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Defects in trafficking bridge Parkinson's disease pathology and genetics

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Parkinson's disease is a debilitating, age-associated movement disorder. A central aspect of the pathophysiology of Parkinson's disease is the progressive demise of midbrain dopamine neurons and their axonal projections, but the underlying causes of this loss are unclear. Advances in genetics and experimental model systems have illuminated an important role for defects in intracellular transport pathways to lysosomes. The accumulation of altered proteins and damaged mitochondria, particularly at axon terminals, ultimately might overwhelm the capacity of intracellular disposal mechanisms. Cell-extrinsic mechanisms, including inflammation and prion-like spreading, are proposed to have both protective and deleterious functions in Parkinson's disease.

Parkinson's disease (PD) is the most common neurodegenerative movement disorder, affecting about 1% of people aged 65 or older worldwide[1]. Clinical manifestations of PD include motor deficits such as rigidity, slowness in movement (bradykinesia), postural instability and a characteristic tremor at rest[2]. The motor symptoms of PD result from the selective loss of dopaminergic neurons in the pars compacta of the substantia nigra (SN) in the midbrain, as well as their axon terminals, which project to the dorsal striatum[3]. Dopamine replacement therapies, such as the dopamine precursor levodopa, typically lead to the relief of symptoms but lose their potency as the disease progresses.

A neuropathological hallmark of PD is the presence of intraneuronal proteinaceous inclusions, termed Lewy bodies (LBs) or Lewy neurites. These structures are enriched in filamentous forms of the synaptic protein α-synuclein[4,5] as well as the small regulatory protein ubiquitin. Ubiquitin monomers or polyubiquitin chains are required for the proper intracellular trafficking and disposal of many proteins in cells, and the accumulation of ubiquitin is therefore consistent with defects in trafficking and disposal pathways[6]. Although LBs in the SN are a defining feature of PD in postmortem analysis, typically, these structures are also found more broadly in the PD brain[7]. The location of LBs seems to follow a specific pattern of progression: tissue from people with only mild clinical symptoms is typified by the presence of LBs mainly in lower regions of the brainstem such as the dorsal motor nucleus of the vagus nerve; by contrast, in people whose symptoms become increasingly severe, LBs are found in more rostral brain regions, including the SN, and ultimately the forebrain regions[7]. The systematic progression of LB pathology has been used as evidence to support the hypothesis that prion-like mechanisms of spreading might contribute to PD.

Genetic causes of PD

Although PD was historically considered to be a sporadic disorder of unknown aetiology, it has become clear in the past two decades that a considerable proportion of cases (about 5–10%) are caused by familial genetic mutations[8]. Initial genetic studies focused on rare, inherited forms of PD in large families and mapped the causative mutations[9–14]. Exome and whole-genome sequencing approaches that use next-generation sequencing methods have enabled a further wave of rare, inherited causative mutations to be identified.

Genome-wide association studies (GWAS), in which genetic variants are queried across the entire human genome in large cohorts of affected and unaffected people, have been transformative. For example, a large-scale meta-analysis of more than 19,000 people with PD and 100,000 controls that queried the role of about 8 million common genetic single nucleotide polymorphisms (SNPs) across the human genome identified 24 loci that are linked to an altered risk of developing the disease[15]. Although the effect size of each variant is typically modest (less than a 30% alteration in risk), together the variants have considerable impact[16].

Genetic discoveries are beginning to illuminate cellular pathways and functions that are involved in the development of PD[15], and the impairment of intracellular trafficking is emerging as a mechanistic link between many PD-associated genes. Here, we review the converging evidence that points to defects in the endosomal trafficking machinery, particularly at neuronal synapses, in the disruption of trafficking to the lysosome and in lysosomal dysfunction as pathological processes that contribute to PD, and we describe the putative role of α-synuclein protein in these processes. We also relate these defects in intracellular trafficking to new data suggesting that extracellular mechanisms, including inflammation and the spread of disease-related proteins between cells, play important parts in the development of PD.

α-Synuclein and intracellular trafficking

The first gene to be linked to familial PD was *SNCA*, which encodes the protein α-synuclein[9]. Missense mutations in this gene, which alter the amino-acid sequence of the resulting 140-residue protein, cause rare, autosomal dominant inherited forms of PD-related diseases[9]. Similarly, genetic duplication or triplication of the *SNCA* locus can lead to familial, autosomal dominant forms of PD[17,18]. In a striking convergence of genetics and pathology, aggregates of α-synuclein were discovered to be the building blocks of LBs, the pathological hallmark of PD[5,19].

As well as the identification of missense mutations in α-synuclein in rare, familial forms of PD, common genetic variants (typically defined as being found in more than 1% of a population) at the α-synuclein locus have been associated with an increased lifetime risk of PD. For instance, in large population-based gene-association studies of people with PD and unaffected individuals, some genetic variations at the *SNCA* locus, including SNPs, were found to be enriched in those with PD, although modestly, which suggests that these variants increase the likelihood of developing the disease in the general population[15,16,20]. Such disease-associated SNPs do not seem to alter protein-coding sequences and their proposed mode of action is the modification of gene expression[17,21,22].

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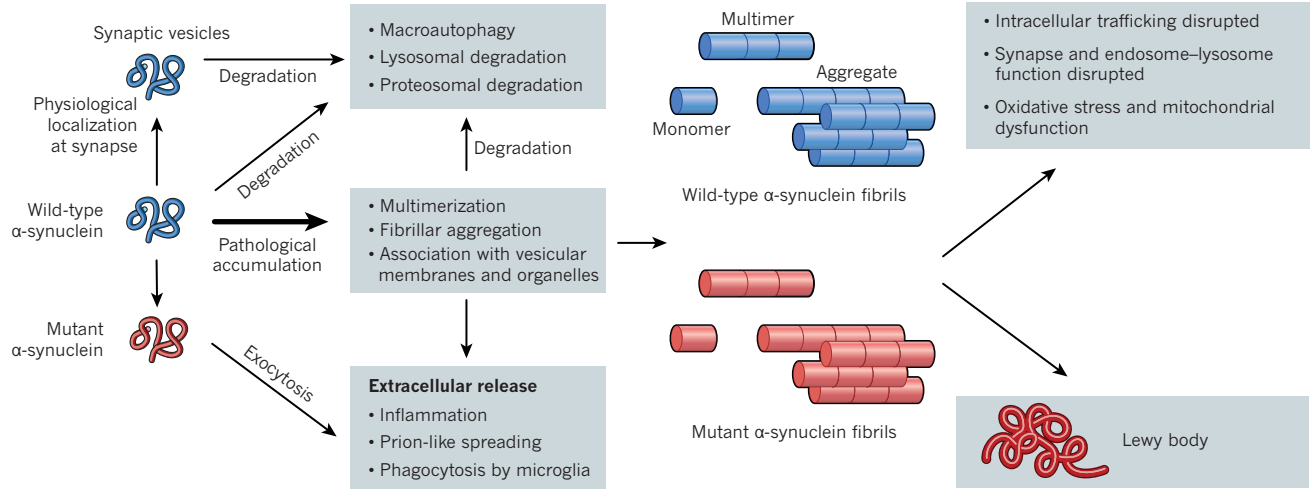


