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Invited Mini Review

# LRRK2 and membrane trafficking: nexus of Parkinson's disease

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Recent evidence from genetics, animal model systems and biochemical studies suggests that defects in membrane trafficking play an important part in the pathophysiology of Parkinson's disease (PD). Mutations in *leucine-rich repeat kinase 2* (*LRRK2*) constitute the most frequent genetic cause of both familial and sporadic PD, and LRRK2 has been suggested as a druggable target for PD. Although the precise physiological function of LRRK2 remains largely unknown, mounting evidence suggests that LRRK2 controls membrane trafficking by interacting with key regulators of the endosomal-lysosomal pathway and synaptic recycling. In this review, we discuss the genetic, biochemical and functional links between LRRK2 and membrane trafficking. Understanding the mechanism by which LRRK2 influences such processes may contribute to the development of disease-modifying therapies for PD. [BMB Reports 2019; 52(9): 533-539]

## INTRODUCTION

Neurons are long-lived cells that are not replaced during the entire lifespan of the organism, and continuous exchange of materials is essential for neuronal survival and function throughout the lifespan. In addition, neurons are the most morphologically complex cells and are highly polarized. Thus, neurons can be extremely vulnerable to disruption of intracellular trafficking, and it is no coincidence that defects in vesicle or membrane trafficking have emerged as hallmarks of most, or perhaps all, neurodegenerative diseases. The link between impaired trafficking and disease progression is more evident in Parkinson's disease (PD). A number of PD-related genes, both familial and sporadic, such as *SNCA* (a gene encoding α-synuclein), *LRRK2*, *VPS35*, *ATP13A2*, *ATP6AP2*, *GBA*, *PINK1*, *RAB29*, *RAB39B*, and *SYNJ1* can be mapped to the membrane and/or synaptic vesicle trafficking pathway (1). The motor symptoms of PD are primarily attributed to the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc). Anatomical analysis of individual dopaminergic neurons in SNc has revealed that a single dopaminergic neuron exhibits an extremely high level of arborization (average total length of an axon from a single neuron is ∼50 cm in rats) and forms a massive number of synapses (100,000-250,000 synapses per neuron in rats), which are orders of magnitude greater than those in other neurons (2, 3). The exceptional cellular morphology of dopaminergic neurons imposes a phenomenal burden on the molecular machinery that controls intracellular trafficking. Herein, we review recent genetic, biochemical, and functional studies that suggest a role of leucine-rich repeat kinase 2 (LRRK2) in intracellular vesicle trafficking. LRRK2 has also been implicated in extracellular or transcellular mechanisms, such as inflammation and disease propagation between cells, but here the focus will be on intracellular mechanisms.

## LRRK2

Mutations in *LRRK2* (also known as *PARK8*) gene constitute the most frequent cause of familial PD (4-6), and genome-wide association studies (GWAS) have linked common genetic variants in the *LRRK2* locus to sporadic PD (7). Autosomal dominant missense mutations in *LRRK2* account for 1% to 2% of all cases of PD and reach 30% to 40% in certain ethnic groups (8, 9). Familial PD cases carrying mutations in *LRRK2* manifest clinical symptoms and neuropathological features that are indistinguishable from idiopathic PD cases (10, 11). Therefore, understanding the physiological and pathological roles of LRRK2 might provide critical molecular insights into the mechanism of neurodegeneration and help the development of therapeutic strategies for both inherited and sporadic PD cases.

The *LRRK2* gene encodes a large 280 kDa protein consisting

of a serine/threonine kinase domain, Ras of complex (ROC) GTPase domain, and the C-terminal of ROC (COR) domain surrounded by multiple protein-protein interaction domains (Fig. 1). The N-terminal region contains armadillo, ankyrin, and leucine-rich repeat motifs, and the C-terminal region contains WD40 repeat domains. The presence of both the kinase and the GTPase enzymatic domains and multiple protein-protein interaction motifs suggests that LRRK2 plays diverse roles and may also act as a scaffold for the assembly of protein complexes to integrate and coordinate a wide range of cellular signaling pathways. Indeed, various studies have suggested that LRRK2 plays a role in multiple fundamental cellular processes, including mitochondrial function, autophagy, cytoskeletal remodeling, and protein synthesis (12-15).

