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Review Article

LRRK2 along the Golgi and lysosome connection: a jamming situation

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Parkinson's disease (PD) is an age-related neurodegenerative disorder, clinically characterized by bradykinesia, rigidity, and resting tremor. Leucine-Rich Repeat Kinase 2 (LRRK2) is a large, multidomain protein containing two enzymatic domains. Missense mutations in its coding sequence are amongst the most common causes of familial PD. The physiological and pathological impact of LRRK2 is still obscure, but accumulating evidence supports a role for LRRK2 in membrane and vesicle trafficking, mainly functioning in the endosome-recycling system, (synaptic) vesicle trafficking, autophagy, and lysosome biology. LRRK2 binds and phosphorylates key regulators of the endomembrane systems and is dynamically localized at the Golgi. The impact of LRRK2 on the Golgi may reverberate throughout the entire endomembrane system and occur in multiple intersecting pathways, including endocytosis, autophagy, and lysosomal function. This would lead to overall dysregulation of cellular homeostasis and protein catabolism, leading to neuronal dysfunction and accumulation of toxic protein species, thus underlying the possible neurotoxic effect of LRRK2 mutations causing PD.

Introduction

Leucine-Rich Repeat Kinase 2 (LRRK2) is a large, multidomain protein containing two enzymatic domains (GTPase and kinase) where many mutations linked to familial Parkinson's disease (PD) reside [1]. The most common mutation is the G2019S substitution, located in the kinase domain as the I2020T. A mutational hotspot is found in the GTPase Roc domain, with the rarer R1441G/C/H mutations. Altogether, LRRK2 mutations are amongst the most common causes of familial PD. LRRK2 mutations have an incomplete penetrance that depends on ageing and present a clinical progression similar to idiopathic PD but with pleomorphic pathology [2]. The G2019S mutation is linked to alpha-synuclein (aSyn) neuropathology in ~50% of carriers, albeit several patients present tauopathy, which could be even more relevant than Lewy bodies (LBs) [3]. This spectrum of similar pathological presentations is intriguing and muddies the waters when attempting to elucidate the underlying molecular mechanisms. Biochemically, the PD mutations in LRRK2 generally lead to an increase in kinase activity, with the G2019S presenting the clearest consensus in enhancing this enzymatic function across independent studies [4,5].

