Published in final edited form as:

Adv Neurobiol. 2017 ; 14: 71–88. doi:10.1007/978-3-319-49969-7_4.

Understanding the GTPase Activity of LRRK2: Regulation, Function, and Neurotoxicity

An Phu Tran Nguyen and Darren J. Moore

Center for Neurodegenerative Science, Van Andel Research Institute, 333 Bostwick Ave NE, Grand Rapids, MI 49503, USA

Abstract

Mutations in the *leucine-rich repeat kinase 2 (LRRK2)* gene are the most frequent cause of Parkinson's disease (PD) with late-onset and autosomal-dominant inheritance. LRRK2 belongs to the ROCO superfamily of proteins, characterized by a Ras-of-complex (Roc) GTPase domain in tandem with a C-terminal-of-Roc (COR) domain. LRRK2 also contains a protein kinase domain adjacent to the Roc-COR tandem domain in addition to multiple repeat domains. Disease-causing familial mutations cluster within the Roc-COR tandem and kinase domains of LRRK2, where they act to either impair GTPase activity or enhance kinase activity. Familial LRRK2 mutations share in common the capacity to induce neuronal toxicity in cultured cells. While the contribution of the frequent G2019S mutation, located within the kinase domain, to kinase activity and neurotoxicity has been extensively investigated, the contribution of GTPase activity has received less attention. The GTPase domain has been shown to play an important role in regulating kinase activity, in dimerization, and in mediating the neurotoxic effects of LRRK2. Accordingly, the GTPase domain has emerged as a potential therapeutic target for inhibiting the pathogenic effects of LRRK2 mutations. Many important mechanisms remain to be elucidated, including how the GTPase cycle of LRRK2 is regulated, whether GTPase effectors exist for LRRK2, and how GTPase activity contributes to the overall functional output of LRRK2. In this review, we discuss the importance of the GTPase domain for LRRK2-linked PD focusing in particular on its regulation, function, and contribution to neurotoxic mechanisms.

Keywords

LRRK2; GTPase; ROCO protein; Roc; COR; Roc-COR; Dimerization; Parkinson's disease; PARK8

Introduction

Missense mutations in the *leucine-rich repeat kinase 2 (LRRK2)* gene cause late-onset, autosomal-dominant Parkinson's disease (PD) and represent the most common cause of familial PD accounting for 5–15 % of dominant PD [1–4]. Moreover, genome-wide association studies have identified common variants in the LRRK2 genomic locus that are

Correspondence to: Darren J. Moore.

Conflict of Interest The author declares no conflicts of interest.

associated with risk for idiopathic PD [5–7]. Several putative variants in the LRRK2 gene have been identified with at least seven missense mutations (i.e., N1437H, R1441C, R1441G, R1441H, Y1699C, G2019S, and I2020T) considered to be truly pathogenic based upon segregation with disease in PD families (Fig. 4.1, [8, 9]). The most frequent mutation is G2019S which is responsible for up to 40 % of familial PD depending on ethnicity and 1–2 % of idiopathic PD [2, 8]. The presence of the G2019S variant in idiopathic cases has been attributed to age-dependent but incomplete penetrance [2, 10]. Interestingly, familial mutations tend to cluster within the catalytic triad of the LRRK2 protein composed of a Ras-of-complex (Roc) GTPase domain (i.e., N1437H, R1441C, R1441G, R1441H) and a protein kinase domain (i.e., G2019S, I2020T) separated by a C-terminal-of-Roc domain (COR; i.e., Y1699C) (Fig. 4.1, [11]). In the past decade since the first discovery of LRRK2 mutations, the kinase activity of LRRK2 has been investigated intensively largely in the context of the most common G2019S mutation which has been shown to produce a hyperactive kinase. Although a large number of familial mutations are located within the Roc-COR tandem domain, the role of GTPase activity in regulating the normal function of LRRK2 has received considerably less attention and remains incompletely understood. However, as will be discussed, the GTPase domain is likely key for understanding LRRK2 function and neurotoxic mechanisms, and therefore the GTPase domain represents an important therapeutic target for the treatment of PD.

LRRK2 Domain Architecture

LRRK2 encodes a multi-domain protein of 2527 amino acids that exists predominantly in a dimeric form in cells and tissues [11–15]. At present, the structure of LRRK2 has not been determined related largely to technical issues of purifying sufficient quantities of soluble full-length recombinant human LRRK2 protein. X-ray crystallography has so far been applied to isolated LRRK2 domains, including the kinase domain and the Roc domain revealing a controversial domain-swapped dimeric conformation [16, 17]. LRRK2 is predicted to contain multiple domains including a central Roc-COR tandem domain, a tyrosine kinase-like protein kinase domain, and at least four repeat domains located within N-terminal (armadillo, ankyrin, and leucine-rich repeats) and C-terminal (WD40 repeats) regions (Fig. 4.1, [18]). The Roc-COR tandem domain classifies LRRK2 as a member of the ROCO protein superfamily which represents a unique multi-domain family of Ras-like G proteins [19–21]. Important structural and functional understanding of LRRK2 has been inferred from other ROCO protein members in bacteria and *Dictyostelium discoideum* where the ROCO family was first described [17, 22, 23]. ROCO proteins are characterized by the presence of a Roc-COR tandem domain often (but not always) in association with a kinase domain [20, 24]. The Roc domain contains five G-box motifs that are required for guanine nucleotide binding and hydrolysis (Fig. 4.1, [11, 19, 25]). In mammals, four ROCO proteins have been identified including LRRK1, LRRK2, MASL1 (malignant fibrous histiocytoma-amplified sequence with leucine-rich tandem repeats 1), and DAPK1 (death-associated protein kinase 1) [24]. LRRK1 and LRRK2 share the closest sequence homology and differ only in the N-terminal region which is ~650 amino acids longer in LRRK2. LRRK1, LRRK2, and DAPK1 possess a kinase domain, whereas MASL1 does not. The evolutionary conservation of the Roc-COR tandem domain, independent of a kinase domain,

implies that GTPase activity is most likely the primary functional output of ROCO proteins with the kinase domain potentially serving to regulate the GTPase domain. However, it is not yet known whether LRRK2 conforms to the classic model of a ROCO protein since there is evidence that the kinase domain may serve to regulate the intrinsic GTPase domain as well as extrinsic protein substrate phosphorylation. Interestingly, only LRRK2 and DAPK1 have so far been linked to human disease (PD and cancer, respectively) [24, 25].

GTPase Domain and Activity

Genetic Mutations Located in Roc and COR Domains Cause Familial PD

Several familial mutations of LRRK2 are located within the Roc-COR tandem domain suggesting that GTPase activity plays an important role in the development of PD [11]. Although the Roc GTPase domain comprises only a small fraction (residues 1335–1510) of full-length LRRK2 protein (~7 % of total), this domain represents something of a hotspot for mutations perhaps best exemplified by the identification of three PD mutations at a single R1441 residue (i.e., R1441C, R1441G, and R1441H) [8, 11]. The R1441G variant is frequent in PD families from the Basque region of Spain (~20 % of familial PD) but rare elsewhere, whereas the R1441C and R1441H variants are found in many populations but are not frequent [8]. Another variant nearby in the Roc domain, N1437H, has been identified in a large Norwegian family with autosomal-dominant PD [9]. Additional variants in the Roc domain such as I1371V are found in individual PD cases but have not yet been confirmed by segregation analyses in families [8, 26]. The protective R1398H variant is associated with PD in certain populations suggesting that variation within the GTPase domain may also be beneficial [27–29]. The familial Y1699C mutation, identified in German-Canadian and UK families, is the only known disease-causing variant located within the COR domain (residues 1510–1850) [4, 30]. Human genetic studies highlight the importance of the Roc-COR tandem domain to the development of PD with multiple independent mutations located within this region. In contrast, only two mutations, G2019S and I2020T, located in adjacent residues of the kinase activation loop, are known to unambiguously cause familial PD [8]. Pathogenic mutations located outside of the catalytic triad are either rare or their pathogenicity has not been confirmed. The major challenge now becomes how can we best reconcile the functional effects of disease-causing mutations in the Roc-COR tandem and kinase domains for understanding their effects on overall LRRK2 function.