Figure 1 | The proposed physiological and PD-associated pathological functions of α-synuclein in neurons. When generated at a moderate level, wild-type α-synuclein (blue) associates with synaptic vesicles at axon terminals. Typically, α-synuclein undergoes degradation either through lysosomal or proteasomal-led degradation. The pathological accumulation of wild-type α-synuclein can result from an increase in its production — through high-risk, common genetic variants at the α-synuclein gene that modify gene expression or through rare, familial gene multiplications — or from ineffective degradation or trafficking pathways. These defective mechanisms have also been associated with rare, familial PD-associated mutations in α-synuclein (red) as well as mutations in trafficking-associated genes linked to PD and environmental factors such as ageing (not shown). An excess of α-synuclein in the form of monomers, multimers or aggregates can disrupt intracellular trafficking and synaptic function and contributes to the formation of LBs. Furthermore, the extracellular release of α-synuclein through exocytosis might lead to inflammation and the spread of PD-associated lesions such as LBs through prion-like mechanisms.

An important question concerns how the accumulation of α-synuclein contributes to PD pathogenesis. In other words, why is α-synuclein toxic to cells? Studies in model systems that range from yeast cells to transgenic mice have shown that elevated levels of α-synuclein disrupt numerous essential intracellular trafficking steps, including those at the endoplasmic reticulum[23], the early and late endosomes[24] and the lysosome[25,26] (Fig. 1 and Box 1). The increased accumulation of α-synuclein in long-term cultures of human induced pluripotent stem (iPS) cell-derived dopamine neurons, owing to genetic duplication or viral overexpression, leads to insufficient protein degradation as a consequence of the defective trafficking of important enzymes to lysosomes[27]. Upregulation of Ras-related protein Rab-1A, a prototypical regulator of vesicle trafficking (Box 1), is sufficient to rescue these trafficking deficits, which is reminiscent of the ability of a yeast homologue of Rab-1A to suppress α-synuclein toxicity in yeast[23]. Elevated levels of α-synuclein in neurons might therefore disrupt protein trafficking through vesicles or the endosome and, in particular, to the lysosome for degradation[28]. α-Synuclein has also been implicated in a separate intracellular pathway for trafficking proteins to the lysosome, termed macroautophagy (Box 2).

The actions of α-synuclein on vesicular transport processes might reflect its biophysical properties. α-Synuclein in solution is mostly a disordered monomer with a propensity to bind lipid membranes[29,30], which could underlie its role in the disruption of vesicular trafficking, as well as its physiological functions in regulating the release of synaptic vesicles. In certain contexts, α-synuclein can also assume a variety of soluble or insoluble oligomeric forms[30–32], although the precise physiological or pathological roles of such structures are contentious. Insoluble α-synuclein aggregates and fibrils with β-sheet secondary structure are a main constituent of LBs and seem to be neurotoxic[33,34].

In model systems such as neurons derived from human embryonic stem cells, the presence of PD-causing mutations in α-synuclein has been associated with the accumulation of reactive oxygen and nitrogen species[25,35]. This could reflect the build-up of defective mitochondria, which are essential for the production of ATP, the energy currency of the cell, but also produce reactive species as a by-product. Mitochondria are disposed of typically through a process termed mitophagy, in which these organelles are encompassed by double-membraned structures, forming autophagosomes that later fuse to lysosomes for degradation[36,37]. Overexpression of α-synuclein in dopamine neurons of the mouse midbrain has been reported to impede mitophagy[38]. Several genes involved in familial PD, including *PARK2* (encoding the protein parkin), *PINK1*, *FBXO7* and *PARK7* (also known as *DJ-1*), have important roles in the execution of mitophagy (Fig. 2), which are reviewed elsewhere[39].

α-Synuclein is released from cells — particularly when accumulated in excess — through various pathways, including those dedicated to secretion and those involved in the exocytosis of lysosomes[40–43]. Such extracellular release of α-synuclein could serve as a backup disposal mechanism to complement intracellular lysosomes. Neighbouring cells such as innate-immune microglia are thought to have an enhanced capacity for phagocytosis and lysosomal degradation. But released α-synuclein might also have detrimental roles by inducing inflammation directly[44–46], by acting as a chemoattractant that draws inflammatory microglia towards damaged neurons[47] or by helping to spread PD pathology.

Many studies have sought to identify the physiological functions of α-synuclein in neurons and to relate these to the pathology of PD. For example, knockout mice that are deficient in α-synuclein show potentiated vesicular transmitter release[48]. *In vitro* studies suggest that α-synuclein might normally function as a chaperone to modulate the activity of the synaptic-vesicle fusion machinery[49,50]. And the affinity of α-synuclein for phospholipids, which are enriched on vesicle membranes[51,52], probably facilitates its role as a regulator of vesicle trafficking.

In a broader sense, perhaps the toxic and normal functions of α-synuclein are intimately linked. At physiological levels, α-synuclein might function as a regulator of vesicle fusion and neurotransmitter release at the synapse (Fig. 3). However, its accumulation beyond a certain threshold (for example, in people with SNPs that elevate the level of α-synuclein) could lead to the inappropriate deployment of this regulatory function at the synapse or the promiscuous inhibition of other steps in vesicular trafficking[53] (Fig. 3), including those in the endosome–lysosome pathway.

