Contribution of GTPase activity to *LRRK2*-associated Parkinson disease

Elpida Tsika and Darren J. Moore*

Laboratory of Molecular Neurodegenerative Research; Brain Mind Institute; School of Life Sciences; Ecole Polytechnique Fédérale de Lausanne (EPFL); Lausanne, Switzerland

Mutations in the *leucine-rich repeat kinase 2* (*LRRK2, PARK8,* OMIM 607060) gene represent the most common known cause of hereditary Parkinson's disease (PD) with late-onset and dominant inheritance. LRRK2 protein is composed of multiple domains including two distinct enzymatic domains, a kinase and a Ras-of-complex (Roc) GTPase, connected by a C-terminal-of-Roc (COR) domain, and belongs to the ROCO protein family. Disease-causing mutations located in the kinase domain enhance kinase activity (i.e., G2019S) whereas mutations clustering within the Roc-COR tandem domain impair GTPase activity (i.e., R1441C/G and Y1699C). Familial LRRK2 mutations commonly induce neuronal toxicity that, at least for the frequent G2019S variant, is dependent on kinase activity. The contribution of GTPase activity to LRRK2-dependent neuronal toxicity is not yet clear. Therefore, both GTPase and kinase activity may be important for mediating neurodegeneration in PD due to familial *LRRK2* mutations. At present, the physiological function of LRRK2 in the mammalian brain and the regulation of its enzymatic activity are incompletely understood. In this review, we focus on the GTPase domain of LRRK2 and discuss the recent advances in elucidating its function and its interplay with the kinase domain for the regulation of LRRK2 activity and toxicity. GTPase activity is an important feature of LRRK2 biology and pathophysiology and represents an underexplored yet potentially tractable therapeutic target for treating *LRRK2*-associated PD.

Introduction

Parkinson disease (PD) is a devastating neurodegenerative movement disorder affecting 1 to 2% of the population above 60 y of age.[1,2] Typical motor symptoms of PD are resting tremor, brady-kinesia, muscular rigidity and postural instability. The manifestation of the motor symptoms coincides with a marked reduction of dopamine levels, which results from the pronounced loss of dopaminergic neurons in the substantia nigra pars compacta and the degeneration of their projections to the caudate-putamen.[2] A characteristic patho-anatomical feature of PD is the deposition of protein inclusions in the soma or processes of surviving brainstem

neurons, termed Lewy bodies and neurites, respectively, which are composed mainly of fibrillar α-synuclein protein.[3] The etiology of PD is unknown and most cases occur sporadically; however, since 1997 several mutations in genes of no apparent connection to each other have been identified to cause hereditary PD.[4] The occurrence of mutations in the genes of *SNCA* (α-synuclein), *PARK2* (parkin), *DJ-1*, *PINK1*, *ATP13A2*, *VPS35* (vacuolar protein sorting 35), *EIF4G1* (eukaryotic initiation factor 4G1) and *LRRK2* (leucine-rich repeat kinase 2) are relatively rare. However, understanding the impact of pathogenic mutations on the physiological function of these gene products is of major importance in order to elucidate the molecular pathways underlying neurodegeneration in PD.

LRRK2 has attracted significant attention since genetic studies revealed that missense mutations in the protein represent the most common cause of familial PD.[5-7] Moreover, genome-wide association studies have identified common variation in the *LRRK2* gene as a risk factor for sporadic PD.[8,9] From the numerous putative pathogenic variants in *LRRK2* that have been identified, at least seven missense mutations—R1441C, R1441G, R1441H, N1437H, Y1699C, G2019S and I2020T—are considered truly pathogenic since they segregate with disease in families with PD.[1,10] The G2019S mutation is the most frequent *LRRK2* mutation, reported within a range of 5 to 40% of familial cases, depending on the ethnicity of the patient population.[10] *LRRK2* mutations are inherited in a dominant fashion and homozygous carriers exhibit the same phenotype and age of disease onset as heterozygous carriers indicating that pathogenic mutations most likely act through a toxic gain-of-function mechanism.[11] Although the clinical characteristics of the disease in *LRRK2* mutation carriers are similar to sporadic PD, the neuropathological findings are partly heterogeneous. The majority of *LRRK2*-linked cases show typical Lewy body pathology; however, some reported cases, mostly related to mutations in the GTPase domain, lack Lewy body pathology and exhibit either ubiquitin-positive inclusions, tauopathy or the absence of pathological inclusions.[7,12-14] The reasons and implications for such pathological heterogeneity in *LRRK2*-linked PD cases are not clear at this juncture.

