Alterations in late endocytic trafficking related to the pathobiology of LRRK2-linked Parkinson's disease

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Abstract

Mutations in the *leucine-rich repeat kinase 2 (LRRK2)* gene comprise the most common cause of familial Parkinson's disease (PD), and variants increase the risk for sporadic PD. LRRK2 displays kinase and GTPase activity, and altered catalytic activity correlates with neurotoxicity, making LRRK2 a promising therapeutic target. Despite the importance of LRRK2 for disease pathogenesis, its normal cellular function, and the mechanism(s) by which pathogenic mutations cause neurodegeneration remain unclear. LRRK2 seems to regulate a variety of intracellular vesicular trafficking events to and from the late endosome in a manner dependent on various Rab proteins. At least some of those events are further regulated by LRRK2 in a manner dependent on two-pore channels (TPCs). TPCs are ionic channels localized to distinct endosomal structures and can cause localized calcium release from those acidic stores, with downstream effects on vesicular trafficking. Here, we review current knowledge about the link between LRRK2, TPC- and Rab-mediated vesicular trafficking to and from the late endosome, highlighting a possible cross-talk between endolysosomal calcium stores and Rab proteins underlying pathomechanism(s) in LRRK2-related PD.

Introduction

Parkinson's disease (PD) is a prominent neurodegenerative disorder without a cure. Mutations in leucine-rich repeat kinase 2 (LRRK2) comprise the most common known genetic cause of PD, causing clinical features similar to late-onset sporadic PD [1]. In addition, sequence variations at the LRRK2 locus are associated with an increased risk for developing sporadic PD [2,3], thus implicating LRRK2 in both disease entities. Penetrance of LRRK2 mutations for PD is age-related and incomplete, such that genetic and/or environmental influences probably further modulate mutant LRRK2 toxicity.

LRRK2 is a large ubiquitous protein containing N-terminal and C-terminal protein interaction domains, and a central region composed of a Ras-of-complex protein (ROC) GTPase, C-terminal of ROC (COR) and kinase domain, respectively (Figure 1). Point mutations in the GTPase and kinase domains of LRRK2 segregate with familial disease in an autosomal-dominant manner, indicating that these enzymatic activities are important for disease development (Figure 1). Indeed, the most common pathogenic mutation G2019S has been consistently shown to increase the kinase activity of LRRK2 [4], and kinase activity, possibly with concomitant downstream effects on protein stability, appears crucial for LRRK2-mediated toxicity [5,6]. Since efficient and rather specific pharmacological kinase inhibition is feasible, this finding has spurred great hope for novel PD drug development approaches. However, the effects of other pathogenic LRRK2 mutations on kinase activity are less clear [4] and leave open the possibility of kinase-independent mechanisms underlying LRRK2 toxicity.

Indeed, LRRK2 also displays low levels of intrinsic GTPase activity, which is reduced by some pathogenic mutants, implicating altered GTPase activity in LRRK2 pathology. If altered GTPase activity, or indeed GTP binding, impinge back on to the kinase activity, the latter may be the common output of the various pathogenic LRRK2 mutations [7–9]. Alternatively, kinase activity may regulate GTPase activity, possibly via autophosphorylation of the ROC domain, such that alterations in GTPase activity may cause LRRK2 toxicity [10]. LRRK2 activity also seems to be further regulated by various upstream kinases, GTP-binding proteins or GTPases [8,11], highlighting a complex interplay

Key words: autophagy, endocytosis, leucine-rich repeat kinase 2 (LRRK2), nicotinic acid adenine dinucleotide phosphate (NAADP), Parkinson's disease, Rab protein.

Abbreviations: COR, C-terminal of ROC; GAP, GTPase-activating protein; GDI, guanine nucleotide dissociation inhibitor; GEF, guanine nucleotide exchange factor; LRRK2, leucine-rich repeat kinase 2; NAADP, nicotinic acid adenine dinucleotide phosphate; PD, Parkinson's disease; ROC, Ras-of-complex protein; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; TGN, *trans*-Golgi network; TPC, two-pore channel.

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between kinase and GTPase activities, and a current lack of our understanding as to the enzymatic readout of the various LRRK2 mutants.