LRRK2, *GBA* and lysosomal trafficking

Autosomal dominant, inherited mutations in the gene *LRRK2* are the most prevalent genetic lesions that underlie familial forms of PD[13,14]. *LRRK2* encodes a large, multidomain protein that contains kinase, GTPase and other protein-interaction domains. The most common

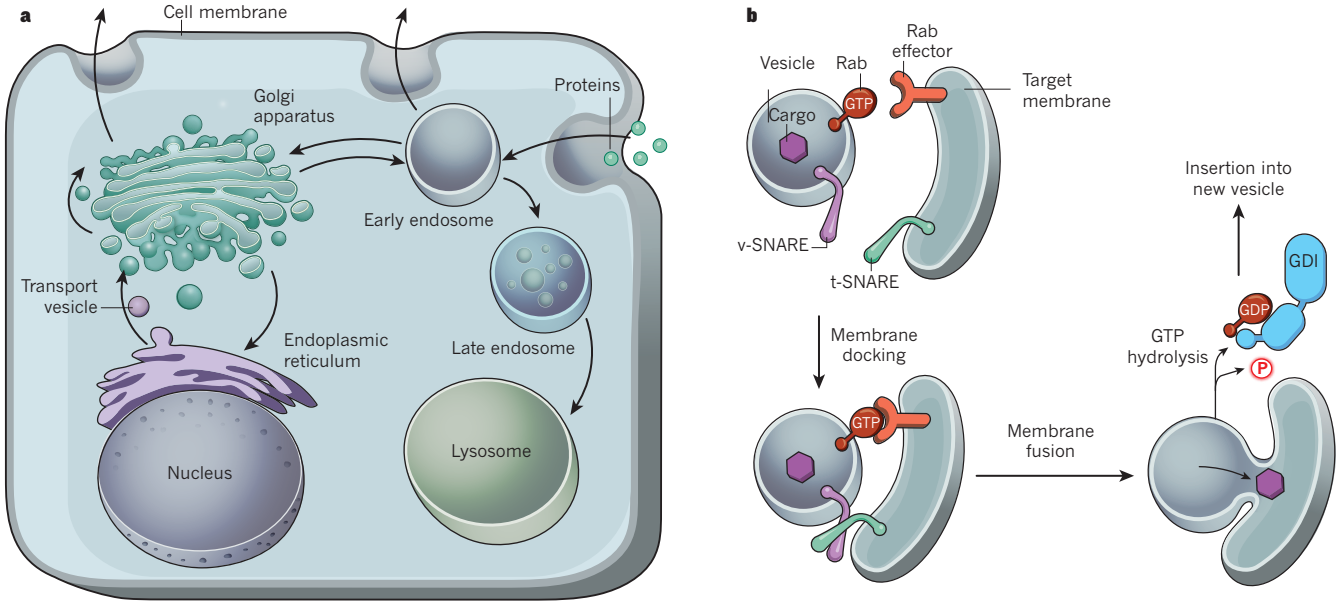
BOX 1

Overview of intracellular trafficking

● **Secretory and endocytic pathways** Select protein cargoes are transported from their site of production, the endoplasmic reticulum, to their final destinations — either the cell-surface membrane or other cellular organelles — through the secretory pathway (Box Fig. a). The cargoes are packaged into transport vesicles, which bud from the membrane and are delivered to the target compartment, where they fuse. As proteins are transported through the Golgi apparatus, some will be modified by the addition of carbohydrates, which provide further information for targeting proteins to different parts of the cell.

● **Endocytosis** Proteins and other molecules are transported into the cell through endocytosis (Box Fig. a). During this process, part of the plasma membrane becomes invaginated and pinches away from the membrane to form a vesicle that incorporates the proteins. Invagination can be enabled by a coating composed of the protein clathrin, which is then removed. The vesicles are delivered to other membrane-bound vesicles called endosomes, where they are sorted. Some proteins are returned to the plasma membrane or sent to the Golgi apparatus, a process that is regulated by the retromer complex, which includes VPS35. Proteins that are destined for degradation are delivered to the late endosome (or a specialized type of endosome known as a multivesicular body) that subsequently fuses with the lysosome. The contents of the endosome are released to be degraded by enzymes in the acidic environment of the lysosome. Lysosomal contents can also be released into the extracellular environment through secretion.

● **Vesicle docking and fusion with target membranes** Small proteins called Rab GTPases are involved in targeting vesicles to the correct membranes[135] (Box Fig. b). A Rab protein on the surface of the vesicle interacts with an adaptor protein (Rab effector) that is located on the target membrane. This interaction causes the vesicle to dock with the appropriate target membrane. Specificity is achieved by using different types of Rab proteins and effectors for each type of vesicle and at each step of the secretory pathway; for instance, RAB1A functions selectively in transport between the endoplasmic reticulum and the Golgi apparatus. After the vesicle has docked with the target membrane, transmembrane proteins called SNAP receptors (SNAREs) interact, which brings the two membranes together to enable fusion. On vesicle fusion, GTP is hydrolysed to GDP, and the Rab protein is extracted from the membrane by the protein Rab GDP dissociation inhibitor (GDI) and reinserted into the membrane of a new vesicle so that the cycle can be repeated. SNAP, soluble NSF attachment protein; t-SNARE, target SNARE; v-SNARE, vesicle SNARE.



mutation associated with *LRRK2* in Western countries is G2019S, which occurs in the kinase domain and is thought to disinhibit the kinase activity — towards at least a subset of substrates[54,55]. The expression of mutant LRRK2 in both cells and animal models leads to defective endosome-to-lysosome trafficking, the accumulation of abnormal lysosomal structures and a reduction in the number of neurite processes in neurons[55–57]. In addition to defective vesicular trafficking to the lysosome, mutant LRRK2 has been implicated in other pathological processes such as dysregulated protein translation[58].

In support of its role in vesicular trafficking, LRRK2 has been shown to interact with several proteins from the Rab family[56,57,59,60], which are important regulators of vesicular intracellular trafficking (Box 1). Among these Rab proteins are Ras-related protein Rab-7L1 (also known as RAB29) (refs 56 and 61), encoded by the *PARK16* locus and therefore associated with the risk of developing PD, and the structurally related protein RAB32, which has been linked to another neurodegenerative disease, amyotrophic lateral sclerosis[59]. These two Rab proteins are part of a subfamily that is implicated in trafficking to the lysosomes and to lysosome-like organelles. Phosphoprotein analyses have also implicated LRRK2 in phosphorylating and modulating other Rab proteins, including RAB3A, RAB8A, RAB10 and RAB12 (ref. 60). Mutant LRRK2 that causes PD might harbour a potentiated kinase activity towards these Rabs, probably leading to altered interactions with downstream effectors of Rab and its regulatory proteins, as well as to perturbations in vesicular transport at several steps (Box 1). Future studies will be required to define whether and how some of these substrates for LRRK2 kinase activity contribute to PD.

Neither transgenic mice that express PD-associated mutant LRRK2 nor *LRRK2*-knockout mice consistently show a robust PD-like phenotype, such as the progressive loss of dopamine neurons. However, *LRRK2*-knockout mice do show the features of age-associated lysosomal pathology — particularly in proximal tubule cells of the kidney and type II pneumocytes of the lung[62–64]. These data reinforce the link between the

BOX 2

Cellular components of macromolecular degradation

● **Lysosome** An acidic organelle that harbours a variety of enzymes for the degradation of proteins, lipids, sugars and other macromolecules. These are trafficked from the external environment (through mechanisms such as endocytosis)[79], from endocytic compartments or from the cytoplasm (by macroautophagy). The fusion of lysosomes with late endosomes or autophagosomes is a process that requires the SNARE complex and RAB7 (as well as the homotypic fusion and protein sorting (HOPS) complex for autophagy)[136] that seems to be impaired by the accumulation of α-synuclein[28,130].