Structure of LRRK2

LRRK2 is a large 2527 amino acid protein of ~280 kDa consisting of multiple domains that is named for its leucine-rich
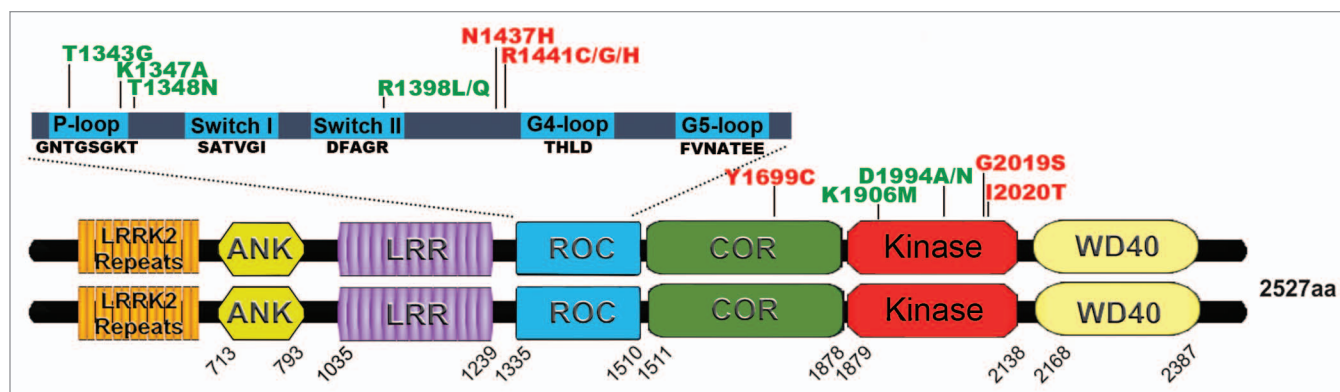
Figure 1. A detailed schematic representation of the GTPase domain of LRRK2 is shown at the top with the conserved motifs between small G proteins shown in blue boxes: the guanine nucleotide phosphate-binding motif (P-loop), switch I and switch II motifs, which undergo conformational changes upon GTP binding, G4 and G5 motifs. The domain architecture of LRRK2 is also depicted with PD-associated mutations which are clustered within the catalytic core (Roc-COR-kinase) of the protein indicated in red and synthetic functional mutations that alter the enzymatic function of LRRK2 within the Roc and kinase domains indicated in green.

repeats and kinase domain. LRRK2 is classified as member of the ROCO family of proteins, a Ras GTPase-related subfamily, which is comprised by large proteins with multiple functional domains. Like other members of this family, LRRK2 contains a Ras-of-Complex (Roc) GTPase domain in tandem with a characteristic domain of unknown function, termed C-terminal-of-Roc (COR), and a serine/threonine-directed protein kinase domain with homology to receptor-interacting protein kinases (RIPKs) and mixed-lineage kinases (MLKs).[15,16] The Roc-COR-kinase catalytic region in the center of the protein is flanked by repeat sequences which comprise putative protein-protein interaction domains: LRRK2-specific, armadillo, ankyrin and leucine-rich repeat motifs in the N-terminal region and WD40 repeats at the C-terminus of the protein (**Fig. 1**).[11]

The ROCO protein family, named for the Roc and COR domains, which are always found in tandem, has a broad evolutionary range being described in plants, prokaryotes, invertebrates and mammals. The first protein belonging to the ROCO family, a cyclic GMP target protein, was discovered in *Dictyostelium discoideum*.[17] In mammals, besides LRRK2 the other known ROCO proteins are DAPK1 (death-associated protein kinase 1), MFHAS1 (malignant fibrous histiocytoma amplified sequence 1) and LRRK1. The latter is the only paralog to LRRK2 in humans and shares the closest sequence homology; they differ in the N-terminal region, which is longer (approximately 650 amino acids) in LRRK2. Of the mammalian ROCO proteins, MFHAS1 does not contain a kinase domain while the kinase domains of LRRK1/2 and DAPK1 proteins differ in sequence suggesting that they evolved independently. These two observations may suggest that GTPase activity is the primary enzymatic functional output of ROCO proteins with the kinase domain potentially arising to regulate this function.[15,18,19] Although there is little information on the regulation and physiological function of ROCO proteins they all appear to be commonly involved in regulating cytoskeleton organization.