Even though the distinct pathogenic LRRK2 mutants may produce variable effects on LRRK2 biochemistry, all of them converge on the same cellular endpoint, namely degeneration and death of dopaminergic neurons. Common molecular mechanisms which cause deficits in early cellular events have been reported in some, but not all studies where various pathogenic LRRK2 mutants have been compared side-by-side [12–15]. Studies of this type indicate that pathogenic LRRK2 alters a variety of intracellular vesicular trafficking steps including endocytosis, autophagy, retromer-mediated trafficking from late endosomes to the *trans*-Golgi complex, lysosome size/positioning and Golgi integrity [12,15–20] (Figure 2). Thus, LRRK2 may modulate a variety of targets, localized to different intracellular organelles and involved in distinct membrane trafficking events. Alternatively, as intracellular vesicular trafficking events are highly interconnected and interdependent, the large spectrum of observed changes may be due to a small number of LRRK2-mediated alterations. In either case, changes in intracellular vesicular trafficking events seem to lie at the heart of mechanisms underlying PD, as the products of various other genes, mutations in which cause PD, are also involved in this process [21,22].

The pleiotropic effects of LRRK2 on membrane trafficking are reminiscent of the multiple effects of calcium on various distinct intracellular vesicular membrane transport steps [23]. Interestingly, calcium dyshomoeostasis, promoted by the specific and shared physiological features of neurons at risk of death in PD, has been proposed to underlie disease pathomechanism [24–26]. Although little is known about the possible link between LRRK2 and calcium dyshomoeostasis [27], our recent studies suggest possible LRRK2-mediated alterations in endolysosomal calcium handling which may have an impact upon various vesicular membrane trafficking steps to and from late endosomes [18]. Here, we summarize recent data on the role of LRRK2 in membrane trafficking, and possible mechanistic links related to altered calcium handling in endolysosomal stores.

LRRK2 function at the late endosome

The late endosome acts as a major intracellular trafficking station, receiving input from the endocytic, autophagic as well as exocytic pathways [28] (Figure 2). For example, during endocytosis, vesicles bud off from the plasma membrane to fuse with early endosomes, followed by sorting to the late endosome and the lysosome for subsequent degradation, or recycling back to the cell surface. Similarly, autophagosomes can fuse with late endosomes to generate amphisomes before intraluminal content is finally degraded in lysosomes. The late endosome receives further input from the exocytic pathway via delivery of proteins and enzymes from the *trans*-Golgi network (TGN) which are destined for the lysosome, and such receptor-mediated delivery is followed by recycling of the receptors back to the TGN in a process requiring the action of a protein complex termed retromer.

LRRK2 is localized to the cytosol, as well as to a variety of intracellular structures including endosomes, lysosomes, autophagosomes, and the TGN, consistent with a role in endomembrane trafficking [29–31]. Under conditions which enhance flux through the endocytic system, both overexpressed as well as endogenous LRRK2 can display pronounced colocalization with late endosomes [15,32]. However, the molecular trigger(s) which cause such relocalization, and the targets and mechanisms of action of LRRK2 at the late endosome remain largely unknown.

A number of distinct proteomics approaches indicate that LRRK2 can interact with cytoskeletal components, with proteins implicated in early as well as late endomembrane trafficking steps [33], and with small GTPases including Rac1 [34], Rab7L1/Rab29 [12,16], Rab5b [35], Rab7 [19], Rab9 [18], Rab32 and Rab38 [36]. Although such large array of reported interactions complicates our understanding of LRRK2 function, it may reflect the ability of LRRK2 to modulate distinct vesicular trafficking events dependent on the precise cellular context, such as changes in autophagic or endosomal flux.

LRRK2, calcium, TPCs and late endosomal trafficking

Studies from our laboratory indicate that LRRK2 may interact with TPCs (two-pore channels) [20], especially under conditions which favour autophagic flux. TPCs are a family of cation channels which reside in endolysosomal organelles, with TPC1 important for trafficking from endosomes to the Golgi, and TPC2 for trafficking from endosomes to lysosomes, respectively [37]. Most work indicates that TPCs are activated by the calcium-mobilizing molecule nicotinic acid adenine dinucleotide phosphate (NAADP) [38,39], which triggers calcium release from acidic stores. Interestingly, the observed LRRK2-mediated alterations in autophagy could be mimicked by NAADP and reverted by Ned-19, an NAADP antagonist, further highlighting a functional link between TPCs and LRRK2 [20]. The mechanism by which LRRK2 may modulate TPC function remains to be determined. Multiple scenarios are possible, including direct regulation of TPCs by LRRK2-mediated phosphorylation, or modulation of interactors which regulate TPC activity. Indeed, TPC2 is known to be regulated by multiple protein kinases, as well as by phosphatidylinositol 3,5-bisphosphate, a phosphoinositide known to play important roles in regulating both late trafficking events along the endocytic system as well as retrograde transport from late endosomes to the TGN. In addition, NAADP does not directly bind to TPCs, but to currently unidentified small molecular-weight proteins which then modulate channel action [40]. Thus, LRRK2 may