The role of LRRK2 in the onset and progression of PD and the molecular mechanism that leads to neurodegeneration are not fully understood; however, PD-causing mutations cluster within the ROC GTPase, COR, and the kinase domains of LRRK2. G2019S mutation in the kinase domain is by far the most prevalent, known genetic cause of neurodegeneration (16). The G2019S mutation is located in the kinase activation loop and augments the kinase activity towards generic kinase substrates (17). Importantly, neurotoxicity induced by LRRK2-G2019S in culture and *in vivo* critically depends on the kinase activity (18-21). These lines of evidence suggest that the kinase activity of LRRK2 might be central to PD pathogenesis and the progression of neurodegeneration (22).

## LOCATION OF LRRK2

Endogenous LRRK2 is largely a cytosolic protein associated
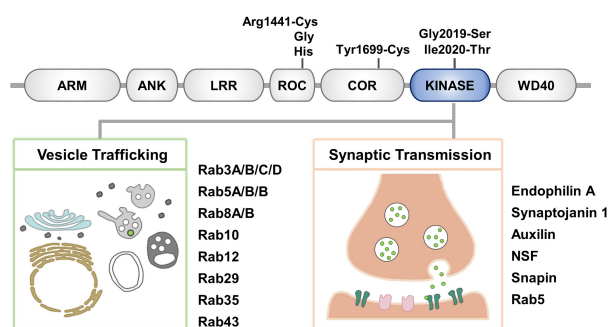


**Fig. 1.** Overview of LRRK2 and its substrates in vesicle or membrane trafficking. (*Top*) Schematic representation of LRRK2 domains, including armadillo repeats (ARM), ankyrin repeats (ANK), leucine-rich repeats (LRR), a ROC domain, a COR domain, a kinase domain, and a C-terminal WD40 domain. Pathogenic mutations are indicated above. (*Bottom*) Two categories of LRRK2 substrates discussed in this review. LRRK2 phosphorylates a subset of Rab GTPases, master regulators of vesicle trafficking (*left*), and key molecules of synaptic transmission (*right*).

with membranous structures but is generally excluded from the nucleus. Notably, LRRK2 was particularly enriched in membrane-associated fractions (23-25). At the ultrastructural level, LRRK2-positive immunolabeling was localized to Golgi apparatus, endoplasmic reticulum, mitochondria, multivesicular bodies (MVBs), and lysosomal and endosomal vesicles (23). Expression of a GFP-reporter construct in cells also located LRRK2 to a variety of different membranous compartments, including the neck of caveolae, microvilli, MVBs and autophagic vacuoles, suggesting a possible role in endolysosomal-autophagic pathway (26). A bioinformatic approach using manually-curated protein-protein interaction datasets reiterated the role of LRRK2 and LRRK2 interactome in intracellular transport and vesicular trafficking (27).

It is not clear how LRRK2 manages to associate with many different types of membranous structures. The possibility that LRRK2 binds directly to each of the membranous structures through an intrinsic affinity for lipids has not been formally excluded. However, the lack of a known membrane-spanning or membrane-targeting signal within LRRK2 together with the localization of LRRK2 in diverse vesicular or membranous structures with different lipid compositions suggest that LRRK2 might be recruited to each target membrane by interacting with specific membrane-bound proteins. This possibility is supported by the presence of several protein-protein interaction motifs in LRRK2 and is further strengthened by the identification of a growing list of membrane-associated, LRRK2-interacting proteins.