In this respect, intense research work addressed the pathways whereby LRRK2 mutations affect neuronal viability and ultimately cause neurodegeneration. This has been hampered so far by the lack of information on the physiological role of LRRK2 itself. Since its genetic discovery, LRRK2 has been implicated in a plethora of cellular and neuronal functions. LRRK2 appears to consistently regulate synaptic transmission and plasticity [6–11], with pathogenic mutations progressively impairing synaptic efficacy [12,13]. Consistent with this, a role for LRRK2 in intracellular organelle and vesicle trafficking has been emerging, with reports of function in the endosome-recycling system, (synaptic) vesicle trafficking, autophagy, and lysosome biology [14–16]. The identification of the exact neuronal function(s) of LRRK2 found an additional hurdle in the uncertainty about the substrates of its kinase
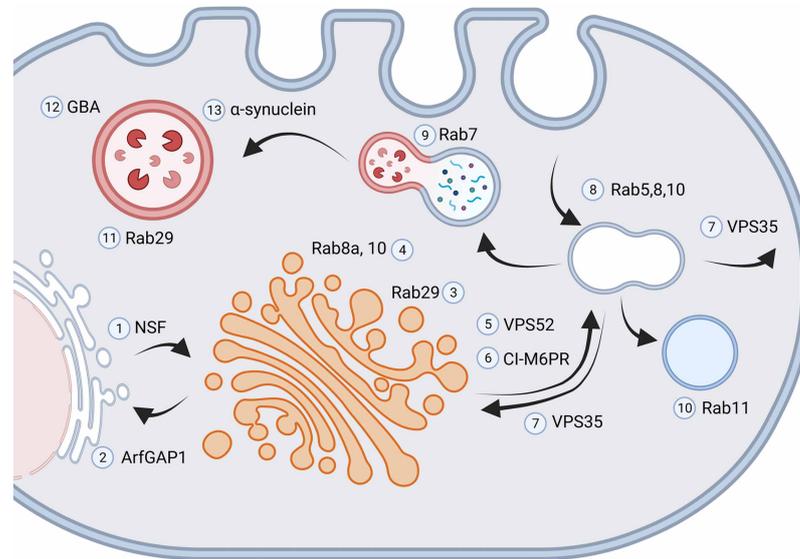
Figure 1. LRRK2 at the endolysosomal system.
LRRK2 interacts with NSF (**1**) and ArfGAP1 (**2**) and promotes ER-Golgi antero- and retrograde trafficking, respectively. Both NSF and ArfGAP1 are substrates of LRRK2 kinase activity. Genetic manipulation of NSF and ArfGAP1 affects the neurite phenotype observed in G2019S-LRRK2 models. Rab29 (**3**) localizes and activates LRRK2 at the trans-Golgi membrane. Rab29-LRRK2 complex triggers the recruitment of Rab10 and Rab8a (**4**) mediating the clearance of Golgi-derived vesicles. The expression of LRRK2 mutants affects Golgi integrity in a kinase-dependent manner. LRRK2 interacts VPS52 (**5**), component of the GARP complex that ensures proper trafficking between TGN and the endosome. The cation-independent mannose 6-phosphate receptor (CI-M6PR, **6**) delivers the lysosomal hydrolases to endosomes. LRRK2 mutations impair CI-M6PR levels and lysosomal sorting. LRRK2 functionally interacts with VPS35 (**7**), the core component of the retromer complex, to regulate endosome-to-Golgi and endosome-to-plasma membrane vesicular trafficking. LRRK2 mutations could affect retromer activity via Rab proteins, such as Rab29 or Rab7A. LRRK2 phosphorylation of Rab5, Rab8 and Rab10 (**8**) impairs the endocytosis of membrane proteins. LRRK2 influences the activity of Rab7 (**9**) and Rab11 (**10**), thus altering the trafficking of late and recycling endosomes. The Rab29-LRRK2 complex acts also at the membrane of stressed lysosomes (**11**). LRRK2 mutants impair the activation of Rab29-LRRK2 healing mechanism. LRRK2 kinase inhibition enhances the activity of the lysosomal enzyme Glucocerebrosidase (GBA, **12**). LRRK2 mutation decreases aSyn (**13**) clearance and enhances aSyn toxicity (created on BioRender.com).

activity. Many cellular targets have been proposed with different methodologies until a robust proteomic approach identified members of the Rab subfamily of GTPases as bona fide phosphorylation targets [17]. Of note, Rabs regulate protein trafficking between the plasma membrane and cellular organelles. This finding further supported a role for LRRK2 in vesicle trafficking.

In this review, we focus on the processes modulated by LRRK2, which could underlie its pathogenic role in PD. Several lines of evidence agree on the negative impact of LRRK2 PD-linked mutations on endosome and autophagy pathways, both impinging on lysosomal function. Interestingly, most intracellular trafficking events involve the Golgi apparatus. We discuss here a cell biology perspective that centers on this organelle to recapitulate the observations reported so far (Figure 1).