LRRK2 Is a Functional GTPase

LRRK2 is known to possess GTPase activity, at least when measured in in vitro assays using recombinant protein. LRRK2 can selectively bind to guanine nucleotides (GDP and GTP) with similar affinity via a phosphate-binding “P-loop” motif (¹³⁴¹GNTGSGKT¹³⁴⁸) within its GTPase domain (Fig. 4.1, [31–35]). This has been measured by binding of LRRK2 or the isolated Roc domain to immobilized or radiolabeled GTP and its non-hydrolyzable analogs (i.e., GTPγS, GppCp), either using purified recombinant LRRK2 or LRRK2 expressed in cell extracts. Competition with an excess of free GTP or GDP can reduce this binding as can synthetic mutations that disrupt key P-loop residues such as K1347A or T1348N, thereby confirming binding specificity. The impact of familial mutations on GTP-binding activity of LRRK2 is somewhat inconsistent but with some evidence that mutations located in the Roc-

COR tandem domain tend to increase GTP binding [9, 35]. LRRK2 also exhibits a low rate of GTP hydrolysis activity in vitro. Interestingly, familial mutations in the Roc-COR tandem domain have been shown to reduce GTPase activity to varying degrees. R1441C, R1441G, R1441H, and Y1699C variants all exhibit decreased GTP hydrolysis compared to wild-type LRRK2 [31–33, 36–38]. Familial mutations located in the kinase domain such as G2019S have no discernible effects on GTP binding or hydrolysis suggesting that their pathogenic effects are solely mediated through altered kinase activity [15, 35, 37]. Oppositely, mutations located in the Roc-COR domain have inconsistent effects on kinase activity with the general consensus across most laboratories being that they do not alter kinase activity [18, 35, 39, 40]. Therefore, a clearer picture has emerged that familial mutations in the Roc-COR domain consistently impair GTP hydrolysis, and some may also correspondingly increase the affinity for GTP binding, whereas mutations located in the kinase domain influence only kinase activity. One interesting exception is the familial N1437H mutation, located in the Roc domain, which is reported to simultaneously increase both GTP-binding and kinase activity [9].

At a functional level, only a handful of key residues within the LRRK2 Roc domain have so far been identified to influence GTPase activity based upon highly conserved residues in related small G proteins. The P-loop null mutants, K1347A and T1348N, are useful tools that effectively disrupt GTP binding, but they also have the undesirable effect of impairing LRRK2 dimerization and compromising protein stability [15, 31, 34, 35]. Therefore, these mutants should be used with some caution when attempting to attribute the contribution of GTP binding to LRRK2 activity or cellular properties. Nonetheless, the effects of P-loop mutants on LRRK2 suggest that either guanine nucleotide binding or the conformation of the P-loop (or Roc domain in general) is important for dimerization and that dimeric LRRK2 is generally more stable than monomeric LRRK2 in cells [15]. Notably, familial PD mutations have not been identified in the P-loop motif. The switch II motif (¹³⁹⁴DFAGR¹³⁹⁸) has been shown to be critical for the GTP hydrolysis activity of LRRK2 (Fig. 4.1, [31]). The R1398 residue in LRRK2 is typically a glutamine (Gln, Q) in the vast majority of small GTPases, whereas likewise the T1343 residue in the P-loop is typically a glycine (Gly, G). Replacing both R1398 with a Gln (R1398Q) and T1343 with a Gly (T1343G) to create a Ras-like form of LRRK2 (R1398Q/T1343G) increases GTPase activity [31]. A Gln → Leu (Q → L) substitution in the switch II region of Ras GTPases oppositely impairs GTP hydrolysis and creates a “GTP-locked” protein that is constitutively bound to GTP. However, introduction of a Leu at 1398 (R1398L) unexpectedly increases the GTP hydrolysis activity (by two-to threefold) of LRRK2, similar to the Ras-like R1398Q/T1343G mutant, creating a functional mutant equivalent to a predominant “GDP-bound” protein [15, 37]. Combining the R1398L mutant with a T1343V mutation in the P-loop (R1398L/T1343V) is now sufficient to create a “GTP-bound” form of LRRK2 with impaired GTPase activity [15], similar to corresponding mutations in some related Ras proteins. The Ras-like R1398Q/T1343G, GDP-bound R1398L, and GTP-bound R1398L/T1343V mutant forms of LRRK2 exhibit normal GTP binding, are relatively stable in cells, and do not influence LRRK2 dimerization, in contrast to mutants disrupting GTP binding (i.e., K1347A, T1348N) [15]. These hypothesis-testing mutations provide important tools for exploring the contribution of GTPase activity to other functions of LRRK2, such as kinase activity,

dimerization, or cellular phenotypes. The availability of GTPase mutations that can create GDP-bound and GTP-bound forms of LRRK2 may also prove useful for identifying GTPase effector proteins that bind in the GTP-bound “on” state as well as guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) that regulate the GTPase cycle, if indeed they exist for LRRK2.

Regulation of GTPase Activity

Members of the Ras GTPase superfamily conventionally function as binary molecular switches cycling between a GDP-bound “off” and GTP-bound “on” state. When small GTPases are bound to GDP, they adopt an inactive conformation, but GTP binding induces conformational changes which allow the enzyme to bind and activate effector proteins and initiate signal transduction cascades. Most small GTPases are regulated by GAPs that promote the hydrolysis of GTP to GDP and render the GTPase inactive (GDP bound) and GEFs that promote the exchange of GDP with GTP leading to an active GTPase (GTP bound). GTPase activation is often coupled to the activation of a kinase effector similar to a classical Ras GTPase/Raf kinase mechanism, and thus parallels have been drawn with LRRK2 which contains an intrinsic kinase domain adjacent to the Roc-COR tandem domain. This has led to the notion that the GTPase and kinase domains of LRRK2 could be linked together via an intramolecular mechanism with GTPase activity serving to regulate kinase activity. However, current evidence is not consistent with such a simple mechanism. For example, GTP binding to LRRK2 does indeed increase kinase activity, whereas disrupting GTP binding by mutating key P-loop residues impairs kinase activity (albeit with the caveat that such mutations also disrupt dimerization) [15, 31, 34, 35]. However, GTP binding to LRRK2 only increases kinase activity in the context of a cell extract, whereas GTP binding directly to purified recombinant LRRK2 has no impact on kinase activity [15, 41]. This observation has led to the suggestion that GTP-binding capacity rather than direct GTP binding per se drives kinase activation and may hint at the requirement for a yet to be identified GTP-dependent accessory protein (presumably only present in cell extracts) [41]. A second inconsistency with a “simple” Ras-/Raf-like mechanism is the observation that GTP hydrolysis unexpectedly appears to contribute to kinase activation rather than serving to terminate kinase activity as might be predicted from such a model. For example, the GTP-locked R1398L/T1343V mutant exhibits markedly reduced kinase activity, whereas ArfGAP1, a GAP-like protein for LRRK2 which promotes its GTP hydrolysis activity, also enhances its kinase activity [15, 42]. Furthermore, while GTP and GppCp binding have been shown to promote LRRK2 kinase activity [15, 41], this effect is attenuated when GTP hydrolysis activity is impaired (via the R1398L/T1343V mutation) [15], suggesting that GTPase activity might be required in part for kinase activation. It is notable that GTP increases kinase activity to a greater extent than non-hydrolyzable GppCp again suggesting a requirement for an actual GTP hydrolysis event (presuming that GTP and GppCp have similar affinity for binding to LRRK2 in this assay) [15]. These observations would be consistent with the effects of ArfGAP1 on promoting kinase activity [42]. While GTP binding can promote LRRK2 kinase activity in an unconventional manner, the GTP hydrolysis event itself may also contribute to kinase activation. Therefore, the intramolecular regulation of GTPase and kinase activities is rather complex, and LRRK2 does not appear to function as a canonical GTPase.

Although LRRK2 is an unconventional GTPase, there is some evidence for the regulation of LRRK2 GTPase activity by GAPs and GEFs. ArhGEF7 was first nominated as a GEF and was shown to interact with LRRK2 in cells and mouse brain and increases its GTPase activity [43]. The familial R1441C mutation causes reduced binding of LRRK2 to ArhGEF7 consistent with the known reduction of GTPase activity in this mutant. ArhGEF7 binding to LRRK2 is regulated by the phosphorylation of LRRK2 by CK1 α , and loss of this constitutive phosphorylation increases ArhGEF7 binding and alters ArhGEF7-mediated LRRK2 GTP binding [44]. LRRK2 also phosphorylates ArhGEF7 in vitro at two threonine residues within its N-terminus [43]. It is unclear whether ArhGEF7 activity imparts any effect on LRRK2 kinase activity and also what impact phosphorylation has on ArhGEF7 activity.