## THE ROLE OF LRRK2 IN VESICLE TRAFFICKING

In addition to the localization of LRRK2 to multiple membranous compartments (23), mounting evidence suggests that regulation of vesicular transport is a proximal event in LRRK2 signaling. LRRK2 has been implicated in the regulation of autophagy, lysosomal maturation and trafficking, and the endosomal pathway (28, 29).

Rab GTPases are the largest subgroup of the Ras-like GTPase superfamily and comprise more than 60 members in the human genome. Rab proteins reside in specific membranous compartments and are master regulators of vesicle trafficking, controlling virtually all aspects of vesicle or membrane traffic (30-32). By cycling between GTP-bound and GDP-bound states, Rab proteins function as molecular switches and orchestrate the formation, maturation, transport, tethering, and fusion of vesicles. Once the Rab protein is inserted into its respective target membrane, a guanine nucleotide exchange factor (GEF) mediates the conversion from the GDP- to the GTP-bound form. The active, GTP-bound Rab recruits effector proteins that specifically control certain aspects of trafficking and is hydrolyzed back to its inactive state, a process which is facilitated by a GTPase-activating protein (GAP). GDP-bound Rab becomes a substrate of GDP dissociation inhibitor (GDI), which extracts Rab in its GDP-bound state from the

membrane. Rab bound to GDI can then be reinserted into a membrane to start the cycle again.

Many lines of evidence have suggested physical, genetic and functional interactions between LRRK2 and several members of the Rab GTPases (29, 33-36). In yeast-two-hybrid screening, LRRK2 has been shown to interact with Rab5, Rab32 and Rab38 (34, 37). The *Drosophila* homolog of LRRK1 and LRRK2, *dLRRK* interacts with Rab7 and a mutation in dLrrk corresponding to the G2019S mutation disturbs Rab7-dependent perinuclear localization of the lysosome (29).

Recently, we and others have identified a subset of Rab GTPases as authentic substrates of LRRK2 (38-40). Steger *et al.* performed large-scale phosphoproteomic screens using fibroblasts from knock-in mice carrying either the LRRK2-G2019S mutation or the kinase inhibitor-resistant LRRK2-A2016T mutation, which led to the identification of a single substrate of LRRK2, Rab10. Further analysis in HEK293FT cells with group I Rabs showed that LRRK2 also phosphorylated Rab1b and Rab8a (39). In a following study, Rab3a/b/c/d, Rab5a/b/c, Rab8a/b, Rab10, Rab12, Rab29, Rab35 and Rab43 were phosphorylated by LRRK2 (41). By performing *in vitro* LRRK2 kinase assays with forty-five human Rab GTPases, we also found that Rab1a, Rab3c, Rab8a, and Rab35 were phosphorylated by LRRK2 at a conserved Thr residue in the switch II motif, which is known to mediate the binding with interactors responsible for vesicle transport (38). Moreover, we showed that the phosphorylation of Rab1a, Rab3c, Rab8a, and Rab35 was enhanced by LRRK2-G2019S compared with wild-type LRRK2, while it was decreased in *Lrrk2* knockout mice and in cells treated with LRRK2 kinase inhibitors (38). Importantly, dysregulation of Rab phosphorylation in the LRRK2 site induced neurotoxicity in primary neuronal cultures. Furthermore, intracranial injection of the adeno-associated virus (AAV) expressing either nonphosphorylatable (AAV-Rab35-T72A) or phosphomimetic (AAV-Rab35-T72D) mutant of Rab35 into the SN induced substantial degeneration of dopaminergic neurons *in vivo* (38).