LRRK2 and the endolysosomal system

Accumulating evidence localizes LRRK2 within the endolysosomal system. LRRK2 is localized to endosomes of the degradative pathway, where it interacts with clathrin and modulates actin remodeling [18]. Studies in the brains of iPD subjects revealed endolysosomal pathology in nigral DA neurons, such as the accumulation of Rab5-positive early endosomes, reduction of late endosomes, and depletion of lysosomes [19,20]. Noteworthy, by using proximity ligation assays (PLA), Di Maio and colleagues proposed an increase in LRRK2 kinase activity to underlie endolysosomal alterations. Rotenone exposure in rats triggers LRRK2 activation, as measured by

this technique, and mimics the endolysosomal defects observed in iPD brains, including aSyn accumulation. The treatment with a brain penetrant LRRK2 inhibitor prevented endolysosomal pathology and DA toxicity observed upon rotenone insult [20]. Our group has obtained similar results in mice injected in the midbrain with AAV-A53T-aSyn, which causes an increase in PLA signal reflecting LRRK2 kinase activity in DA neurons (*BioRxiv 10.1101/2020.10.21.348144*). Nevertheless, additional validation from independent laboratories is warranted. Indeed, LRRK2 interacts with several proteins active along the endolysosomal pathway, such as AP3B1, ArfGAP1, N-ethylmaleimide-sensitive fusion (NSF), and the markers Rab proteins (reviewed in [21]). The genetic or pharmacological manipulation of LRRK2 kinase activity directly impacts vesicle trafficking and protein clearance, but the underlying mechanisms are not yet fully clarified. One plausible explanation may arise from the physical and functional interaction between LRRK2 and Rab proteins, such as Rab5, Rab7, Rab8a, Rab10, Rab12, and Rab29 [17,22] (and reviewed in [23,24]). Rabs orchestrate a molecular fingerprint ensuring membrane identity and act as key players for the transport between organelles through vesicles. Specific Rab proteins are active on endosome and lysosome membranes. Rab5 decorates endosomes, but when early endosomes mature into late endosomes, Rab7 replaces Rab5. Further Rab proteins, namely Rab4, Rab11, and Rab14, support the recycling pathways at early endosomes, while Rab9 supports the exchange between the Golgi and the lysosomes (reviewed in [25]).

While a clear LRRK2-dependent Rab phosphorylation has been measured in complementary LRRK2-PD rodent models [17,26–28], the link between the increased Rab phosphorylation and endolysosomal defects and, eventually, neuronal death is still missing. Three studies focusing on the trafficking of the epidermal growth factor receptor (EGFR) showed that overexpression of PD-linked mutant LRRK2 hampered endosomal trafficking of EGFR by decreasing Rab7, Rab8, and Rab10 activity [29–31]. In particular, by comparing mutants mimicking active Rab variants to specific knockdown studies, the authors concluded that pathogenic LRRK2 inhibits Rabs function via their phosphorylation and thus alters membrane trafficking events. Likely, LRRK2 impacts the endolysosomal system through interaction with, or direct phosphorylation of, several Rab proteins. Eventually, functional deficits in Rabs that regulate the late endocytic step may alter the lysosomal degradative processes.

LRRK2 and the Golgi network

Early studies demonstrated that genetic ablation of LRRK2 causes Golgi fragmentation and disrupts vesicular trafficking homeostasis in human renal proximal tubule epithelial cells [32].

