK19	DNAJC6	Auxilin	Co-chaperone	Edvardson et al., 2012 Köro lu et al., 2013
PARK20	SYNJ1	Synaptojanin-1	Phosphoinositide phosphatase	Krebs et al., 2013 Quadri et al., 2013
PARK21	DNAJC13	RME-8	Co-chaperone	Vilarinho-Güell et al., 2014
PARK23	VPS13C	VPS13C	Retromer, Lipid transporter	Lesange et al., 2014, Schormair et al., 2018
Risk Genes		Glucocerebrosidase 1	Glucosidase	Sidransky et al., 2009
	GBA	Cyclin-G Associated Kinase	Co-chaperone	Nalls et al., 2014 Chang et al., 2017
	GAK	Endophilin A1	Membrane bending protein	Nalls et al., 2014 Chang et al., 2017
	SH3GL2			

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