Among LRRK2 substrates, *Rab29* (also known as *Rab7L1*) is one of the five genes located in the *PARK16* locus linked to PD (42). Previously, an unbiased search for interactors of LRRK2 and a brain transcriptomics approach found that LRRK2 interacted with Rab29, and dysfunction of LRRK2-Rab29 signaling caused dysregulation of the autophagy-lysosome system (26, 33, 35, 43). More recent studies have suggested that Rab29 recruits LRRK2 to the *trans*-Golgi network (TGN) and enhances its kinase activity, leading to the phosphorylation of other LRRK2 substrates, Rab8 and Rab10 (40, 44). Rab29 has also been shown to recruit LRRK2 to stressed lysosomes, which, in turn, induced lysosomal accumulation of other LRRK2 substrate Rabs, such as Rab3, Rab8, Rab10, and Rab35 (45). It will be interesting to investigate if recruitment of LRRK2 to discrete membranous compartments by specific Rab proteins applies more generally to tether and activate LRRK2 at distinct subcellular locations

and enable phosphorylation of a specific subset of substrates.

The first gene identified in familial PD was *SNCA*, which encodes a synaptic vesicle-associated protein, α-synuclein. Aggregates of α-synuclein are major constituents of Lewy bodies and Lewy neurites, which are cardinal pathological features of PD. Several Rab GTPases have been identified as modulators of α-synuclein toxicity, and notably, the same Rab proteins are also implicated in LRRK2 signaling. Aggregation of α-synuclein in long-term cultures of human induced pluripotent stem cell (iPSC)-derived dopamine neurons resulted in lysosomal dysfunction, and upregulation of Rab1a, a substrate of LRRK2, could restore protein trafficking and lysosomal function (46). These results are reminiscent of the ability of Ypt1 (the yeast homolog of Rab1) to alleviate impaired ER-to-Golgi transport caused by overexpression of α-synuclein in yeast (47). Moreover, Rab1, Rab3a and Rab8a, all of which are LRRK2 substrates, prevented α-synuclein-induced degeneration of dopaminergic neurons in a nematode model of PD and in rat primary midbrain cultures (48). Furthermore, a previous study showed that α-synuclein from A30P α-synuclein transgenic mice (Tg5093) interacted with Rab3a, Rab5, and Rab8 (49). Vesicular compartments and trafficking events regulated by these Rab proteins may represent intracellular locations or pathways where the interplay between α-synuclein and LRRK2 take place.

## THE ROLE OF LRRK2 IN SYNAPTIC VESICLE RECYCLING

Neurons undergo high rates of synaptic transmission, which requires efficient membrane trafficking at the presynaptic axon terminal to recover and recycle membranes that have fused with the plasma membrane during neurotransmitter release. Postmortem studies of PD brains support the notion that synaptic dysfunction is an early event in PD (1), and increasing lines of evidence suggest that LRRK2 plays a role in clathrin-dependent endocytosis and recycling of synaptic vesicles (Fig. 2). Clathrin-dependent synaptic vesicle endocytosis (SVE) is initiated by recruiting clathrin at the plasma membrane via adaptor protein 2 (AP-2). Clathrin-coated vesicle (CCV) then invaginates from the plasma membrane by the actions of proteins that sense and induce membrane curvature, such as endophilin. CCV then physically separates from the plasma membrane by the action of the GTPase dynamin. Shedding of the adaptors depends on phosphatidyl inositol hydrolysis by synaptojanin, and the ATPase Hsp70 and its cofactor auxilin remove the clathrin lattice after fission, and thereby, produce synaptic vesicles that can be reused (50).