Indeed, LRRK2 plays a crucial role in the Golgi compartment, where it influences integrity and vesicle trafficking. The expression of LRRK2 mutants affected Golgi integrity via a LRRK2 kinase-dependent mechanism in a number of PD-relevant models, such as immortalized cell lines, primary neuron cultures, and striatal tissue [33–36]. Recent evidence demonstrated that Rab29 (a.k.a. Rab7L1) recruits LRRK2 to specific cellular compartments, including the trans-Golgi network (TGN). The binding of Rab29 at the LRRK2 N-terminal domain boosts its kinase activity in a GTPase-dependent fashion and triggers the recruitment of Rab10 and Rab8a to the membrane of specific organelles, such as enlarged lysosomes, TGN or phagosomes [35,37–40]. However, these results have been obtained upon Rab29 overexpression. A recent study indicates that endogenous levels of Rab29 do not significantly modulate LRRK2 kinase activity [41]. Further investigations on this aspect are thus required. Of note, Rab10, Rab8a, and Rab29 themselves are substrates of LRRK2 kinase activity [34,36,38]. Phosphorylation operated by LRRK2 may stabilize the Rabs at the TGN membrane and influence their interactions with downstream targets [38]. This Rab29-LRRK2 pathway promotes the clearance of Golgi-derived vesicles through an autophagy-dependent mechanism [33]. The recently described interaction with VPS52, a member of the Golgi-associated retrograde protein (GARP) complex, brings further support to a role for LRRK2 within Golgi physiology [42]. The LRRK2-GARP complex stabilizes the interaction of VPS52 with the SNARE protein STX-6 and eventually modulates both anterograde and retrograde trafficking. ArfGAP1 acts as a GTPase-activating protein for Arf1, a cis-Golgi resident protein involved in the Golgi-ER retrograde pathway [43,44]. LRRK2 interacts with ArfGAP1 within the cytoplasm and at the membrane surface of the Golgi complex and Golgi-derived vesicles [36]. *In vitro*, ArfGAP1 sustains LRRK2 GTPase and kinase activities and acts as a LRRK2 substrate [36,45]. Genetic manipulation of ArfGAP1 affects the phenotype observed in G2019S-LRRK2 models, such as neurite shortening [36] and DA neuron loss [45]. In particular, ArfGAP1 silencing can revert the effect of G2019S-LRRK2, suggesting the occurrence of an ArfGAP1-LRRK2 mechanism underlying neuronal toxicity.

Lastly, the function of LRRK2 at the Golgi may involve the NSF protein. NSF is an ATPase that facilitates SNARE complex disassembly, thus promoting intracellular membrane dynamics [46]. NSF catalyzes vesicle trafficking within the Golgi [47] and from the endoplasmic reticulum to the Golgi [48]. Eventually, it promotes

the reorganization of the Golgi upon mitosis [49]. NSF acts as an interactor and substrate for LRRK2, which increases NSF ATPase activity *in vitro* upon phosphorylation [50] and leads to the formation of toxic aggregates that induce neurite shortening [11].

Clearly, since the Golgi apparatus sits as a major hub within the endomembrane system, its dysfunctions can affect downstream organelles and processes, such as endosome and lysosome function, synaptic vesicle trafficking and, eventually, neuronal plasticity and maintenance.

The addition of lipids and proteins to target membranes is required for neuronal dendritic growth and takes place through the Golgi (reviewed in [51]). Not surprisingly, genetic alteration of the LRRK2 interactors VPS52, ArfGAP1, and NSF affects neurite outgrowth [36,52,53]. Thus, the Golgi dysfunction observed upon expression of PD mutant LRRK2 may explain dendritic shortening. Instead, the membrane sources allowing axonal growth are less characterized. However, complementary observations suggest a role for late and recycling endosomes via mechanisms involving several Rab proteins, including the LRRK2 substrates Rab8 and Rab5 (albeit evidence is weaker for the latter; reviewed in [54]).

Lysosomes are the last step of the endocytic pathway. Internalization of extracellular and membrane proteins through endosomes culminates in the fusion of late endosomes with lysosomes, forming endolysosomes that degrade the endocytic cargo. Alterations in this pathway could critically imbalance the recycling and degradation of specific protein substrates, further progressing cellular dysfunction [14,16]. The cation-independent mannose 6-phosphate receptor (CI-M6PR, also known as IGF-IIR) delivers the lysosomal hydrolases to endosomes [55]. To execute this function, the receptor must efficiently move from the TGN to the endosomes. Beilina and colleagues brought evidence that LRRK2 mutations impair CI-M6PR levels and trafficking, along with its cargo cathepsin D, implying a lysosomal alteration that eventually resulted in DA neurotoxicity [42]. Noteworthy, postmortem analysis of frontal cortex specimens from PD patients harboring G2019S and I2020T LRRK2 mutations found a robust reduction in CI-M6PR protein expression [56].