The physiological function of LRRK2 is unclear at present. Its distribution within neuronal cells under basal conditions is diffuse and cytoplasmic in close proximity with various intracellular membranous and vesicular structures. Subcellular fractionation experiments and confocal and electron microscopy studies have revealed the association of LRRK2 with the following intracellular structures: mitochondrial outer membrane, endosomes, lysosomes, multivesicular bodies, microtubule-associated vesicles, lipid rafts, the Golgi complex and the endoplasmic reticulum.[11,20,21] From the subcellullar localization of LRRK2 and evidence acquired so far from cellular and animal models, it is suggested to play a role in organization of cytoskeletal and microtubule networks, vesicular trafficking and membrane dynamics as well as in molecular signaling events such as the Wnt pathway and microRNA processing.[11,22-25] It has not yet been clarified how the kinase, GTPase and protein-protein interaction domains of LRRK2 regulate these cellular processes under physiological conditions and consequently how pathogenic mutations affect the enzymatic activity and overall functional output of LRRK2 upon these processes.

Enzymatic Activity of LRRK2

The GTPase domain of LRRK2 comprises only a small fraction (amino acids 1335–1510) of the full length protein (~7% of total); however, it harbors many of the mutations that clearly segregate with PD. The R1441 residue is a particular hotspot for mutations with three confirmed variants (R1441C/G/H) known to cause PD suggesting that this residue is important for the proper function of LRRK2. Recently, another variant within the GTPase domain of LRRK2, N1437H, was identified to cause autosomal dominant PD.[26] The GTPase domain of LRRK2 is composed of motifs that are conserved among all small G proteins: the guanine nucleotide phosphate-binding motif (P-loop), switch I and switch II motifs, which undergo conformational changes upon GTP binding, G4 and G5 motifs (**Fig. 1**).

Several in vitro biochemical studies have unequivocally confirmed that LRRK2 can bind to guanine nucleotides and possesses weak intrinsic GTPase activity.[27-32] The precipitation of

exogenous LRRK2 from cell lysates with GTP-coupled resin, or binding experiments with radiolabeled non-hydrolyzable GTP analogs, have confirmed the GTP binding capacity of LRRK2 while competition with free GTP or GDP can prevent this binding.[27-29,31-33] Familial mutations in the Roc-COR domains, R1441C/G and Y1699C, exhibit decreased GTP hydrolysis when compared with wild-type (WT) LRRK2, but evidence of their effects on the steady-state levels of GTP binding has proved inconsistent with reports of both increased or unaltered binding compared with WT LRRK2.[28,29,32-34] The recently described N1437H mutation has also been reported to increase LRRK2 GTP binding.[26] Based on sequence similarities with the GTPase domain of Rab-like proteins, it is possible to identify synthetic functional mutations that should alter the ability of LRRK2 to bind and hydrolyze GTP.[27] For example, mutations in the conserved Lys1347 and Thr1348 residues within the P-loop dramatically reduce the binding affinity of LRRK2 for guanine nucleotides, therefore the K1347A and T1348N mutations fail to bind GTP or GDP.[27,31,32,35] Such functional mutations have provided important biochemical insight into LRRK2 regulation and activity, and they also serve as useful specificity controls to distinguish LRRK2 GTPase activity from contaminating GTPase proteins co-purified from mammalian cells in certain assays since recombinant full-length LRRK2 has been difficult to purify for enzymatic analyses. A number of studies have consistently shown that the K1347A and T1348N P-loop mutations markedly impair kinase activity indicating that an intact GTPase domain is critically required for kinase activation. Furthermore, the in vitro kinase activity of LRRK2 can be enhanced by addition of GTP (or its analogs) to cell lysates prior to the immunopurification of LRRK2 whereas direct binding of GTP to purified LRRK2 in vitro does not apparently influence kinase activity.[32,35,36] These experiments suggest that kinase activity is dependent on the capacity for GTP binding rather than GTP binding per se. One could speculate that the GTP-dependent interaction of LRRK2 with an unknown guanine nucleotide-binding protein could be important for the regulation of kinase activity; however, evidence for such a mechanism is lacking at present.[32,36]