● **Macroautophagy** An evolutionarily conserved process by which certain constituents of the cytoplasm such as defective proteins, aggregates or organelles are engulfed by double-membrane-bound phagophores, which envelop cargoes to form autophagosomes that can fuse to lysosomes[137]. The initiation of autophagy is negatively regulated by the mTOR signalling pathway and inhibitors of this pathway have therefore been considered as potential therapeutic drugs. However, the accumulation of α-synuclein and other mechanisms associated with the development of PD might impair the fusion of autophagosomes with lysosomes, and increased initiation could therefore be harmful.

● **Mitophagy** The macroautophagic degradation of defective mitochondria. On the depolarization of mitochondria, PINK1 becomes stabilized and able to recruit the ubiquitin ligase parkin[39] as well as autophagy receptors such as optineurin[132,138].

● **Proteasome** A multiprotein complex that degrades short-lived cytoplasmic proteins typically marked by polyubiquitin chains[6].

● **Ubiquitin** A short polypeptide that can be attached to cellular proteins by ligases. Ubiquitin can be assembled into polyubiquitin chains on target proteins that serve as postal codes for various destinations, including the proteasome or lysosome, that are determined partly by the molecular nature of the inter-ubiquitin linkages[6].

function of LRRK2 and lysosome-associated trafficking. Because people with mutations in *LRRK2* eventually accumulate α-synuclein in their brains, it has been proposed that α-synuclein represents a cargo that is relevant for LRRK2-associated trafficking. Alternatively, the effect of *LRRK2* mutations on the accumulation of α-synuclein might be indirect, the result of more general endosome and lysosome dysfunction. Although many studies support a model in which PD-associated mutations in *LRRK2* lead to the disinhibition of kinase activity, and therefore a gain of function, it is possible that PD is caused by a reduction in kinase activity; for instance, kinase activity towards a subset of substrates might be reduced in the context of disinhibition, or the non-kinase activities of LRRK2 could be modified instead. The gain-of-function versus loss-of-function issue is even more vexing with respect to the impact of common genetic variants associated with the risk of developing PD, such as those found at the *LRRK2* locus, because typically these variants do not change the coding sequence of the gene and might only alter expression subtly.

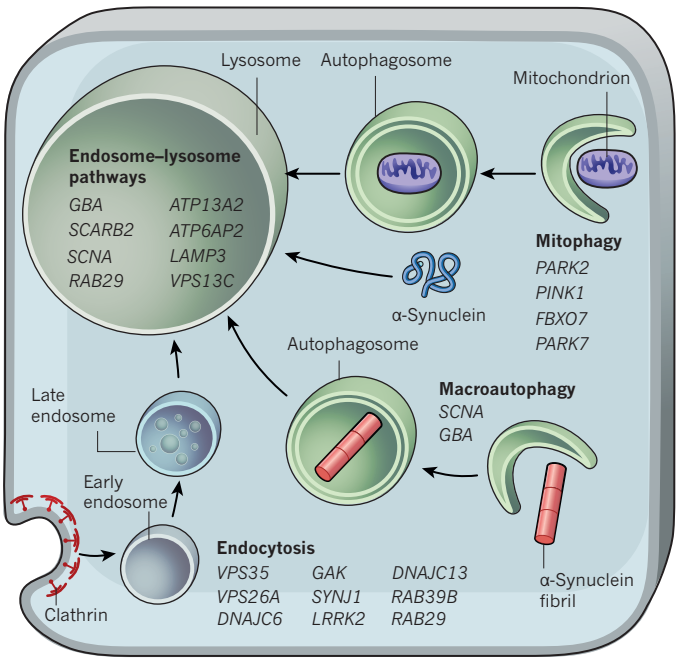


Figure 2 | PD-related genes associated with trafficking to the lysosome. Genes that encode intracellular trafficking components are associated with common sporadic and familial forms of PD, as well as related syndromes that share some of the clinical features of PD. Most of these genes are known to affect trafficking to the lysosome in the context of late endosome-to-lysosome pathways, clathrin-dependent endocytosis, macroautophagy or mitophagy. Wild-type α-synuclein (blue) can also enter lysosomes through chaperone-mediated autophagy[118].

Autosomal recessive mutations in the gene *GBA*, which encodes a lysosomal hydrolase called glucocerebrosidase, lead to the defective breakdown of the glycosphingolipid glucoceramide (also known as glucosylceramide (GlcCer)) into ceramide and glucose, and cause Gaucher's disease, a lysosomal storage disorder with neurological features that include parkinsonism as well as a variety of other clinical findings[65]. Heterozygous carriers of *GBA* mutations have a considerably higher risk of developing PD (about threefold to eightfold greater)[66,67]. A deficiency in glucocerebrosidase can lead to neurodegeneration, either indirectly through general lysosome dysfunction and the failure of endosome–lysosome or autophagosome–lysosome fusion[68] or through a more direct link between the accumulation of GlcCer and α-synuclein. For instance, GlcCer has been shown to stabilize α-synuclein oligomers. Glucocerebrosidase deficiency and the consequent accumulation of GlcCer might also damage cells through the over-activation of the endoplasmic-reticulum-associated degradation (ERAD) pathway and the disruption of other cellular homeostatic mechanisms such as stress-associated calcium release[68]. And a reduction in glucocerebrosidase activity might disrupt the generation of sphingolipids in the central nervous system (CNS) through the ceramide synthesis pathway, leading to further membrane-associated dyshomeostasis[26,68].

Human iPS-cell-derived neuronal models of PD that are associated with a mutation in *GBA* show an increased accumulation of both α-synuclein[26,68] and GlcCer. Furthermore, neuronal cells that overexpress α-synuclein have a defect in the trafficking of glucocerebrosidase to the lysosome[26,27], which points to a positive feedback loop. Notably, a cofactor required for the import of glucocerebrosidase into the lysosome, lysosome membrane protein 2 (LIMP-2), which is encoded by the gene *SCARB2*, has also been linked by GWAS to the risk of developing PD[69]. A deficiency in LIMP-2 leads to the defective transport of glucocerebrosidase, as well as lysosomal dysfunction and α-synuclein accumulation[15,70].