VPS35 is a core component of the retromer complex that also includes VPS29 and VPS26. The retromer facilitates both endosome-to-Golgi and endosome-to-plasma membrane transport, thus allowing the recycling of transmembrane protein cargo [57,58]. The retromer is also crucial to target acidic hydrolases to the endosomes that eventually mature in lysosomes. Genetic evidence links VPS35 to familial PD: after the first reports describing the functional impact of the D620N mutation [59,60], other mutations located within the VPS35 gene have been identified in PD patients (reviewed in [61]). VPS35-associated PD is rare, with an estimated frequency of 0.4% of all PD cases. Structural insights suggest that the D620N-VPS35 mutation may impact VPS35 protein interactions [62]. Although the pathological cascade is still obscure, the data gathered so far are supportive for both a gain and loss of function mechanism (reviewed in [63]). LRRK2 functionally interacts with VPS35 in regulating intracellular vesicular trafficking. VPS35 overexpression rescues the sorting defects and neurite shortening observed in G2019S-LRRK2 models. Conversely, the expression of mutant LRRK2 reduces the levels of retromer proteins, including VPS35, in rodent models [39]. However, the investigation of specimens from the frontal cortex of G2019S-LRRK2 PD brains did not enlighten a substantial VPS35 reduction [64]. LRRK2 mutations could affect retromer activity via the phosphorylation of Rab proteins, such as Rab29. To further complicate the picture, D620N-VPS35 indirectly enhances LRRK2 kinase activity towards Rab8A, Rab10, and Rab12, while genetic ablation of VPS35 reduces LRRK2-dependent Rab10 phosphorylation [62]. Insights from D620N-VPS35 models suggest that retromer dysfunctions may induce neurodegeneration potentially via three main pathways: defective autophagy, disruption of neuronal receptor trafficking, and altered mitochondrial plasticity (reviewed in [65]). Accordingly, LRRK2 mutations can affect autophagy (reviewed in [66]), synaptic receptor trafficking [67–69], and mitochondrial physiology (reviewed in [70]). Thus, VPS35 and LRRK2 may participate in the same pathway and control retromer-dependent sorting to different cellular compartments, including the lysosomes. It is important to note here that the vast majority of the studies reported above employ overexpression methods, leaving the exact mechanics of the physiological conditions still to be accurately determined. Still, the data reported above suggests that alteration of Golgi-mediated protein trafficking toward the lysosome has a role in LRRK2-related PD.

The endosome and autophagy systems: convergence of LRRK2 on the lysosome

The common neuropathological feature of neurodegenerative diseases is the accumulation of intracellular protein aggregates, such as the LBs in PD [71]. The autophagy-lysosome pathway (ALP) has been causally

implicated in the onset of proteinopathies [72] and in the clearance of aSyn (the main component of LBs) [73]. Indeed, blockade of lysosome function impairs the degradation of WT aSyn [74]. Lysosome abnormalities are recognized as a feature of PD [75], and the efficacy of autophagy declines with aging [66,76], possibly contributing to the risk of neurodegeneration. The ALP is one of the processes mostly studied in the context of LRRK2 pathophysiology. Early studies localized LRRK2 to autophagic vesicles and multivesicular bodies [77]. A wealth of reports indicates a link between ALP dysfunction and LRRK2-PD onset and progression [15,66,76,78,79]. Correct autophagy function is required for neuronal survival and its impairment causes neurodegeneration, including loss of midbrain DA neurons and proteinopathy [80,81]. The alterations of autophagy due to LRRK2 mutants might underlie the pathogenic role of LRRK2 in PD. In addition, it has been reported that the impairment of chaperone-mediated autophagy (CMA) operated by mutant LRRK2 impedes the degradation of aSyn [82], providing a direct link to neuropathology. Autophagy is important for the clearance of other proteins prone to amyloid formation, including Tau [83]. Tauopathy is relatively common in LRRK2 PD, and this could indicate the protein catabolism as a common denominator for different neuropathologies. Nevertheless, it remains mysterious what factors determine the impairment in the degradation of one specific protein substrate.