GAP-like proteins for LRRK2 have also been identified. Deletion of *GCSI* (an ortholog of mammalian ArfGAP1) in yeast was originally identified as a suppressor of LRRK2-induced toxicity [37]. LRRK2 interacts with ArfGAP1 in human cells and in rodent brain, and mutations within the Roc-COR domain that alter GTPase activity modulate the interaction with ArfGAP1 [42, 45]. GTP hydrolysis activity of LRRK2 is markedly enhanced by ArfGAP1 in vitro, in a manner dependent on the GAP domain, consistent with a role for ArfGAP1 as a GAP-like protein for LRRK2 [42, 45]. The impact of ArfGAP1 on LRRK2 kinase activity is unclear with reports of either increased or reduced activity [42, 45]. Unexpectedly, ArfGAP1 also serves as a robust substrate of LRRK2-mediated phosphorylation, with multiple putative sites of phosphorylation identified, although the impact of phosphorylation on ArfGAP1 activity and function is not yet clear [42, 45]. In rodent primary neurons, silencing of ArfGAP1 expression consistently rescues G2019S LRRK2-induced toxicity [42, 45], similar to findings in yeast [37], supporting a critical role for ArfGAP1 in mediating toxicity downstream of LRRK2. Whether this toxic pathway involves the phosphorylation and/or GAP activity of ArfGAP1, or ArfGAP1-dependent effects on Golgi vesicle sorting, is not yet clear. In a *Drosophila* model, the overexpression of mutant LRRK2 or ArfGAP1 alone induces dopaminergic neuronal loss and motor deficits, yet surprisingly their co-expression ameliorates these neurotoxic effects through an unknown mechanism [45], a finding that is difficult to reconcile with yeast and neuronal culture data. Whether ArfGAP1 is required for mutant LRRK2-dependent phenotypes in rodent models remains to be determined. A second putative GAP-like protein, RGS2, has also been identified for LRRK2. RGS2 was originally identified in a *C.elegans* screen as a genetic modifier of the susceptibility of LRRK2 transgenic worms to rotenone-induced dopaminergic neuronal loss [46]. RGS2 was subsequently shown to interact with LRRK2, enhance its GTP hydrolysis activity in vitro, and also serve as a modest substrate of LRRK2 kinase activity [46]. However, opposite to the effects of ArfGAP1, RGS2 reduces LRRK2 kinase activity in vitro, and RGS2 overexpression rescues LRRK2-induced neuronal toxicity suggesting a neuroprotective capacity [46]. How these distinct GAPs both acting upon the GTPase domain can have opposing effects on LRRK2 kinase activity and neuronal toxicity remains to be clarified. However, it is not yet clear whether ArfGAP1 or RGS2 serves as authentic physiological GAPs for LRRK2 in vivo or whether they may serve to modulate GTPase activity via an alternative mechanism perhaps, for example, by acting as GTPase effector proteins or by stabilizing LRRK2 dimers. At this juncture, while LRRK2 can

interact with known GEFs and GAPs that can modify its GTPase activity at least in vitro, whether these proteins modulate the GTPase cycle of LRRK2 in a conventional manner requires additional investigation.

An alternative mechanism for the regulation of the LRRK2 GTPase cycle has been proposed based upon the structure of simpler ROCO proteins. LRRK2 is suggested to function as a G protein activated by nucleotide-dependent dimerization (GAD) [22, 23]. GADs do not require GEFs or GAPs but instead rely upon nucleotide binding and dimerization to regulate the GTPase cycle. For the related ROCO proteins from *Chlorobium tepidum* and *Methanosarcina barkeri*, constitutive dimerization is mediated through the COR domains where upon GTP binding a conformational shift induces the juxtaposition of the adjacent Roc domains of the dimer so that they complement each other to form an active GTPase [23, 47]. Subsequent GTP hydrolysis, presumably coupled to the activation of an effector protein, restores the Roc domains to the inactive GDP-bound conformation [22, 23, 47]. Critical for this noncanonical mechanism is COR domain-mediated dimerization with the COR domain dimer interface being the most highly conserved region among ROCO proteins [23, 47]. LRRK1 and LRRK2 share a great deal of sequence conservation with simpler ROCO proteins within the COR domain suggesting that a potential dimer interface may also be present in LRRK2 [23, 47]. While LRRK2 has been shown to predominantly exist as a homodimeric protein in cells [12–15], in the absence of high-resolution structural data, domain mapping interaction studies have highlighted potential roles for the Roc or WD40 repeat domains as the putative dimer interface [13, 16, 36, 38, 48]. A recent study has provided evidence for an interaction between the isolated COR domains of LRRK2, but the relative strength of this interaction compared to those between other domains (i.e., Roc-Roc or Roc-COR) is not yet clear [47]. An intermolecular interaction between the Roc domains, albeit potentially weaker, is to be expected based upon the proposed GAD mechanism. Recent studies have shown that the isolated Roc domain of LRRK2 can form stable monomeric or dimeric conformations in solution that are catalytically active [38, 49], suggesting that dimerization is not essential for activity although whether this applies in the context of the full-length LRRK2 protein is not yet clear. One of the most robust interactions within LRRK2 appears to be between the Roc and COR domains, being more robust than the interaction between Roc domains [13, 36]. The interaction between isolated Roc and COR domains is strengthened by the familial Y1699C mutation that is suggested to occupy an intramolecular interaction interface and limit the conformational flexibility of these domains within a monomer [36], thereby potentially explaining how this mutation impairs GTPase activity [36, 37]. Such an effect of this mutation would be consistent with a GAD mechanism for LRRK2 based on similar studies with *C. tepidum* ROCO protein using mutations analogous to R1441C and Y1699C [23]. At present, it is not known which domains and residues of LRRK2 are important for dimerization in the full-length protein since most studies have relied upon isolated domains, therefore highlighting the need for structural data. One inconsistency with the GAD model is that guanine nucleotide binding does not appear to regulate LRRK2 dimerization [36]. Instead, functional mutations that disrupt the P-loop (i.e., K1347A or T1348N), pharmacological kinase inhibition, and association with cellular membranes have been reported to modulate LRRK2 dimerization [12–15, 47]. It is possible that dimerization alone is sufficient to regulate the GTPase cycle

of LRRK2. While a GAD mechanism for LRRK2 is an attractive hypothesis, there is currently limited evidence to support such a mechanism, and additional biochemical and structural studies will be required to further understand how the GTPase cycle is regulated.

While there is some evidence that GTPase activity can regulate LRRK2 kinase activity in an unconventional manner, there is also emerging evidence that kinase activity may reciprocally serve to regulate GTPase activity. The mapping of in vitro autophosphorylation sites within LRRK2 by mass spectrometry reveals that many of these sites tend to cluster within the Roc domain at multiple serine and threonine residues including key P-loop residues (T1343, T1348), S1403, T1404, T1410, T1491, and T1503 [50–53]. How phosphorylation at each of these sites regulates GTP binding and hydrolysis activity is not yet clear. However, in full-length LRRK2, disrupting kinase activity has no appreciable effect on GTPase activity suggesting that phosphorylation is likely to have rather subtle or dynamic effects on GTPase activity depending upon the combination of sites modified in a single dimer [15].