Figure 1 | **Domain structure of LRRK2**

Full-length LRRK2 is indicated with its distinct domains, and clearly pathogenic mutations indicated above. ARM, armadillo repeats; ANK, ankyrin repeats; COR, C-terminal of ROC domain; LRR, leucine-rich repeats.

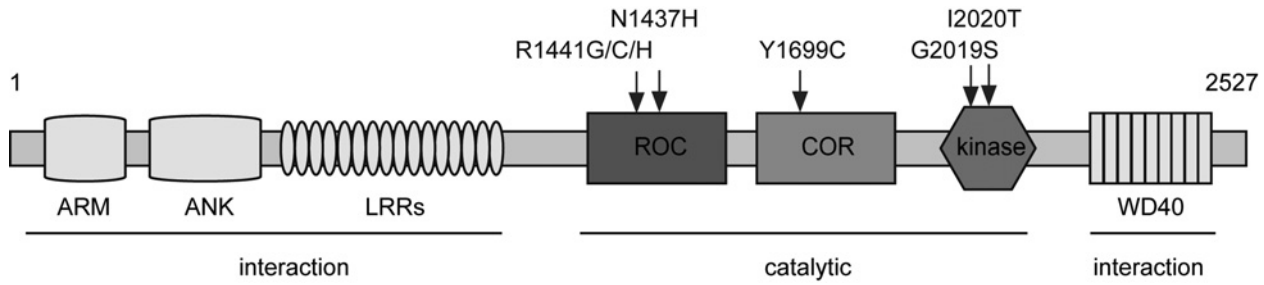
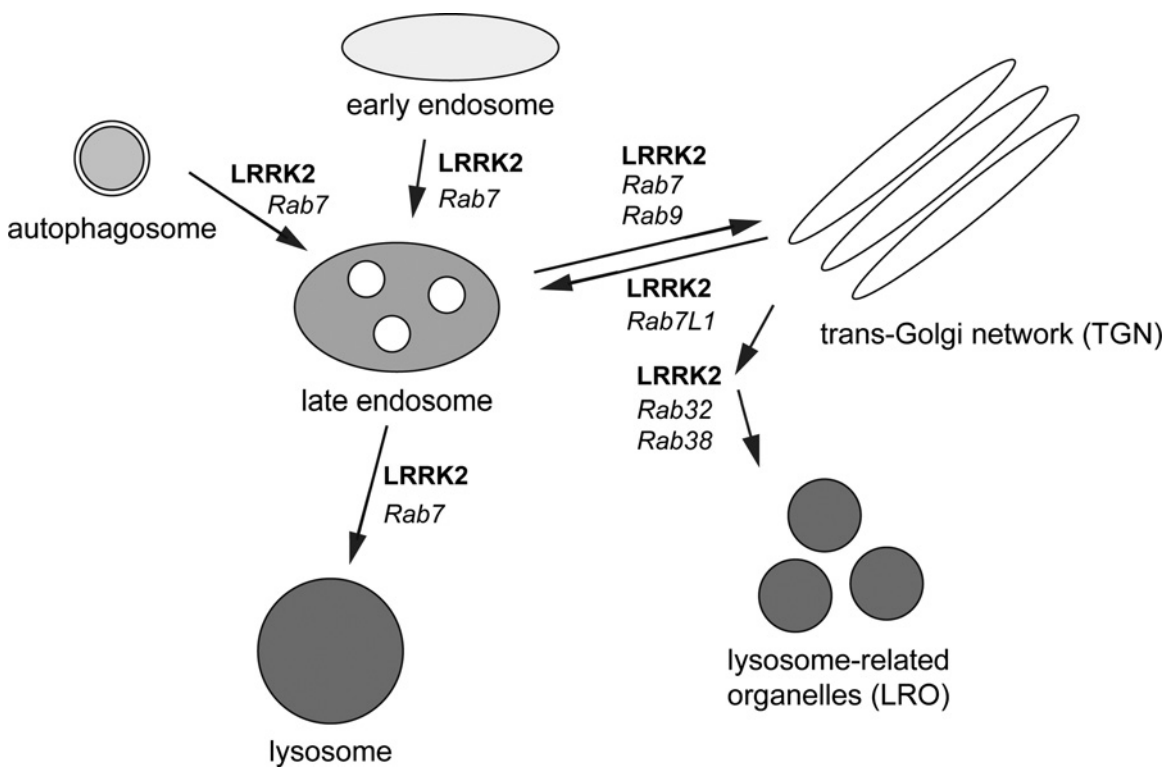