Despite the flourishing of studies, controversies on the specific effect(s) of LRRK2 on autophagic flux still exist. Inhibition of LRRK2 kinase activity can stimulate the autophagic process [84]. The effects of PD-linked mutations appear more complex and might depend on several parameters, including cell type [85]. Of note, LRRK2 expression increases in activated microglia and localizes to autophagosomes. Stimulation of microglial cells induces autophagy, and LRRK2 could be involved in this process and modulate a neuroinflammatory response. In concordance, LRRK2 silencing in microglia impairs autophagy, indicating it is required for a correct autophagic response [86–88]. On the other hand, the physiological role of LRRK2 in neuronal autophagy is more complex to unequivocally define. Recent evidence indicates an increase in lysosomal activity in LRRK2 KO primary neurons [89], contrasting with previous evidence suggesting the loss of LRRK2 in animals did not affect autophagy in the brain [90].

It is generally accepted that the G2019S mutation causes abnormalities in both macroautophagy and CMA, albeit it is not clear at which step of the ALP these take place. LRRK2 might phosphorylate p62, with G2019S increasing this process and thus impacting autophagy initiation and neurotoxicity [87]. However, the regulation of phagophore biogenesis and autophagosome formation by LRRK2 is straightforward, as reviewed previously [85].

LRRK2 appears to consistently modulate lysosome function, maybe regulating the ALP downstream of its initial stages. The Rab29-LRRK2 complex described above also occurs at the lysosomal membrane. In particular, Rab29 promotes the recruitment of LRRK2 onto overloaded and thus enlarged lysosomes. The subsequent LRRK2-dependent phosphorylation and lysosomal targeting of several Rab proteins, including Rab8a and Rab10, restores the functionality of this organelle. The expression of LRRK2 mutants prevented lysosomal enlargement, thus possibly impairing the activation of the Rab29-LRRK2 healing mechanism [37]. This pathway constitutes a stress-response mechanism to attenuate the consequences of lysosomal overload [37]. However, the participation of Rab29 to this pathway has been recently queried [41]. In addition, LRRK2 inhibition promotes the activity of Glucocerebrosidase (GBA), a critical lysosomal enzyme genetically linked to PD [91]. This suggests a possible link between LRRK2 and lysosomal activity. We and others reported that G2019S-LRRK2 negatively affects lysosome activity via different mechanisms [82,91–94]. These findings indicate a functional relationship between LRRK2 and lysosomes, and suggest that LRRK2 kinase inhibition may improve lysosomal function in PD patients.

LRRK2-dependent modulation of aSyn processing

The observations that aSyn is degraded by lysosomes [95] and that LRRK2 affects autophagy and lysosome activity [82,84] sparked the exploration of a possible role for LRRK2 in aSyn handling and accumulation. Indeed, LB pathology is a prominent feature of LRRK2-PD, albeit not exclusive [96]. However, recent evidence suggested that endogenous LRRK2 may be hyperactive in idiopathic PD presenting with aSyn pathology [19], further supporting a functional interaction between these proteins. Preclinical studies first demonstrated that genetic LRRK2 deletion is protective against aSyn neurotoxicity elicited by viral overexpression [97]. The same authors later reinforced their findings showing that G2019S-LRRK2 sensitizes animals to aSyn-induced neurodegeneration and inflammation [98], which is consistent with the expression and function of LRRK2 in immune cells [87]. Early studies indicated increased aSyn neuropathology in aSyn and LRRK2 double