Phosphorylation at individual sites is not particularly abundant and varies between sites and may modify only a small proportion of LRRK2 at any given time [51, 53]. For this reason it has so far been difficult to confirm the phosphorylation at individual sites occurring in cells and tissues using phospho-specific antibodies [53], with the exception of phosphorylation at S1292 located between the leucine-rich repeat and Roc domains [54]. Studies of the functional impact of individual phospho-sites within the Roc domain are limited, but one study suggests that the T1503 residue may regulate the GTP binding and kinase activity of LRRK2 [53]. A recent study using the isolated Roc domain from LRRK2 suggests that autophosphorylation enhances the rate of GTP hydrolysis and promotes the formation of Roc dimers, potentially by altering the conformation of the P-loop structure [49]. P-loop phosphorylation appears to be common to many GTPases suggesting a novel mechanism for the control of GTPase activity by kinases [49]. Although these studies are insightful, it remains to be determined how autophosphorylation regulates GTPase activity in the context of full-length LRRK2 protein. GTPase activity may also be regulated by extrinsic kinases. PKA has been shown to phosphorylate LRRK2 at S1444, and this modification is reduced by the familial mutations R1441C/R1441G/R1441H which occupy a consensus PKA recognition site [55]. Phosphorylation at S1444 by PKA serves as a 14-3-3 docking site, and binding leads to decreased kinase activity in vitro, whereas inhibition of S1444 phosphorylation impairs 14-3-3 binding and increases kinase activity [55]. The mechanism by which S1444 phosphorylation influences kinase activity is not yet clear, and the impact of phosphorylation and 14-3-3 binding on the GTPase cycle of LRRK2 has not been determined. Intrinsic and extrinsic phosphorylation may therefore provide an additional level of regulation of the LRRK2 GTPase cycle potentially by altering P-loop structure or by the recruitment of accessory proteins.

Contribution of GTPase Activity to LRRK2-Induced Toxicity

While familial mutations in the kinase domain, such as G2019S, have been extensively shown to induce cellular toxicity in culture models in a kinase-dependent manner [34, 56–59], there is still uncertainty about the contribution of GTPase activity to cellular toxicity. Studies in a yeast model expressing the catalytic core of human LRRK2 have highlighted a critical requirement of the GTPase domain and GTPase activity for cellular toxicity. While

mutations that disrupt GTP binding and hydrolysis (i.e., K1347A or T1348N) cause a dramatic increase in yeast toxicity, enhancing GTP hydrolysis (i.e., R1398L or R1398Q/T1343G) improves viability [37]. Similar effects of these LRRK2 synthetic mutants on the viability of primary neuronal models have been confirmed. LRRK2-induced toxicity in yeast correlates with severe defects in endosomal trafficking to the vacuole and the accumulation of autophagosomes [37]. Genetic suppressors of LRRK2 toxicity also restore endosomal trafficking suggesting a causal role. Similar cellular phenotypes have been observed with full-length mutant LRRK2 in mammalian cells or neurons [58, 60–64]. Consistent with the importance of GTPase activity for LRRK2 toxicity, the GTP-locked R1398L/T1343V mutation enhances neuronal toxicity induced by LRRK2 in primary cultures comparable to the effects of the pathogenic G2019S mutation [15]. Interestingly, however, GTPase-hyperactive R1398L or GTPase-impaired R1398L/T1343V mutations are not able to modify the elevated kinase activity or neurotoxic effects of G2019S LRRK2 [15], suggesting that the G2019S variant may act independent of GTPase activity. A prior study suggested that disruption of GTP binding (via K1347A) can rescue neuronal toxicity induced by G2019S LRRK2 [34], although it is likely that neuroprotection may result instead from the impaired dimerization and destabilization of LRRK2 known to be caused by the K1347A variant in neurons [15]. As mentioned above, ArfGAP1 expression is required for G2019S LRRK2-induced neuronal toxicity, whereas RGS2 is neuroprotective [42, 46]. It is not known whether these GAPs act upon LRRK2 GTPase or kinase activity to regulate neurotoxicity or whether they act in pathways downstream of LRRK2 potentially as GTPase effectors or kinase substrates. Aside from the G2019S mutation, it is not yet known whether GTPase activity contributes to neuronal toxicity induced by familial mutations in the Roc-COR domain such as R1441C and Y1699C, although some evidence suggests that kinase activity may be required for the toxicity of the R1441C mutant [34, 56, 59]. A number of model organisms with interesting phenotypes have been developed based upon familial mutations in the Roc-COR tandem domain of LRRK2 although mechanistic insight into the contribution of GTPase activity is so far lacking [65]. For example, R1441C or Y1699C LRRK2 selectively inhibit axonal transport and cause locomotor deficits in neuronal and *Drosophila* models that may result from their preferential association with deacetylated microtubules [66]. Increasing microtubule acetylation prevents the association of mutant LRRK2 with microtubules and restores axonal transport [66]. Transgenic or knockin mouse models expressing R1441G or R1441C LRRK2 exhibit a combination of motor deficits, impaired dopaminergic neurotransmission, axonopathy, tau pathology, altered autophagy, or abnormal nuclear envelope architecture [63, 67–70]. Understanding how GTPase activity contributes to these LRRK2-dependent phenotypes will be challenging and may rely in the future upon genetic or pharmacological manipulation of the GTPase domain.

Conclusion and Future Perspectives

LRRK2 is a central player in PD and an attractive target for therapeutic development. However, LRRK2-related mechanisms leading to neurotoxicity remain incompletely understood. So far, most studies have highlighted the kinase activity of LRRK2 as a key therapeutic target since the most common G2019S mutation elevates kinase activity and induces neuronal toxicity in a kinase-dependent manner [18, 34, 40, 57]. Genetic or

pharmacological inhibition of kinase activity has been proven to be protective in viral-based G2019S LRRK2 rodent models [57, 71] and also protects against neurodegeneration induced by human α -synuclein or LPS-induced neuroinflammation in rat models [72]. A robust in vivo substrate of LRRK2 kinase activity is still lacking to be able to fully explain the neuroprotective effects of kinase inhibition, although one such substrate could be LRRK2 itself via auto-phosphorylation [54].

LRRK2 contains an evolutionarily conserved Roc-COR tandem domain, and many familial mutations are clustered within the Roc and COR domains and impair GTPase activity. Therefore, GTPase activity is clearly important for LRRK2 function, for regulating kinase activity, and for the development of PD. Potential therapeutic strategies for targeting the Roc-COR tandem domain might include (1) inhibition of GTP binding, (2) modulation of GTP hydrolysis, (3) disrupting dimerization, (4) kinase inhibition, or (5) targeting GAPs, GEFs, or GTPase effectors. The validation of each of these strategies is now required in disease-relevant models and may rely upon the future development of small-molecule compounds. There have been a paucity of such studies and compounds, although recent studies have reported the development of novel compounds that simultaneously inhibit LRRK2 GTP binding and kinase activity and attenuate LRRK2 toxicity, although the mechanism of action is not yet clear [73, 74]. Yeast LRRK2 models could prove to be a useful tool for screening and identifying small-molecule GTPase modulators since cellular toxicity in this model is fully dependent on GTPase activity [37]. The incorporation of hypothesis-testing mutations that create “GTP-locked” and “GDP-locked” forms of LRRK2 into adenoviral-mediated rodent models expressing mutant LRRK2 may prove informative for understanding how best to modulate GTPase activity to attenuate neurodegenerative phenotypes [15]. Such models produce rapid and robust phenotypes and it is relatively simple to produce viruses containing new LRRK2 variants [75]. A similar approach has been employed to demonstrate that kinase activity is required for neuropathology induced by G2019S LRRK2 [71]. This approach could also be used to evaluate key residues that are important for LRRK2 dimerization or to modulate the expression of GAPs such as ArfGAP1 or RGS2.

The Roc-COR tandem domain and GTPase activity of LRRK2 represent attractive and potentially tractable targets for the development of new therapeutics to treat PD. A deeper mechanistic understanding of how the GTPase cycle is regulated and how GTPase activity modulates kinase activity and neurotoxicity will be critical to fully understand LRRK2 function and its role in the development of PD.

Acknowledgments

The authors are grateful for funding support from the National Institutes of Health (R01 NS091719), the Swiss National Science Foundation (grant no. 31003A_144063), and the Van Andel Research Institute.