Other PD-related genes identified by familial genetic studies or GWAS also support roles for altered trafficking to the lysosome and defective lysosome integrity in the pathogenesis of PD (Fig. 2). Familial autosomal mutations in the gene *ATP13A2*, which encodes a transmembrane P-type ATPase that is localized to lysosomes and late endosomes and enriched in the brain, underlie an early onset form of parkinsonism with dementia

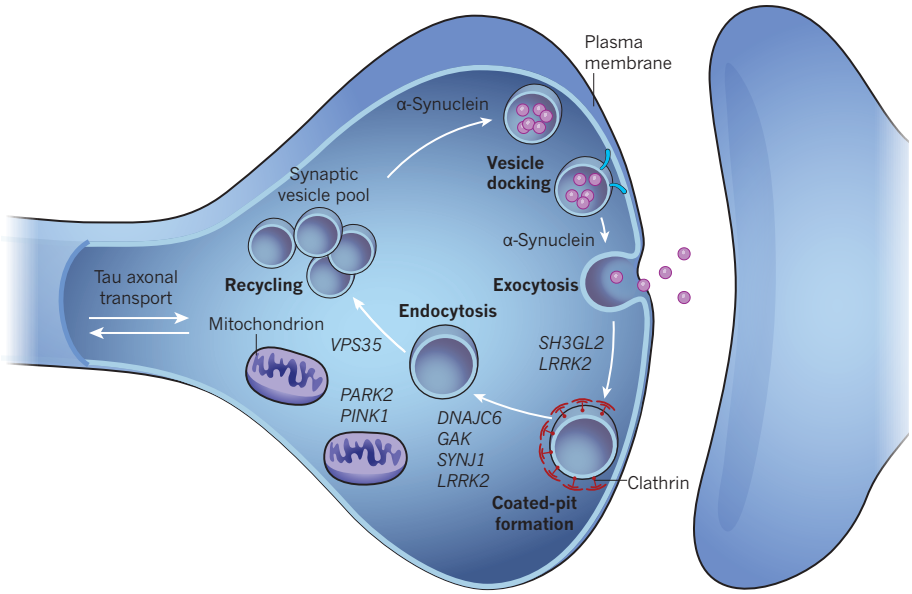


Figure 3 | The synaptic vesicle cycle is implicated in PD. Synaptic vesicles typically cycle through several stages: docking at the plasma membrane of the synaptic terminal; exocytosis through fusion with the plasma membrane in a calcium-dependent manner; the formation of a coated pit at the plasma membrane, which is enabled by a protein coating that is composed of numerous clathrin proteins and other protein components; endocytosis; recycling into a readily releasable pool of synaptic vesicles; and, re-docking with the plasma membrane[133]. In vesicle cycling, mitochondria are essential for generating ATP, buffering calcium and enabling other functions at the synaptic terminal, and proper axonal trafficking must also be maintained. Many PD-related genes are implicated in these processes (red). α-Synuclein usually localizes to synaptic vesicles and might modulate the pool of vesicles and vesicle docking with the membrane. In PD, α-synuclein accumulates inappropriately and too robustly interferes with the priming of synaptic vesicles, which leads to a decrease in the size of the pool of releasable vesicles.

and other symptoms[71]. Mutations in *ATP13A2* lead to a reduction in the function of lysosomes and to the accumulation of abnormal lysosomes; they also sensitize cells to oxidative stress, mitochondrial dysfunction and manganese toxicity[72]. In cell models, mutations in this gene have been reported to cause the accumulation of α-synuclein, however, such accumulation was not seen in animal models that were deficient in *ATP13A2* (ref. 73). ATP13A2 might interact with synaptotagmin-11 (encoded by the gene *SYT11*), which has been implicated in lysosomal function and exocytosis[74,75] and associated by GWAS with the risk of developing PD[16]. Mutations in *ATP6AP2*, an essential accessory component of the vacuolar-type H^+ ATPase that is required for lysosome acidification and function, have been associated with an X-linked form of parkinsonism with spasticity[76]. In animal models, depletion of the ATP6AP2 protein led to neurodegeneration with evidence of defective protein degradation through the autophage–lysosome pathway[77]. Rare, autosomal recessive loss-of-function mutations in *VPS13C*, a gene encoding a component of the vesicular machinery that was specifically implicated in sorting to the lysosome on the basis of the analysis of a yeast orthologue, have been associated with familial PD[78], and common variants at this locus have also been associated with a risk of developing sporadic PD[15].

Such studies, driven by genetic associations with PD and related syndromes, point to altered trafficking to lysosomes and altered lysosomal function as potent mechanisms that underlie the loss of neurons in PD.

Endocytosis and retromer dysfunction in PD

The endocytosis of membrane proteins proceeds stepwise from the cell-surface plasma membrane, through clathrin-coat-mediated or clathrin-independent routes[79], to early endosome and late-endosome vesicles and eventually leads either to the recycling of cargo — such as through the retromer pathway to the Golgi apparatus — or to the degradation of cargo in the lysosome (Box 1). Mutations in several components of the endosome–lysosome trafficking machinery have been associated with familial forms of PD. The discovery of autosomal dominant mutations in the gene *VPS35*, which encodes a component of the retromer complex, has implicated retromer dysfunction in familial PD[80,81]. The retromer protein complex is required for the recycling of certain cargo proteins in early endosomes to the *trans*-Golgi network or plasma membrane. The cargo-selection function of the retromer complex is orchestrated by a trimer that is comprised of protein subunits VPS35, VPS26A or VPS26B and VPS29, and a dimer of sorting nexin proteins, including SNX1 and SNX2, which bind and curve endosomal membranes, presumably to enable the sequestration of selected cargo[82,83].

At a cellular level, PD-associated mutations in *VPS35* have been linked to defects in vesicular trafficking and neuronal toxicity[56,84–86]. Several retromer cargo proteins, such as the cation-independent mannose-6-phosphate receptor, are essential for the delivery of the main component enzymes of lysosomes, and retromer dysfunction therefore leads to the disruption of lysosomal trafficking and integrity[56,87], which is reminiscent of the findings seen in the context of mutations in the genes *LRRK2* and *GBA*.

When part of the multimeric retromer complex, VPS35 might function as a scaffold that binds to the complex and links it to other trafficking machinery[88], and PD-associated mutations in *VPS35* possibly disrupt these interactions. For instance, the retromer complex associates with the multicomponent WASH complex, and together these enable the sequestration of cargo in the early endosomes and its sorting to appropriate destinations. PD-associated mutations in *VPS35* have been shown to disrupt interactions of the WASH complex[84,89], leading to proteotoxic stress[89].

VPS35 and retromer dysfunction have also been connected directly to the pathological effects of α-synuclein, as a loss of VPS35 function can sensitize cells to the accumulation of α-synuclein by interfering with the degradation machinery in a range of model systems, including yeast and transgenic mice[85,90,91]. Potentiated retromer function might suppress the altered trafficking and toxicity that is associated with mutations in *LRRK2* (ref. 56) or the overexpression of α-synuclein[85], which suggests a potential therapeutic avenue.