Figure 2 | **Schematic intracellular membrane trafficking pathways altered by LRRK2**

LRRK2 has been shown to regulate vesicular trafficking from early to late endosomes, fusion of autophagosomes with endosomes and/or lysosomes, trafficking from late endosomes to lysosomes, lysosome biogenesis and maintenance, retromer-mediated trafficking between the late endosome and the TGN, integrity of the TGN, and possibly the generation of lysosome-related organelles. The Rab proteins implicated in the various vesicular trafficking steps are indicated in italics.



directly regulate TPC2 action, or indirectly through effects on phosphoinositides, NAADP binding proteins or other proteins which regulate TPC2 functioning. In either case, the link between LRRK2 and TPCs highlights the possibility that alterations in intracellular calcium handling around acidic stores, with downstream effects on late endosomal membrane trafficking, may contribute to LRRK2 pathophysiology.

Focally localized, transient calcium signals are important for various intracellular membrane fusion events. Upon Rab-mediated tethering interactions between vesicles and target membranes, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) bridge the opposing membranes to mediate their fusion. Calcium action can be superimposed on to this molecular mechanism to regulate at least some constitutive membrane trafficking events [23]. It may perform a triggering role initiated by intrinsic signalling events, or a more general biophysical role, possibly along with other divalent cations such as magnesium, to

favour membrane bending [41]. In either case, at least some intracellular vesicular trafficking steps seem to be regulated by intraluminal calcium levels, including late endosome-lysosome transport [42] and retrograde transport from late endosomes to the TGN [43], and these are precisely the trafficking events regulated by TPCs as well as by LRRK2. How calcium would act to modulate such trafficking events remains unclear, but one attractive hypothesis is that adequate vesicle tethering and/or docking may result in proper functional coupling to TPCs, triggering luminal calcium release and subsequent vesicle fusion. Interestingly, TPCs have been found to interact with syntaxin 16, a SNARE protein involved in retrograde transport from endosomes to the TGN [44]. In addition, TPCs interact with Rab7a, and TPC2 has been found to require Rab7a activity and interactivity for proper functioning [44]. These findings are in agreement with a model whereby proper vesicle tethering/docking may trigger subsequent luminal calcium release to bring about late endosome-derived vesicular fusion events.

LRRK2, Rab proteins and the late endosomal trafficking

Rab proteins are small GTPases localized to the cytoplasmic surface of specific subcellular compartments across the exocytic and endocytic pathways. There are more than 60 members of the Rab family, and they play important roles in distinct trafficking pathways in a cell type-specific manner. Because Rab proteins do not have high intrinsic guanine nucleotide exchange or hydrolysis activities, they are regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). In their GDP-bound state, Rabs are soluble and bound to guanine nucleotide dissociation inhibitor (GDI). At the acceptor membrane, the Rab–GDI complex interacts with GDI displacement factor, which removes GDI and allows Rab membrane insertion. A GEF then converts the Rab to its GTP-bound, active conformation, allowing it to interact with downstream effectors to control various aspects of membrane trafficking, and upon GAP-assisted GTP hydrolysis, Rabs are extracted from the bilayer by GDI [45]. Thus, GEFs and GAPs act in concert to establish restricted subcellular distributions for each particular active Rab form, serving as landmark for the recruitment of proteins which need to act in this location. Several Rab proteins can be present on a given organelle at a given time, consistent with the idea that organelles display distinct membrane domains co-ordinated by Rabs. At the same time, several multivalent effectors that link multiple Rab GTPase cascades have been discovered, indicating that while Rabs may confer specificity to distinct vesicular trafficking pathways, they may share downstream effectors to bring about similar intracellular events. To understand how Rab proteins can display distinct, but at times also overlapping binding preferences for effector and activator proteins, they have been divided into distinct subfamilies either based on sequence, or a combination of sequence, structure, and differences in electrostatic potentials and hydrophobic fields [46]. Interestingly, based on the latter analysis, Rab7a, Rab7L1, Rab32 and Rab38 are all part of the same Rab subcluster, and thus may share binding partners like GEFs, GAPs and effector proteins. Indeed, all these Rab proteins have been reported to interact with LRRK2.