References

1. Gasser T. Mendelian forms of Parkinson's disease. *Biochim Biophys Acta*. 2009; 1792:587–596. [PubMed: 19168133]
2. Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, Brice A, Aasly J, Zabetian CP, Goldwurm S, Ferreira JJ, Tolosa E, Kay DM, Klein C, Williams DR, Marras C, Lang AE,

Wszolek ZK, Berciano J, Schapira AHV, Lynch T, Bhatia KP, Gasser T, Lees AJ, Wood NW. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. *Lancet Neurol.* 2008; 7(7):583–590. DOI: 10.1016/S1474-4422(08)70117-0 [PubMed: 18539534]

3. Paisán-Ruiz C, Jain S, Evans EW, Gilks WP, Simón J, van der Brug M, de Munain AL, Aparicio S, Gil AM, Khan N, Johnson J, Martinez JR, Nicholl D, Carrera IM, Pena AS, de Silva R, Lees A, Martí-Massó JF, Pérez-Tur J, Wood NW, Singleton AB. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron.* 2004; 44(4):595–600. DOI: 10.1016/j.neuron.2004.10.023 [PubMed: 15541308]
4. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, Kachergus J, Hulihan M, Uitti RJ, Calne DB, Stoessl AJ, Pfeiffer RF, Patenge N, Carbajal IC, Vieregge P, Asmus F, Müller-Myhsok B, Dickson DW, Meitinger T, Strom TM, Wszolek ZK, Gasser T. Mutations in LRRK2 cause autosomal-dominant Parkinsonism with pleomorphic pathology. *Neuron.* 2004; 44(4):601–607. DOI: 10.1016/j.neuron.2004.11.005 [PubMed: 15541309]
5. Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, Kawaguchi T, Tsunoda T, Watanabe M, Takeda A, Tomiyama H, Nakashima K, Hasegawa K, Obata F, Yoshikawa T, Kawakami H, Sakoda S, Yamamoto M, Hattori N, Murata M, Nakamura Y, Toda T. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat Genet.* 2009; 41(12):1303–1307. [PubMed: 19915576]
6. Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, Paisan-Ruiz C, Lichtner P, Scholz SW, Hernandez DG, Kruger R, Federoff M, Klein C, Goate A, Perlmutter J, Bonin M, Nalls MA, Illig T, Gieger C, Houlden H, Steffens M, Okun MS, Racette BA, Cookson MR, Foote KD, Fernandez HH, Traynor BJ, Schreiber S, Arepalli S, Zonozi R, Gwinn K, van der Brug M, Lopez G, Chanock SJ, Schatzkin A, Park Y, Hollenbeck A, Gao J, Huang X, Wood NW, Lorenz D, Deuschl G, Chen H, Riess O, Hardy JA, Singleton AB, Gasser T. Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat Genet.* 2009; 41(12):1308–1312. [PubMed: 19915575]
7. Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, DeStefano AL, Kara E, Bras J, Sharma M, Schulte C, Keller MF, Arepalli S, Letson C, Edsall C, Stefansson H, Liu X, Pliner H, Lee JH, Cheng R, International Parkinson's Disease Genomics Consortium (IPDGC), Parkinson's Study Group (PSG) Parkinson's Research: The Organized GENetics Initiative (PROGENI), 23andMe, GenePD, NeuroGenetics Research Consortium (NGRC), Hussman Institute of Human Genomics (HIHG), Ashkenazi Jewish Dataset Investigator, Cohorts for Health and Aging Research in Genetic Epidemiology (CHARGE), North American Brain Expression Consortium (NABEC), United Kingdom Brain Expression Consortium (UKBEC), Greek Parkinson's Disease Consortium, Alzheimer Genetic Analysis Group. Ikram MA, Ioannidis JP, Hadjigeorgiou GM, Bis JC, Martinez M, Perlmutter JS, Goate A, Marder K, Fiske B, Sutherland M, Xiromerisiou G, Myers RH, Clark LN, Stefansson K, Hardy JA, Heutink P, Chen H, Wood NW, Houlden H, Payami H, Brice A, Scott WK, Gasser T, Bertram L, Eriksson N, Foroud T, Singleton AB. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet.* 2014; 46(9):989–993. DOI: 10.1038/ng.3043 [PubMed: 25064009]
8. Biskup S, West AB. Zeroing in on LRRK2-linked pathogenic mechanisms in Parkinson's disease. *Biochim Biophys Acta.* 2009; 1792(7):625–633. DOI: 10.1016/j.bbadis.2008.09.015 [PubMed: 18973807]
9. Aasly JO, Vilarinho-Güell C, Dachsel JC, Webber PJ, West AB, Haugarvoll K, Johansen KK, Toft M, Nutt JG, Payami H, Kachergus JM, Lincoln SJ, Felic A, Wider C, Soto-Ortolaza AI, Cobb SA, White LR, Ross OA, Farrer MJ. Novel pathogenic LRRK2 p.Asn1437His substitution in familial Parkinson's disease. *Mov Disord.* 2010; 25(13):2156–2163. DOI: 10.1002/mds.23265 [PubMed: 20669305]
10. Hulihan MM, Ishihara-Paul L, Kachergus J, Warren L, Amouri R, Elango R, Prinjha RK, Upmanyu R, Kefi M, Zouari M, Sassi SB, Yahmed SB, El Euch-Fayeche G, Matthews PM, Middleton LT, Gibson RA, Hentati F, Farrer MJ. LRRK2 Gly2019Ser penetrance in Arab-Berber patients from Tunisia: a case-control genetic study. *Lancet Neurol.* 2008; 7(7):591–594. DOI: 10.1016/S1474-4422(08)70116-9 [PubMed: 18539535]
11. Tsika E, Moore DJ. Contribution of GTPase activity to LRRK2-associated Parkinson disease. *Small GTPases.* 2013; 4(3):164–170. DOI: 10.4161/sgtp.25130 [PubMed: 24025585]

12. Berger Z, Smith KA, LaVoie MJ. Membrane localization of LRRK2 is associated with increased formation of the highly active LRRK2 dimer and changes in its phosphorylation. *Biochemistry*. 2010; 49(26):5511–5523. DOI: 10.1021/bi100157u [PubMed: 20515039]
13. Greggio E, Zambrano I, Kaganovich A, Beilina A, Taymans J-M, Daniëls V, Lewis P, Jain S, Ding J, Syed A, Thomas KJ, Baekelandt V, Cookson MR. The Parkinson disease-associated leucine-rich repeat kinase 2 (LRRK2) is a dimer that undergoes intramolecular autophosphorylation. *J Biol Chem*. 2008; 283(24):16906–16914. DOI: 10.1074/jbc.M708718200 [PubMed: 18397888]
14. Sen S, Webber PJ, West AB. Dependence of leucine-rich repeat kinase 2 (LRRK2) kinase activity on dimerization. *J Biol Chem*. 2009; 284(52):36346–36356. DOI: 10.1074/jbc.M109.025437 [PubMed: 19826009]
15. Biosia A, Trancikova A, Civiero L, Glauser L, Bubacco L, Greggio E, Moore DJ. GTPase activity regulates kinase activity and cellular phenotypes of Parkinson's disease-associated LRRK2. *Hum Mol Genet*. 2013; 22(6):1140–1156. DOI: 10.1093/hmg/ddt522 [PubMed: 23241358]
16. Deng J, Lewis PA, Greggio E, Sluch E, Beilina A, Cookson MR. Structure of the ROC domain from the Parkinson's disease-associated leucine-rich repeat kinase 2 reveals a dimeric GTPase. *Proc Natl Acad Sci U S A*. 2008; 105(5):1499–1504. DOI: 10.1073/pnas.0709098105 [PubMed: 18230735]
17. Gilsbach BK, Ho FY, Vetter IR, van Haastert PJ, Wittinghofer A, Kortholt A. Roco kinase structures give insights into the mechanism of Parkinson disease-related leucine-rich-repeat kinase 2 mutations. *Proc Natl Acad Sci U S A*. 2012; 109(26):10322–10327. DOI: 10.1073/pnas.1203223109 [PubMed: 22689969]
18. Greggio E, Cookson M. Leucine-rich repeat kinase 2 mutations and Parkinson's disease: three questions. *ASN Neuro*. 2009; 1(1):e00002. [PubMed: 19570025]
19. Marín I. The parkinson disease gene LRRK2: evolutionary and structural insights. *Mol Biol Evol*. 2006; 23(12):2423–2433. DOI: 10.1093/molbev/msl114 [PubMed: 16966681]
20. Marín I, van Egmond WN, van Haastert PJM. The Roco protein family: a functional perspective. *FASEB J*. 2008; 22(9):3103–3110. DOI: 10.1096/fj.08-111310 [PubMed: 18523161]
21. Bosgraaf L, Van Haastert PJM. Roc, a Ras/GTPase domain in complex proteins. *Biochim Biophys Acta*. 2003; 1643(1–3):5–10. DOI: 10.1016/j.bbamcr.2003.08.008 [PubMed: 14654223]
22. Gasper R, Meyer S, Gotthardt K, Sirajuddin M, Wittinghofer A. It takes two to tango: regulation of G proteins by dimerization. *Nat Rev Mol Cell Biol*. 2009; 10(6):423–429. [PubMed: 19424291]
23. Gotthardt K, Weyand M, Kortholt A, Van Haastert PJM, Wittinghofer A. Structure of the Roc-COR domain tandem of *C. tepidum*, a prokaryotic homologue of the human LRRK2 Parkinson kinase. *EMBO J*. 2008; 27(16):2239–2249. [PubMed: 18650931]
24. Civiero L, Dihanich S, Lewis PA, Greggio E. Genetic, structural, and molecular insights into the function of ras of complex proteins domains. *Chem Biol*. 2014; 21(7):809–818. DOI: 10.1016/j.chembiol.2014.05.010 [PubMed: 24981771]
25. Gilsbach BK, Kortholt A. Structural biology of the LRRK2 GTPase and kinase domains: implications for regulation. *Front Mol Neurosci*. 2014; 7:32.doi: 10.3389/fnmol.2014.00032 [PubMed: 24847205]
26. Giordana MT, D'Agostino C, Albani G, Mauro A, Di Fonzo A, Antonini A, Bonifati V. Neuropathology of Parkinson's disease associated with the LRRK2 Ile1371Val mutation. *Mov Disord*. 2007; 22(2):275–278. DOI: 10.1002/mds.21281 [PubMed: 17149743]
27. Tan EK, Peng R, Teo YY, Tan LC, Angeles D, Ho P, Chen ML, Lin CH, Mao XY, Chang XL, Prakash KM, Liu JJ, Au WL, Le WD, Jankovic J, Burgunder JM, Zhao Y, Wu RM. Multiple LRRK2 variants modulate risk of Parkinson disease: a Chinese multicenter study. *Hum Mutat*. 2010; 31(5):561–568. DOI: 10.1002/humu.21225 [PubMed: 20186690]
28. Ross OA, Soto-Ortolaza AI, Heckman MG, Aasly JO, Abahuni N, Annesi G, Bacon JA, Bardien S, Bozi M, Brice A, Brighina L, Van Broeckhoven C, Carr J, Chartier-Harlin MC, Dardiotis E, Dickson DW, Diehl NN, Elbaz A, Ferrarese C, Ferraris A, Fiske B, Gibson JM, Gibson R, Hadjigeorgiou GM, Hattori N, Ioannidis JP, Jasinska-Myga B, Jeon BS, Kim YJ, Klein C, Kruger R, Kyrtzi E, Lesage S, Lin CH, Lynch T, Maraganore DM, Mellick GD, Mutez E, Nilsson C, Opala G, Park SS, Puschmann A, Quattrone A, Sharma M, Silburn PA, Sohn YH, Stefanis L, Tadic V, Theuns J, Tomiyama H, Uitti RJ, Valente EM, van de Loo S, Vassilatis DK, Vilarino-