transgenic mice, suggesting a role for LRRK2 in regulating PD pathology and progression [99]. Unfortunately, these results were not replicated by other groups, who failed to identify regulation of aSyn pathology by varied levels of LRRK2 expression [100,101]. It is important to note that these early studies relied on the use of transgenic animal models where expression is regulated differently than the physiological situation. Indeed, the advent of more subtle modeling paradigms, such as knock-in (KI) models and aSyn pre-formed fibrils (PFFs), led to increased literature on the topic. Progressive aSyn neuropathology elicited in neuronal cultures and experimental rodents by exogenous aSyn PFFs [102,103] is worsened by G2019S-LRRK2 expression and ameliorated with pharmacological LRRK2 kinase inhibition [104]. Importantly, brain silencing of LRRK2 using an antisense oligonucleotide strongly prevented PFF-induced aSyn neuropathology in non-transgenic animals, further supporting a permissive role of endogenous LRRK2 in the onset and progression of aSyn proteinopathy [105]. In parallel, KI mouse models expressing PD-causing LRRK2 mutations at physiologic levels became available [8,106–108]. The G2019S-LRRK2 KI mice appear more sensitive to neurodegeneration and neuropathology caused by virally expressed aSyn [109]. In addition, while not showing overt PD-like pathologies, different LRRK2 KI mouse models display age-dependent accumulation of oligomeric and phosphorylated aSyn [110,111]. Indeed, the influence of LRRK2 on the activity of the lysosomal enzyme GBA and the modulation of pathologic aSyn levels supports this view [91]. Our groups also recently reported that LRRK2 modulates lysosome function in a kinase-dependent fashion with a direct impact on pS129-aSyn levels [93]. The field is constantly expanding and providing more detailed information on this complex mechanistic relationship, albeit a 'full circle' that comes back to modulation of lysosomal function seems to be preponderant [82]. Future studies will address with increased specificity the direct mechanistic link between LRRK2 kinase activity, lysosomal degradation, and accumulation of aSyn (and possibly other aggregation-prone proteins, such as Tau [3]). Indeed, LRRK2 dysfunction caused by PD-linked mutations is hypothesized to promote proteinopathy via impaired aSyn degradation. Identifying the lysosome as an important cellular substrate of LRRK2 supports the view of intracellular vesicle trafficking as a critical event in PD pathogenesis. For this reason, understanding the impact of LRRK2 mutation on the ALP appeared crucial for the development of efficacious therapies.

Perspectives

- LRRK2 is dynamically localized at the Golgi where it organizes protein complexes; LRRK2 mutations affect Golgi functionality and integrity.

- The impact of LRRK2 on the Golgi may reverberate throughout the entire endolysosomal system and occur in multiple intersecting pathways, including endocytosis, autophagy and lysosomal function.

- Lysosomes appear to be particularly sensitive to changes in LRRK2 expression and kinase activity. Impaired lysosomal degradation capacity and impaired autophagy are emerging as recurring themes in distinct PD models. Of note, lysosomes are druggable targets.

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Abbreviations

ALP, autophagy-lysosome pathway; AP3B1, adaptor related protein complex 3 subunit beta 1; ArfGAP1, ADP ribosylation factor GTPase activating protein 1; aSyn, alpha-synuclein; CI-M6PR, cation-independent mannose 6-phosphate receptor; CMA, chaperone-mediated autophagy; DA, dopamine; EGFR, epidermal growth factor receptor; GARP, Golgi-associated retrograde protein; GTPase, guanosine triphosphatase; iPD, idiopathic Parkinson's disease; KI, knock-in; KO, knock-out; LB, Lewy bodies; LRRK2, leucine-rich repeat kinase; NSF, N-ethylmaleimide-sensitive fusion; PD, Parkinson's disease; PFFs, aSyn pre-formed fibrils; SNARE, soluble NSF attachment protein receptor; STX-6, syntaxin 6; TGN, trans-Golgi network; VPS35, vacuolar protein sorting ortholog 35; VPS52, vacuolar protein sorting-associated protein 52 homolog; WT, wild-type.

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