LRRK2 has been suggested to play a role in synaptic vesicle recycling via phosphorylation of endophilin A1, an essential molecule involved in clathrin-mediated SVE (51). Endophilin contains an N-terminal amphipathic α-helices followed by the Bin/amphiphysin/Rvs (BAR) domain and a C-terminal SH3 domain. Endophilin promotes early steps of SVE by generating
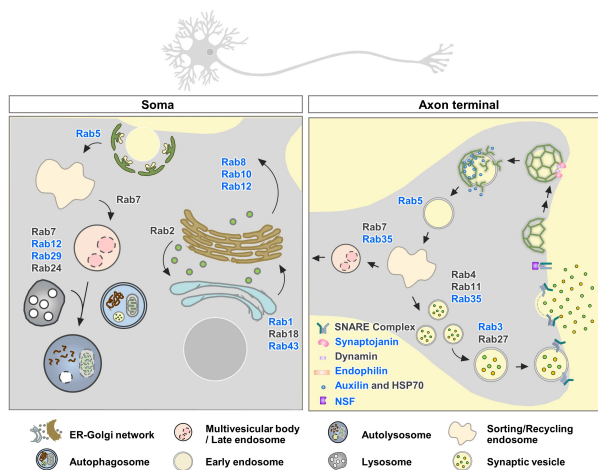
**Fig. 2.** LRRK2 substrates involved in vesicle trafficking and synaptic transmission. LRRK2 phosphorylates a subset of Rab GTPases that control the endolysosomal-autophagy pathway and key molecules involved in synaptic vesicle endocytosis and recycling. Proteins that are suggested as direct substrates of LRRK2 are depicted in blue.

membrane curvature at the plasma membrane and facilitates later steps via clathrin-uncoating (52-55). Membrane curvature can be generated by (i) the BAR domain, which forms a crescent-shaped dimer that interacts with and forces membranes to conform to their own intrinsic protein shape (scaffolding mechanism) and (ii) the amphipathic α-helices, which embed in the lipid bilayer and thereby generate a wedging force (wedging mechanism) (56). It has been suggested that the generation of vesicles and tubes requires different structures and mechanisms of endophilin A1 and the transition between the two membranous structures can be regulated via LRRK2-mediated phosphorylation (56). The LRRK2 phosphorylation site S75 is shallowly inserted on vesicles but is deeply embedded upon tubulation. In contrast to wild-type and the nonphosphorylatable mutant (S75A) of endophilin A1 that increased tubulation, the phosphomimetic mutant (S75D) destabilized tubulation and favored vesiculation by preventing deep insertion of the helical wedges (56). Therefore, it is possible that LRRK2-mediated phosphorylation of endophilin A1 regulates how endophilin structurally interacts with and curves the membrane. This function might play a role at the endocytic neck, a structure which is transiently generated and rapidly destabilized. It remains to be determined whether pathogenic LRRK2 mutants via its aberrant kinase activity disrupt the balance of different types of membrane curvature.

In addition to generating membrane curvature at early stages of SVE, endophilin recruits dynamin and synaptojanin (57, 58). Synaptojanin-1 has been identified as a substrate of LRRK2 via phosphoproteomic analysis of *Drosophila* brains over-

expressing human LRRK2-R1441C (59). Synaptojanin-1 is a lipid phosphatase, dephosphorylating phosphatidylinositol 4,5-bisphosphate ($PIP_2$) to phosphatidylinositol 4-phosphate and to phosphatidylinositol via its dual phosphatase activity. Activation of synaptojanin-1 releases AP-2 from CCVs and promotes synaptic vesicle uncoating during endocytosis. The LRRK2 phosphorylation sites, T1131 and S1142 in synaptojanin are located within the C-terminal proline-rich domain, which is known to mediate the binding with the SH3 domain of endophilin A. Therefore, LRRK2-dependent phosphorylation of synaptojanin-1 may modulate the interaction with endophilin, and accordingly endophilin-dependent SVE. It is also possible that the enzymatic activity of synaptojanin-1 is regulated by phosphorylation. A functional consequence of synaptojanin-1 phosphorylation by LRRK2 has yet to be determined.