Guell C, White LR, Wirdefeldt K, Wszolek ZK, Wu RM, Farrer MJ, Genetic Epidemiology of Parkinson's Disease (GEO-PD) Consortium. Association of LRRK2 exonic variants with susceptibility to Parkinson's disease: a case-control study. *Lancet Neurol.* 2011; 10(10):898–908. DOI: 10.1016/S1474-4422(11)70175-2 [PubMed: 21885347]

29. Heckman MG, Soto-Ortolaza AI, Aasly JO, Abahuni N, Annesi G, Bacon JA, Bardien S, Bozi M, Brice A, Brighina L, Carr J, Chartier-Harlin MC, Dardiotis E, Dickson DW, Diehl NN, Elbaz A, Ferrarese C, Fiske B, Gibson JM, Gibson R, Hadjigeorgiou GM, Hattori N, Ioannidis JP, Boczarska-Jedynak M, Jasinska-Myga B, Jeon BS, Kim YJ, Klein C, Kruger R, Kyratzi E, Lesage S, Lin CH, Lynch T, Maraganore DM, Mellick GD, Mutez E, Nilsson C, Opala G, Park SS, Petrucci S, Puschmann A, Quattrone A, Sharma M, Silburn PA, Sohn YH, Stefanis L, Tadic V, Theuns J, Tomiyama H, Uitti RJ, Valente EM, Van Broeckhoven C, van de Loo S, Vassilatis DK, Vilarino-Guell C, White LR, Wirdefeldt K, Wszolek ZK, Wu RM, Hentati F, Farrer MJ, Ross OA, Genetic Epidemiology of Parkinson's Disease (GEO-PD) Consortium. Population-specific frequencies for LRRK2 susceptibility variants in the Genetic Epidemiology of Parkinson's Disease (GEO-PD) Consortium. *Mov Disord.* 2013; 28(12):1740–1744. DOI: 10.1002/mds.25600 [PubMed: 23913756]
30. Khan NL, Jain S, Lynch JM, Pavese N, Abou-Sleiman P, Holton JL, Healy DG, Gilks WP, Sweeney MG, Ganguly M, Gibbons V, Gandhi S, Vaughan J, Eunson LH, Katzenschlager R, Gayton J, Lennox G, Revesz T, Nicholl D, Bhatia KP, Quinn N, Brooks D, Lees AJ, Davis MB, Piccini P, Singleton AB, Wood NW. Mutations in the gene LRRK2 encoding dardarin (PARK8) cause familial Parkinson's disease: clinical, pathological, olfactory and functional imaging and genetic data. *Brain J Neurol.* 2005; 128(Pt 12):2786–2796. DOI: 10.1093/brain/awh667
31. Ito G, Okai T, Fujino G, Takeda K, Ichijo H, Katada T, Iwatsubo T. GTP binding is essential to the protein kinase activity of LRRK2, a causative gene product for familial Parkinson's disease. *Biochemistry.* 2007; 46(5):1380–1388. DOI: 10.1021/bi061960m [PubMed: 17260967]
32. Lewis PA, Greggio E, Beilina A, Jain S, Baker A, Cookson MR. The R1441C mutation of LRRK2 disrupts GTP hydrolysis. *Biochem Biophys Res Commun.* 2007; 357(3):668–671. DOI: 10.1016/j.bbrc.2007.04.006 [PubMed: 17442267]
33. Li X, Tan Y-C, Poulose S, Olanow CW, Huang X-Y, Yue Z. Leucine-rich repeat kinase 2 (LRRK2)/PARK8 possesses GTPase activity that is altered in familial Parkinson's disease R1441C/G mutants. *J Neurochem.* 2007; 103(1):238–247. DOI: 10.1111/j.1471-4159.2007.04743.x [PubMed: 17623048]
34. Smith WW, Pei Z, Jiang H, Dawson VL, Dawson TM, Ross CA. Kinase activity of mutant LRRK2 mediates neuronal toxicity. *Nat Neurosci.* 2006; 9(10):1231–1233. [PubMed: 16980962]
35. West AB, Moore DJ, Choi C, Andrabi SA, Li X, Dikeman D, Biskup S, Zhang Z, Lim K-L, Dawson VL, Dawson TM. Parkinson's disease-associated mutations in LRRK2 link enhanced GTP-binding and kinase activities to neuronal toxicity. *Hum Mol Genet.* 2007; 16(2):223–232. DOI: 10.1093/hmg/ddl471 [PubMed: 17200152]
36. Daniëls V, Vancraenenbroeck R, Law BMH, Greggio E, Lobbestael E, Gao F, De Maeyer M, Cookson MR, Harvey K, Baekelandt V, Taymans J-M. Insight into the mode of action of the LRRK2 Y1699C pathogenic mutant. *J Neurochem.* 2011; 116(2):304–315. DOI: 10.1111/j.1471-4159.2010.07105.x [PubMed: 21073465]
37. Xiong Y, Coombes CE, Kilaru A, Li X, Gitler AD, Bowers WJ, Dawson VL, Dawson TM, Moore DJ. GTPase activity plays a key role in the pathobiology of LRRK2. *PLoS Genet.* 2010; 6(4):e1000902. [PubMed: 20386743]
38. Liao J, Wu CX, Burlak C, Zhang S, Sahm H, Wang M, Zhang ZY, Vogel KW, Federici M, Riddle SM, Nichols RJ, Liu D, Cookson MR, Stone TA, Hoang QQ. Parkinson disease-associated mutation R1441H in LRRK2 prolongs the "active state" of its GTPase domain. *Proc Natl Acad Sci U S A.* 2014; 111(11):4055–4060. DOI: 10.1073/pnas.1323285111 [PubMed: 24591621]
39. Jaleel M, Nichols RJ, Deak M, Campbell DG, Gillardon F, Knebel A, Alessi DR. LRRK2 phosphorylates moesin at threonine-558: characterization of how Parkinson's disease mutants affect kinase activity. *Biochem J.* 2007; 405(2):307–317. DOI: 10.1042/bj20070209 [PubMed: 17447891]