Remarkably, at least five further genes that are associated with the endocytic pathway have been linked to PD. Three functionally related proteins — DnaJ homolog subfamily C member 13 (also known as RME-8), encoded by the gene *DNAJC13* (ref. 92), putative tyrosine-protein phosphatase auxilin (auxilin 1), encoded by *DNAJC6* (ref. 93), and cyclin-G-associated kinase (auxilin 2), encoded by *GAK*[15] — play integral parts in clathrin-mediated endocytosis at the plasma membrane by helping to uncoat clathrin, including at the synaptic terminal (Fig. 3). And the genes that encode these proteins have either been associated with familial PD (probably through loss-of-function mutations in *DNAJC13* (ref. 92) and *DNAJC6* (ref. 93) or with the risk of developing sporadic PD (through common variants in *GAK*[15]). VPS35 and RME-8 have both been shown to interact with the WASH complex[84,89], which further links the PD-associated genes that encode them to a common pathway. The gene *SYNJ1* (refs 94 and 95) encodes the protein synaptojanin-1, which is required for the shedding of clathrin adaptors in the endosomal pathway, and mutations in *SYNJ1* are linked to defects in late endosomal and lysosomal trafficking and to a familial syndrome with Parkinson's disease and seizures[94,95]. Loss-of-function mutations in the gene *RAB39B*, which encodes a Rab-family GTPase that localizes to early endosomes, have

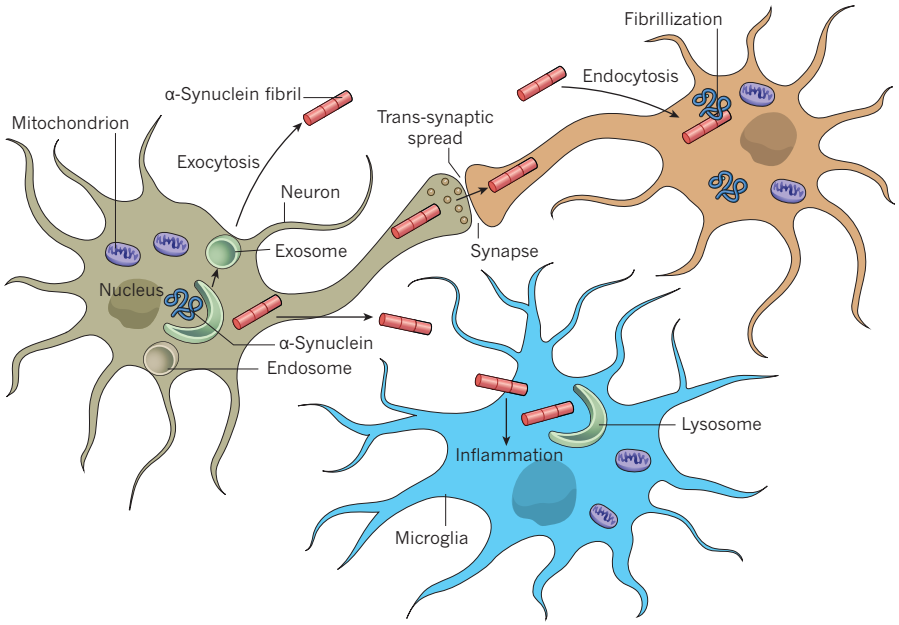


Figure 4 | Extracellular α-synuclein and the prion hypothesis. α-Synuclein can be released into the extracellular environment from neurons, especially in the context of its excessive accumulation, which can result from defective lysosomal function. Monomers, multimers or fibrillar insoluble aggregates of α-synuclein can be released through several routes, including lysosomal exocytosis, trans-synaptic spreading or the death of neurons (not shown). Extracellular fibrillar α-synuclein can then gain entry to the cytoplasm of distant neurons by directly penetrating the plasma membrane, through bulk endocytosis[134] or by other means, where it nucleates the fibrillization of other native α-synuclein molecules that are present throughout the cells. Extracellular α-synuclein might also induce inflammation through the activation of Toll-like receptors found on the surface of innate immune cells such as microglia. Innate immune cells are capable of clearing extracellular α-synuclein through phagocytosis and lysosomal degradation.

been described in familial forms of parkinsonism with cognitive impairment and α-synuclein pathology[96]. Furthermore, RAB39B was specifically implicated in the accumulation of α-synuclein in neurons. Together, these human-genetics findings point to defective endocytic trafficking and sorting, culminating in lysosomal dysfunction and defects in proteostasis, as convergent mechanisms that underlie PD or clinically related syndromes.

Important questions remain regarding the nature of the cargo with impaired transport that underlies PD. What is the normal fate of this cargo? Why does its missorting lead to lysosomal dysfunction and toxicity? And where does such cargo accumulate when trafficking is impaired? In some contexts, such as with mutations in *SCARB2* or *VPS35*, the delivery of essential lysosomal components is proposed to be selectively disrupted. Alternatively, missorted proteins might accumulate in toxic forms or cause a more general disruption in trafficking.

Synaptic trafficking is at the epicentre of PD

Postmortem studies of the brain support the idea that the generation of defects at axon processes and terminals is an early event in PD, and many PD-associated genes are implicated in synaptic function and trafficking at axon terminals (Fig. 3). α-Synuclein is localized mostly to presynaptic terminals in the normal brain and it has a regulatory function in synaptic vesicle release[50,53,97]. *LRRK2* has been specifically implicated in the presynaptic regulation of endophilin A1, which facilitates endocytosis through clathrin uncoating at the synaptic terminal[98]. VPS35 and LRRK2 both seem to be necessary for proper presynaptic function and have been implicated in the trafficking of regulatory components at the synaptic terminal[99]. It is conceivable that these proteins also modulate α-synuclein activity or localization in some contexts. Mutations in the gene that encodes TMEM230, a transmembrane protein enriched at synaptic and recycling vesicles in neurons, have been described in autosomal dominant familial PD[100].

As well as synaptic terminal dysfunction, findings from human genetics and functional studies[28] have also implicated defective axonal transport to and from synaptic terminals in PD (Fig. 3). Several GWAS have strongly linked the risk of developing PD to common variants at the *MAPT* locus, which encodes the microtubule-associated protein tau[15,101,102]. Although aggregates of tau are associated historically with Alzheimer's disease and frontotemporal dementia, the accumulation of tau has also been reported in LBs and in brains affected by PD[103].

Trafficking, spreading and inflammation

Aside from impairments in intracellular transport, transcellular mechanisms, including inflammation and prion-like spreading, have emerged as potential drivers of PD. These cell-extrinsic mechanisms might serve to propagate intracellular trafficking defects.