After fission, auxilin induces clathrin uncoating of the CCVs by recruiting the ATPase Hsc70. The pathological linkage between *DNAJC6* (auxilin) and PD has been suggested through GWAS studies in patients with juvenile and early-onset atypical parkinsonism (60, 61). In iPSC-derived dopaminergic neurons obtained from reprogrammed PD patient fibroblasts harboring the LRRK2-R1441C mutation, the levels of several SVE proteins, such as auxilin, AP-2, endophilin A1, dynamin 1, and synaptojanin were reduced, and activity-dependent endocytic capacity was also decreased when compared with those derived from healthy controls (62). In addition, LRRK2 physically interacted with auxilin and induced the phosphorylation of auxilin at S627 residue located in the clathrin-binding domain. LRRK2-mediated phosphorylation of auxilin has thus been suggested to control auxilin and clathrin binding and the CCV uncoating process during SVE. In the LRRK2-R1441G patient-derived dopaminergic neurons, synaptic vesicle density was reduced and cytosolic dopamine levels were elevated (62). In the synaptic terminal, dopamine should be rapidly packaged into synaptic vesicles. Accumulation of cytosolic dopamine can be oxidized and reach toxic levels. The accumulation of oxidized dopamine, increased α-synuclein levels, and decreased lysosomal glucocerebrosidase activity in the LRRK2-R1441G patient-derived dopaminergic neurons could all be suppressed by overexpressing wild-type auxilin, suggesting that LRRK2-mediated auxilin dysfunction disrupts SVE and generates toxic, oxidized dopamine, which might contribute to dopaminergic neurodegeneration in LRRK2-linked PD (62).

In addition to SVE, LRRK2 is involved in synaptic vesicle fusion by regulating the SNARE complex dissociation via phosphorylation of NSF (63). Vesicular SNARE (v-SNARE) located on the synaptic vesicle membrane and the target SNARE (t-SNARE) present on the plasma membrane assemble into a highly stable alpha-helical SNARE complex that juxtaposes the two membranes together to catalyze membrane fusion. After synaptic vesicle fusion, the SNARE complex is dissociated for reuse. At the transition period of exocytosis and

endocytosis, the disassembly of SNARE complex is initiated by the formation of SNARE-SNAP-NSF complex, followed by NSF-catalyzed ATP hydrolysis and release of individual SNAREs (64). LRRK2 phosphorylates NSF at T645 located within the D2 domain of NSF. T645 is predicted to be a key residue for protein oligomerization, which regulates the ability of the D1 domain to hydrolyze ATP (65). LRRK2 phosphorylation of NSF enhanced the ATPase activity of NSF and increased the rate of SNARE complex disassembly *in vitro* (63).

Snapin, known as SNAP-25 (synaptosomal-associated protein 25) binding protein, is another LRRK2 kinase substrate (66). SNAP-25 is a core component of the SNARE complex. Inhibition of the interaction between snapin and SNAP-25 blocks the association of SNARE complex with synaptotagmin. Snapin interacts with multiple vesicle trafficking-related proteins, and calcium-dependent exocytosis is impaired in snapin null mice (67). It has been shown that LRRK2 phosphorylates snapin at T117. The phosphomimetic snapin-T117D decreases its interaction with SNAP-25 and prevents the association of synaptotagmin with SNARE complex and reduces the number of readily releasable pool of synaptic vesicles. These results suggest that LRRK2 may be involved in neurotransmitter release.

## CONCLUSION

Analysis of postmortem human brain tissue from PD brain donors using correlative light and electron microscopy (CLEM), serial block-free scanning electron microscopy (SEFSEM), and stimulated emission depletion (STED)-based super resolution microscopy showed that α-synuclein immunopositive inclusions were crowded with fragmented membranes, organelles and vesicular structures (68), supporting the notion that defects in membrane trafficking are a potential driver of pathogenesis of PD. Here we have discussed that intracellular localization of LRRK2, the effects of *LRRK2* mutations, and the newly identified substrates of LRRK2 together strengthen the possibility that vesicular trafficking is a proximal event of LRRK2 signaling. Although our understanding of LRRK2 function in membrane trafficking has greatly advanced in recent years, much remains to be learned. Understanding of how LRRK2 and PD-linked gene mutations affect membrane trafficking and how defects in vesicular or synaptic trafficking cause dopaminergic dysfunction will not only advance the field of membrane trafficking but also may have strong implications for developing disease-modifying treatments for PD.

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## CONFLICTS OF INTEREST

The authors have no conflicting interests.

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