Interestingly, most Rab proteins currently described to interact with LRRK2 regulate vesicular transport steps to and/or from the late endosome (Figure 2). For example, Rab7a regulates late endocytic transport, is required for the progression of the autophagic pathway, for the biogenesis and maintenance of lysosomes, and may also contribute to retromer-mediated trafficking between the late endosome and the TGN [47]. Rab9 and Rab7L1/Rab29 play a role in transport of the mannose 6-phosphate receptor between the late endosome and the TGN [48], and Rab32 and Rab38 regulate the biogenesis of lysosome-related organelles [49]. Together, these findings highlight the important role for LRRK2 in late endosome function, possibly in a Rab-dependent manner.

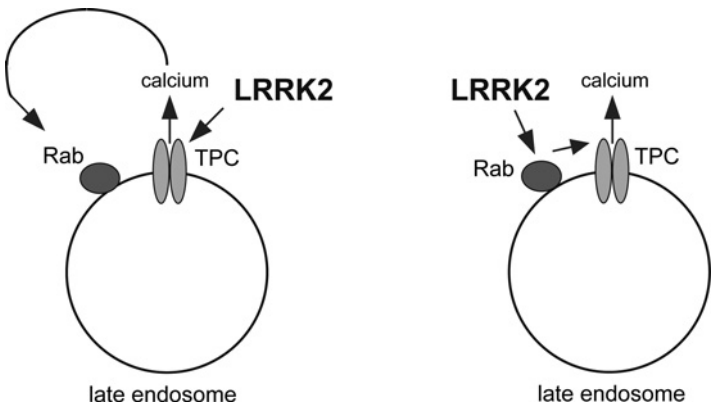
How LRRK2 may regulate the function of the various Rab proteins remains to be determined. At least for Rab7a, our recent studies indicate that distinct pathogenic LRRK2 mutants cause a decrease in Rab7a activity [15]. It will be interesting to determine whether this is due to direct phosphorylation of Rab7, or to LRRK2-mediated alterations in Rab7 GEFs or GAPs. In addition, future studies will be necessary to determine whether the other LRRK2-interacting Rab proteins also display decreased GTPase activity, which would highlight a shared mechanism of action.

Outlook: Linking LRRK2, Rab and TPC action along endomembrane trafficking

The combined observations of a link between LRRK2 and TPC [20], between LRRK2 and Rab7 [15], and between Rab7 and TPC [44] allow for the formulation of at least two testable hypotheses to explain the action of LRRK2 at the late endosome (Figure 3). Under conditions of enhanced autophagic or endocytic flux, LRRK2 may function at the late endosome. It may directly regulate TPC functioning, causing alterations in calcium release from acidic stores with concomitant downstream effects on Rab activity. Recent studies have reported a calcium-dependent down-regulation of Rab11 activation by a vacuolar ion channel to allow vacuole fusion, suggesting that at least in principle, a calcium-mediated modulation of Rab activity through localized channel-mediated release of intraluminal calcium is feasible [50]. Alternatively, LRRK2 may down-regulate Rab activity, causing decreased coupling to TPCs [44] and resulting deficiencies in trafficking to and from the late endosome. Although further epistasis-type experiments will be necessary to distinguish between those two possibilities, the current data clearly pinpoint towards an important link between LRRK2, Rab functioning and TPC action, and

Figure 3 | Two hypothetical models for the mechanistic link between pathogenic LRRK2, TPC channels, Rab and vesicle fusion

Left: LRRK2 may directly regulate TPC channels, and the resultant alterations in acidic store calcium efflux may alter Rab activity with downstream effects on vesicle fusion. Right: LRRK2 may regulate Rab activity, which in turn may modulate TPC channel function to alter calcium release from acidic stores, followed by vesicle fusion.



thus the relevance of acute and/or chronic alterations in intraluminal calcium content in the context of late endosome-derived vesicular trafficking events. Thus, modulation of NAADP-regulated membrane trafficking pathways may provide promising alternative therapeutic targets for LRRK2-related PD.

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