Prion-like spreading of α-synuclein pathology

A concept that has the potential to transform research into neurodegenerative diseases is the hypothesis that proteins linked to neurodegeneration such as α-synuclein and tau might undergo prion-like spreading[104,105]. Prions are well established as the protein-based infectious agents that underlie the transmissible spongiform encephalopathies (for example, bovine spongiform encephalopathy in cattle and Creutzfeldt–Jakob disease in humans). In these rare but devastating diseases, the major prion protein (known as PrP) converts from the normal soluble form to an aggregated, self-templating infectious form. This process initiates an inexorable spread of aggregates and contingent neurodegeneration throughout the brain. Accruing evidence suggests that this phenomenon might extend to more common neurodegenerative diseases such as PD and Alzheimer's disease.

Several studies have focused on the mechanisms of α-synuclein spreading using *in vitro* and *in vivo* model systems (Fig. 4). Notably, the spread of α-synuclein-containing aggregates to endogenous brain neurons seems to require the expression of intracellular α-synuclein: spreading was not observed in α-synuclein-knockout mice[33], which is consistent with a model in which exogenously injected fibrils spread by templating the aggregation of endogenous α-synuclein in a prion-like manner. Notably, in parallel to these α-synuclein-based models, studies focusing on other neurodegenerative proteins such as tau also point to pathological spreading as a mechanism for disease progression[106].

Key questions remain about the precise mechanisms by which α-synuclein aggregates are released from and enter neurons *in vivo*. Dysfunction in intracellular vesicular trafficking that is associated with PD might also affect the spread of α-synuclein between cells. Emerging data support this idea: for instance, mutations in *GBA* seem to promote the exocytosis of α-synuclein[107], and mutations in *ATP13A2* affect the spread of α-synuclein through exosome biogenesis[42,43] whereas those in *VPS35* might promote the endocytosis of aggregated α-synuclein[85]. It is important to emphasize that the *in vivo* model systems in which neuron-to-neuron spread of PD pathological features (for example, α-synuclein aggregates) have been reported are inherently non-physiological because they are based on the exogenous injection of α-synuclein protein. Whether such spreading actually occurs in humans and, if so, whether it has a causative role in PD, remains unresolved[108].

Inflammation is a double-edged sword in PD

Inflammation has also been implicated as a mechanism of spreading α-synuclein pathology. Although inflammatory activation in the CNS is classically induced in the context of infections, it has also been associated with neurodegenerative disorders such as PD[109]. As a first line of defence, innate immune cells in the CNS, including resident microglia and infiltrating peripheral macrophages, express pattern-recognition receptors such as Toll-like receptors that recognize evolutionarily conserved pathogen-associated molecular patterns (PAMPs)[110]. As well as pathogenic species, innate immune cells can also detect endogenous danger-associated molecular patterns (DAMPs), such as cytoplasmic or lysosomal contents that have been released into the extracellular space from injured cells or through exocytotic mechanisms[111].

Signs of inflammation have long been noted in postmortem examinations in the context of PD ; such features include morphologically altered microglia with fewer ramified processes, the induction of certain cell-surface markers and the release of inflammatory cytokines and chemokines, including interleukin-1β, tumour-necrosis factor-α and interferon-γ[109]. However, there is debate about the extent to which inflammation serves protective or disease-causing roles in PD. Innate immune cells can actively clear debris, including protein aggregates such as α-synuclein, in the context of models of PD such as glucosylceramidase insufficiency[107,112]. It has been suggested that α-synuclein might function as a DAMP in the context of PD, leading to increased inflammation[113] (Fig. 4). And if such inflammation causes the further release of α-synuclein, the process could perpetuate itself.

Innate immune cells are professional phagocytic cells with a high capacity for internalizing and degrading cell debris and protein aggregates through the lysosome pathway[45,114]. It is therefore reasonable to postulate that at the point in PD when neurons become over-burdened with proteotoxic stress, the lysosomal degradation machinery in adjoining innate immune cells can have an important supportive role[115]. However, after the lysosomal degradation machinery of even the innate immune cells has been overwhelmed by cell debris such as α-synuclein aggregates, microglia and other innate immune cells probably become over-activated and cause damage to neurons[109,116]. Almost all of the trafficking-related genes

Table 1 | Intracellular trafficking genes that contribute to PD

Gene	Familial or risk	Genetic inheritance	Protein	Proposed function of encoded protein	Trafficking steps	References	Therapeutic approaches
SNCA	Both	AD	α-Synuclein	Potential SNARE-complex assembly chaperone	Endosome–lysosome, synaptic	49, 50	Anti-α-synuclein antibody[131], α-synuclein-reducing small molecules, aggregation inhibitors
LRRK2	Both	AD	LRRK2	Kinase, GTPase	Endosome–lysosome, synaptic	60	LRRK2 kinase inhibitors[63,129]
GBA	Risk	NA	Glucocerebrosidase	Lysosomal glucocerebrosidase enzyme	Lysosome, ER, Golgi apparatus	26, 27	GBA enzyme activity enhancement[27,128]
SCARB2	Risk	NA	LIMP2	Chaperone for glucocerebrosidase trafficking	Lysosome	69	None known
ATP13A2	Familial	AR	ATPase 13A2	ATPase cation transporter	Lysosome	43, 71	None known
ATP6AP2	Familial	X	ATP6AP2	ATPase proton transporter	Lysosome	76	None known
SYT11	Risk	NA	Synaptotagmin-11	Transmembrane regulator of lysosome–autophagosome fusion and exocytosis	Lysosome	NA	None known
VPS13C	Both	AR	VPS13C	Endosomal sorting, mitophagy	Endosome–lysosome	15, 78	None known
VPS35	Familial	AD	VPS35	Retromer subunit	Endosome–lysosome, Golgi apparatus	84	Small-molecule retromer activity boosters[130]
DNAJC13	Familial	AD	RME-8	Co-chaperone function in clathrin uncoating during endosomal transport	Endocytosis, synaptic	92	None known
DNAJC6	Familial	AR	Auxilin-1	Co-chaperone function in clathrin uncoating during endosomal transport	Endocytosis, synaptic	93	None known
GAK	Risk	NA	Auxilin-2	Co-chaperone function in clathrin uncoating during endosomal transport	Endocytosis, synaptic	15	None known
SYNJ1	Familial	AR	Synaptojanin-1	Phosphoinositide phosphatase	Endocytosis, synaptic	94, 95	None known
RAB39B	Familial	X	RAB39B	Rab GTPase	Endosome	96	None known
TMEM230	Familial	AD	Transmembrane protein 230	Transmembrane secretory or recycling vesicle protein	Endosome–lysosome, synaptic	100	None known
MAPT	Risk	NA	Tau	Microtubule-associated protein	Axonal transport	15, 101, 102	None known
LAMP3	Risk	NA	Lysosome-associated membrane protein 3	Regulator of protein degradation during cellular unfolded protein response	Lysosome	16	None known
PINK1	Familial	AR	PINK1	Kinase, phosphorylates parkin and ubiquitin to regulate mitophagy	Mitochondria	37, 132	None known
PARK2	Familial	AR	Parkin	Ubiquitin ligase, regulator of mitophagy	Mitochondria	37, 132	None known
RAB29	Risk	NA	RAB7-L1	Rab GTPase	Endosome–lysosome, Golgi apparatus	56	None known