40. West AB, Moore DJ, Biskup S, Bugayenko A, Smith WW, Ross CA, Dawson VL, Dawson TM. Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proc Natl Acad Sci U S A*. 2005; 102(46):16842–16847. [PubMed: 16269541]
41. Taymans J-M, Vancraenenbroeck R, Ollikainen P, Beilina A, Lobbstaël E, De Maeyer M, Baekelandt V, Cookson MR. LRRK2 kinase activity is dependent on LRRK2 GTP binding capacity but independent of LRRK2 GTP binding. *PLoS One*. 2011; 6(8):e23207.doi: 10.1371/journal.pone.0023207 [PubMed: 21858031]
42. Stafa K, Trancikova A, Webber PJ, Glauser L, West AB, Moore DJ. GTPase activity and neuronal toxicity of Parkinson's disease-associated LRRK2 is regulated by ArfGAP1. *PLoS Genet*. 2012; 8(2):e1002526.doi: 10.1371/journal.pgen.1002526 [PubMed: 22363216]
43. Haebig K, Gloeckner CJ, Miralles MG, Gillardon F, Schulte C, Riess O, Ueffing M, Biskup S, Bonin M. ARHGEF7 (beta-PIX) acts as guanine nucleotide exchange factor for leucine-rich repeat kinase 2. *PLoS One*. 2010; 5(10):e13762.doi: 10.1371/journal.pone.0013762 [PubMed: 21048939]
44. Chia R, Haddock S, Beilina A, Rudenko IN, Mamais A, Kaganovich A, Li Y, Kumaran R, Nalls MA, Cookson MR. Phosphorylation of LRRK2 by casein kinase 1alpha regulates trans-Golgi clustering via differential interaction with ARHGEF7. *Nat Commun*. 2014; 5:5827.doi: 10.1038/ncomms6827 [PubMed: 25500533]
45. Xiong Y, Yuan C, Chen R, Dawson TM, Dawson VL. ArfGAP1 is a GTPase activating protein for LRRK2: reciprocal regulation of ArfGAP1 by LRRK2. *J Neurosci*. 2012; 32(11):3877–3886. DOI: 10.1523/jneurosci.4566-11.2012 [PubMed: 22423108]
46. Dusanochet J, Li H, Guillily M, Liu M, Stafa K, Derada Troletti C, Boon JY, Saha S, Glauser L, Mamais A, Citro A, Youmans KL, Liu L, Schneider BL, Aebischer P, Yue Z, Bandopadhyay R, Glicksman MA, Moore DJ, Collins JJ, Wolozin B. A Parkinson's disease gene regulatory network identifies the signaling protein RGS2 as a modulator of LRRK2 activity and neuronal toxicity. *Hum Mol Genet*. 2014; 23(18):4887–4905. DOI: 10.1093/hmg/ddu202 [PubMed: 24794857]
47. Terheyden S, Ho FY, Gilsbach BK, Wittinghofer A, Kortholt A. Revisiting the Roco G-protein cycle. *Biochem J*. 2015; 465(1):139–147. DOI: 10.1042/BJ20141095 [PubMed: 25317655]
48. Jorgensen ND, Peng Y, Ho CC, Rideout HJ, Petrey D, Liu P, Dauer WT. The WD40 domain is required for LRRK2 neurotoxicity. *PLoS One*. 2009; 4(12):e8463.doi: 10.1371/journal.pone.0008463 [PubMed: 20041156]
49. Liu Z, Mobley JA, DeLucas LJ, Kahn RA, West AB. LRRK2 autophosphorylation enhances its GTPase activity. *FASEB J*. 2016; 30(1):336–347. DOI: 10.1096/fj.15-277095 [PubMed: 26396237]
50. Gloeckner CJ, Boldt K, von Zweydt F, Helm S, Wiesent L, Sarioglu H, Ueffing M. Phosphopeptide analysis reveals two discrete clusters of phosphorylation in the N-terminus and the Roc domain of the Parkinson-disease associated protein kinase LRRK2. *J Proteome Res*. 2010; 9(4):1738–1745. DOI: 10.1021/pr9008578 [PubMed: 20108944]
51. Greggio E, Taymans JM, Zhen EY, Ryder J, Vancraenenbroeck R, Beilina A, Sun P, Deng J, Jaffe H, Baekelandt V, Merchant K, Cookson MR. The Parkinson's disease kinase LRRK2 autophosphorylates its GTPase domain at multiple sites. *Biochem Biophys Res Commun*. 2009; 389(3):449–454. DOI: 10.1016/j.bbrc.2009.08.163 [PubMed: 19733152]
52. Kamikawaji S, Ito G, Iwatsubo T. Identification of the autophosphorylation sites of LRRK2. *Biochemistry*. 2009; 48(46):10963–10975. DOI: 10.1021/bi9011379 [PubMed: 19824698]
53. Webber PJ, Smith AD, Sen S, Renfrow MB, Mobley JA, West AB. Autophosphorylation in the leucine-rich repeat kinase 2 (LRRK2) GTPase domain modifies kinase and GTP-binding activities. *J Mol Biol*. 2011; 412(1):94–110. DOI: 10.1016/j.jmb.2011.07.033 [PubMed: 21806997]
54. Sheng Z, Zhang S, Bustos D, Kleinheinz T, Le Pichon CE, Dominguez SL, Solanoy HO, Drummond J, Zhang X, Ding X, Cai F, Song Q, Li X, Yue Z, van der Brug MP, Burdick DJ, Gunzner-Toste J, Chen H, Liu X, Estrada AA, Sweeney ZK, Scarce-Levie K, Moffat JG, Kirkpatrick DS, Zhu H. Ser1292 autophosphorylation is an indicator of LRRK2 kinase activity and contributes to the cellular effects of PD mutations. *Sci Trans Med*. 2012; 4(164):164ra161.doi: 10.1126/scitranslmed.3004485
55. Muda K, Bertinetti D, Gesellchen F, Hermann JS, von Zweydt F, Geerlof A, Jacob A, Ueffing M, Gloeckner CJ, Herberg FW. Parkinson-related LRRK2 mutation R1441C/G/H impairs PKA

phosphorylation of LRRK2 and disrupts its interaction with 14-3-3. *Proc Natl Acad Sci U S A*. 2014; 111(1):E34–E43. DOI: 10.1073/pnas.1312701111 [PubMed: 24351927]

56. Greggio E, Jain S, Kingsbury A, Bandopadhyay R, Lewis P, Kaganovich A, van der Brug MP, Beilina A, Blackinton J, Thomas KJ, Ahmad R, Miller DW, Kesavapany S, Singleton A, Lees A, Harvey RJ, Harvey K, Cookson MR. Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. *Neurobiol Dis*. 2006; 23(2):329–341. [PubMed: 16750377]
57. Lee BD, Shin J-H, VanKampen J, Petrucelli L, West AB, Ko HS, Lee Y-I, Maguire-Zeiss KA, Bowers WJ, Federoff HJ, Dawson VL, Dawson TM. Inhibitors of leucine-rich repeat kinase-2 protect against models of Parkinson's disease. *Nat Med*. 2010; 16(9):998–1000. [PubMed: 20729864]
58. MacLeod D, Dowman J, Hammond R, Leete T, Inoue K, Abeliovich A. The familial Parkinsonism gene LRRK2 regulates neurite process morphology. *Neuron*. 2006; 52(4):587–593. [PubMed: 17114044]
59. Ramsden N, Perrin J, Ren Z, Lee BD, Zinn N, Dawson VL, Tam D, Bova M, Lang M, Drewes G, Bantscheff M, Bard F, Dawson TM, Hopf C. Chemoproteomics-based design of potent LRRK2-selective lead compounds that attenuate Parkinson's disease-related toxicity in human neurons. *ACS Chem Biol*. 2011; 6(10):1021–1028. DOI: 10.1021/cb2002413 [PubMed: 21812418]
60. Manzoni C, Mamais A, Dihanich S, Abeti R, Soutar MP, Plun-Favreau H, Giunti P, Tooze SA, Bandopadhyay R, Lewis PA. Inhibition of LRRK2 kinase activity stimulates macroautophagy. *Biochim Biophys Acta*. 2013; 1833(12):2900–2910. DOI: 10.1016/j.bbamcr.2013.07.020 [PubMed: 23916833]
61. Manzoni C, Mamais A, Dihanich S, McGoldrick P, Devine MJ, Zerle J, Kara E, Taanman JW, Healy DG, Marti-Masso JF, Schapira AH, Plun-Favreau H, Tooze S, Hardy J, Bandopadhyay R, Lewis PA. Pathogenic Parkinson's disease mutations across the functional domains of LRRK2 alter the autophagic/lysosomal response to starvation. *Biochem Biophys Res Commun*. 2013; 441(4):862–866. DOI: 10.1016/j.bbrc.2013.10.159 [PubMed: 24211199]
62. Plowey ED, Cherra SJ 3rd, Liu YJ, Chu CT. Role of autophagy in G2019S-LRRK2-associated neurite shortening in differentiated SH-SY5Y cells. *J Neurochem*. 2008; 105(3):1048–1056. DOI: 10.1111/j.1471-4159.2008.05217.x [PubMed: 18182054]
63. Ramonet D, Daher JP, Lin BM, Stafa K, Kim J, Banerjee R, Westerlund M, Pletnikova O, Glauser L, Yang L, Liu Y, Swing DA, Beal MF, Troncoso JC, McCaffery JM, Jenkins NA, Copeland NG, Galter D, Thomas B, Lee MK, Dawson TM, Dawson VL, Moore DJ. Dopaminergic neuronal loss, reduced neurite complexity and autophagic abnormalities in transgenic mice expressing G2019S mutant LRRK2. *PLoS One*. 2011; 6(4):e18568.doi: 10.1371/journal.pone.0018568 [PubMed: 21494637]
64. Alegre-Abarrategui J, Christian H, Lufino MM, Mutihac R, Venda LL, Ansorge O, Wade-Martins R. LRRK2 regulates autophagic activity and localizes to specific membrane microdomains in a novel human genomic reporter cellular model. *Hum Mol Genet*. 2009; 18(21):4022–4034. DOI: 10.1093/hmg/ddp346 [PubMed: 19640926]
65. Daniel G, Moore DJ. Modeling LRRK2 pathobiology in Parkinson's disease: from yeast to rodents. *Curr Top Behav Neurosci*. 2015; 22:331–368. DOI: 10.1007/7854_2014_311 [PubMed: 24850078]
66. Godena VK, Brookes-Hocking N, Moller A, Shaw G, Oswald M, Sancho RM, Miller CC, Whitworth AJ, De Vos KJ. Increasing microtubule acetylation rescues axonal transport and locomotor deficits caused by LRRK2 Roc-COR domain mutations. *Nat Commun*. 2014; 5:5245.doi: 10.1038/ncomms6245 [PubMed: 25316291]
67. Li Y, Liu W, Oo TF, Wang L, Tang Y, Jackson-Lewis V, Zhou C, Geghman K, Bogdanov M, Przedborski S, Beal MF, Burke RE, Li C. Mutant LRRK2(R1441G) BAC transgenic mice recapitulate cardinal features of Parkinson's disease. *Nat Neurosci*. 2009; 12(7):826–828. [PubMed: 19503083]
68. Tagliaferro P, Kareva T, Oo TF, Yarygina O, Kholodilov N, Burke RE. An early axonopathy in a hLRRK2(R1441G) transgenic model of Parkinson disease. *Neurobiol Dis*. 2015; 82:359–371. DOI: 10.1016/j.nbd.2015.07.009 [PubMed: 26192625]

69. Tong Y, Pisani A, Martella G, Karouani M, Yamaguchi H, Pothos EN, Shen J. R1441C mutation in LRRK2 impairs dopaminergic neurotransmission in mice. *Proc Natl Acad Sci U S A*. 2009; 106(34):14622–14627. DOI: 10.1073/pnas.0906334106 [PubMed: 19667187]
70. Tsika E, Kannan M, Foo CS, Dikeman D, Glauser L, Gellhaar S, Galter D, Knott GW, Dawson TM, Dawson VL, Moore DJ. Conditional expression of Parkinson's disease-related R1441C LRRK2 in midbrain dopaminergic neurons of mice causes nuclear abnormalities without neurodegeneration. *Neurobiol Dis*. 2014; 71:345–358. DOI: 10.1016/j.nbd.2014.08.027 [PubMed: 25174890]
71. Tsika E, Nguyen AP, Dusanochet J, Colin P, Schneider BL, Moore DJ. Adenoviral-mediated expression of G2019S LRRK2 induces striatal pathology in a kinase-dependent manner in a rat model of Parkinson's disease. *Neurobiol Dis*. 2015; 77:49–61. DOI: 10.1016/j.nbd.2015.02.019 [PubMed: 25731749]
72. Daher JP, Abdelmotilib HA, Hu X, Volpicelli-Daley LA, Moehle MS, Fraser KB, Needle E, Chen Y, Steyn SJ, Galatsis P, Hirst WD, West AB. Leucine-rich repeat kinase 2 (LRRK2) pharmacological inhibition abates alpha-synuclein gene-induced neurodegeneration. *J Biol Chem*. 2015; 290(32):19433–19444. DOI: 10.1074/jbc.M115.660001 [PubMed: 26078453]
73. Li T, Yang D, Zhong S, Thomas JM, Xue F, Liu J, Kong L, Voulalas P, Hassan HE, Park JS, MacKerell AD Jr, Smith WW. Novel LRRK2 GTP-binding inhibitors reduced degeneration in Parkinson's disease cell and mouse models. *Hum Mol Genet*. 2014; 23(23):6212–6222. DOI: 10.1093/hmg/ddu341 [PubMed: 24993787]
74. Li T, He X, Thomas JM, Yang D, Zhong S, Xue F, Smith WW. A novel GTP-binding inhibitor, FX2149, attenuates LRRK2 toxicity in Parkinson's disease models. *PLoS One*. 2015; 10(3):e0122461.doi: 10.1371/journal.pone.0122461 [PubMed: 25816252]
75. Dusanochet J, Kochubey O, Stafa K, Young SM Jr, Zufferey R, Moore DJ, Schneider BL, Aebischer P. A rat model of progressive nigral neurodegeneration induced by the Parkinson's disease-associated G2019S mutation in LRRK2. *J Neurosci*. 2011; 31(3):907–912. DOI: 10.1523/jneurosci.5092-10.2011 [PubMed: 21248115]

Fig. 4.1.

Domain architecture, familial mutations, and functional residues of human LRRK2. LRRK2 is shown as a homodimeric multi-domain protein. Individual LRRK2 domains are depicted in the full-length protein with their respective amino acid positions: ARM, armadillo repeat region; ANK, ankyrin repeat region; LRR, leucine-rich repeats; Roc, Ras-of-complex GTPase domain; COR, C-terminal-of-Roc domain; kinase, protein tyrosine kinase-like domain; WD40, WD40 repeat region. Familial pathogenic mutations that segregate with PD are indicated in red that cluster within the Roc-COR-kinase catalytic region, whereas key functional residues that alter enzymatic activity within the Roc GTPase and kinase domains are indicated in green. The Roc GTPase domain (shown at the top) contains five G-box motifs that are conserved in members of the small GTPase superfamily: guanine nucleotide phosphate-binding loop (P-loop) that binds to GDP or GTP, switch I and II motifs that change conformation upon GTP binding and regulate GTP hydrolysis, and G4 and G5 motifs. K1347A and T1348N mutations impair GDP/GTP binding, whereas R1398L or R1398Q/T1343G increases GTP hydrolysis and R1398L/T1343V impairs GTP hydrolysis