AD, autosomal dominant; AR, autosomal recessive; ER, endoplasmic reticulum; NA, not applicable; X, X linked.

associated with PD, including *GBA*, *LRRK2* and *VPS35*, have also been shown to regulate trafficking and vesicular function in innate immune cells. Therefore, disease-causing trafficking dysfunctions in PD might, in part, occur in innate immune cells, leading to proteostasis and inflammation that culminate in non-autonomous neurotoxicity.

Linking trafficking defects to neuronal loss

A remarkable number of familial and sporadic PD genes are involved, directly or indirectly, in endosome–lysosome trafficking (Table 1). Trafficking-related genes are implicated not only in rare, familial forms of PD on the basis of the identification of inherited mutations, but also in non-familial cases of PD through common variants at these genes that modify the risk of developing PD; in fact, PD genes such as *SNCA* and *LRRK2* are linked both to familial PD and to the risk of developing common, sporadic disease.

The proclivity of CNS neurons, and more specifically of midbrain dopamine neurons and their axon terminals, to develop the pathological hallmarks of PD is surprising given that the endosome–lysosome trafficking mechanisms implicated by the genetics of PD genetics are likely to affect many types of cells, and that most known PD-associated genes (Table 1) are expressed broadly. Neurons are forced to preserve homeostasis with ageing through degradation mechanisms because they do not divide, unlike most other eukaryotic cell types. Furthermore, dopamine itself might induce oxidative and nitrosative damage through the production of reactive species[35], which places dopaminegic axon terminals and their synaptic vesicles at considerable risk. Dopamine might interact directly with α-synuclein at axon terminals[117,118] to mediate its toxic effects[119].

The unique architecture of midbrain dopaminergic neurons could present a particular challenge for trafficking owing to the exuberance and vast number of their axons and synaptic terminals: for example, a single human dopaminergic neuron can harbour more than 1 million axon terminals. The onset of PD pathology therefore might be a consequence of ineffective proteostasis at axon terminals as the result of limited endosome–lysosome pathway functions. Historically, axon terminals were considered to be mostly devoid of lysosomes but several studies have challenged that view[120,121]. Interestingly, the familial-PD genes *PARK2* and *PINK1* play an essential part in the recruitment of the autophagic and lysosomal degradation machinery to defective mitochondria to initiate mitophagy at distal axons[121]. Dopaminergic neurons might also be affected by unique physiological stressors: for example, their reliance on particularly efficient types of calcium channels, including $Ca_v1.3$ L-type channels, leads to increased calcium uptake, which probably places further strain on cellular compartments such as mitochondria and lysosomes[122].

Notably, genes and pathways involved in trafficking have also been associated with other neurodegenerative disorders such as Alzheimer's disease, frontotemporal dementia and amyotrophic lateral sclerosis. This convergence points to common biological pathways in cells on which to focus future studies[123–126]. It is important to ask how genes that are implicated in a common pathway could affect seemingly distinct neuropathologies: for example, the retromer-complex genes *VPS35* and *SORL1* are associated with PD and AD, respectively. Distinct cargoes could be involved in the same pathway in different disorders, or particular cell types affected by the disorders might rely disproportionately on specific pathway-associated disease genes.

Potential therapeutic targets

Defective trafficking to the lysosome is the most prominent mechanism of pathogenesis that links PD-associated genetic variants and mutations, and it therefore has high priority as a therapeutic target. However, diverse strategies are being pursued because there is a complex interplay between the various intracellular trafficking pathways. Lysosomal storage disorders, which typically are severe, childhood inherited syndromes caused by defective lysosome function, might be mechanistically related to PD, and PD could therefore represent a mild and late-onset *forme fruste* of a lysosomal storage disorder. Given the overlap between PD and such disorders, some of the same therapeutic strategies might be applicable to both. Drugs that enhance lysosome function or trafficking, including β-cyclodextrins, or the transduction of genes such as *TFEB*, which encodes the transcription factor EB[127], might provide therapeutic benefits in the context of PD. Strategies to selectively improve the function or delivery of glucosylceramidase, including functional activators of the enzyme[27] or molecular chaperones that stabilize its structure have been considered[128], but their clinical efficacy has not been described. Because several of the most common pathogenic mutations in *LRRK2*, including G2019S, result in promiscuous LRRK2 kinase activity[54], approaches to inhibit such activity are being pursued[63,129]. However, the relevance of altered LRRK2 kinase activity to the development of PD is unresolved and, paradoxically, inhibitors of LRRK2 kinase activity have been associated with lysosomal disorders *in vivo*[63]. The emerging role of *VPS35* mutations and retromer-complex function in PD means that approaches that boost retromer activity could be a promising therapeutic avenue[130]. Improved autophagic flux, for example, through inhibition of the serine–threonine-protein kinase mTOR with rapamycin, has been evaluated, but such a strategy could be counterproductive if the main defect is downstream in the pathway at the fusion of autophagosomes to lysosomes.

A particular focus has been placed on therapies that selectively affect α-synuclein accumulation or inhibitors of its fibrillization, although the precise structure of the toxic form of α-synuclein is under debate. A therapeutic small molecule called NAB2, which might broadly suppress defects in endosomal trafficking that occur downstream of an excess of α-synuclein, has been described using a yeast-based screen[25]. Inhibitors of oxidative or nitrosative damage, which might promote toxic structures, have also been proposed[35]. Given the potential role of extracellular α-synuclein in PD — as a promoter of prion-like spreading or inflammation — antibody-based therapeutic drugs for the clearance of α-synuclein are being pursued extensively[121] (as they are for proteins involved in other neurodegenerative diseases), but their value is yet to be validated[128]. Therapeutic efforts that target α-synuclein might be broadly effective if the protein is an essential downstream effector of PD-associated neuron loss, regardless of the initial insult. Alternatively, it is possible that mechanisms that are independent of α-synuclein, and perhaps related to trafficking dysfunction, drive PD-associated neurotoxicity in most cases. ■

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