It is also informative to study the biochemical and cellular properties of functional mutations that promote the supposed GTP-bound "on" or GDP-bound "off" states of LRRK2. Based on sequence similarities with other small GTPases it appears that an Arg1398 residue directly downstream of the catalytic motif (DFAG) in the switch II region is considered critical for GTPase activity.[27] In many small GTPases that most often contain a conserved Gln (Q) at this site, a Q→L substitution results in a constitutively active GTP-locked protein due to impaired GTPase activity. The substitution of Arg to Leu at this position (R1398L) in LRRK2 is expected to result in a GTP-locked 'on' state where the protein is continuously bound to GTP and therefore active due to impaired GTPase activity. However, the R1398L mutation in LRRK2 surprisingly has the opposite effect whereby it exhibits markedly enhanced GTPase activity.[35,37,38] The introduction of a second functional mutation in the P-loop of LRRK2 to generate the double mutant R1398L/T1343V exhibits normal GTP binding but impairs GTP hydrolysis,[35] similar to equivalent

substitutions in related small GTPases, thus representing a GTP-locked form of LRRK2. Unexpectedly, enhancing (R1398L) or impairing (R1398L/T1343V) GTP hydrolysis impairs kinase activity compared with WT LRRK2 indicating that GTP hydrolysis is required to maintain normal kinase activity.[35] In accordance with this observation, treatment of cell lysates with hydrolyzable GTP enhances the kinase activity of LRRK2 to a greater extent than treatment with the non-hydrolyzable GTP analog, GppCp [guanosine-5'-([β,γ]-methyleno)triphosphate], and the enhanced effects of GTP are dependent on the capacity for GTP hydrolysis.[35] Collectively, these studies provide evidence that the kinase activity of LRRK2 is GTPase domain-dependent and requires both GTP binding and GTP hydrolysis for the proper regulation of activity.

Conventional GTPases function as molecular switches cycling between GTP-bound active and GDP-bound inactive states. Nucleotide binding induces conformational changes within the protein that culminate in the activation of effector proteins and the initiation of signal transduction cascades. It has been suggested that a similar mechanism regulates the enzymatic activity of LRRK2. The unusual presence of kinase and GTPase domains within the same protein initially seeded the notion that these two enzymatic activities could be linked together through intramolecular signal transduction whereby the GTPase domain serves to regulate kinase activity. Similar regulatory mechanisms exist for Ras GTPases which directly activate Raf kinases to initiate a phosphorylation-mediated signaling cascade. Current evidence suggests that GTP binding increases kinase activity and functional mutants that cannot bind to GTP (i.e., K1347A or T1348N) consistently show impairment of kinase activity,[27,31,32,35] initially consistent with a classical Ras/Raf-like mechanism. However, the picture is perhaps more complicated than initially conceived since GTP binding capacity rather than direct GTP binding promotes LRRK2 kinase activity implying the requirement for an unknown GTP-dependent accessory protein,[36] whereas the effects of K1347A and T1348N P-loop mutations on disrupting GDP/GTP binding may occur secondary to impaired dimerization, structure and protein stability of LRRK2 making the interpretation of these functional mutations challenging.[35] Furthermore, GTP hydrolysis unexpectedly appears to contribute to LRRK2 kinase activity in addition to GTP binding potentially suggesting that the hydrolysis event itself may in part drive kinase activation.[35] Collectively, current evidence indicates that the intramolecular regulation of GTPase and kinase activities does not conform to a classical Ras/Raf-like mechanism and that LRRK2 may not strictly function as a conventional GTPase.

Biochemical experiments have confirmed that LRRK2 exists as a homodimeric protein.[39-42] Crystallographic studies of the related Roc-COR tandem domain from the *Chlorobium tepidum* ROCO protein suggest that dimerization is mediated through the COR domains.[43] The residues at the COR-COR dimer interface are the most conserved among bacteria ROCO proteins and mammalian LRRK1/2 proteins indicating that dimerization through the COR domains could potentially occur in human LRRK2, although evidence for such an interaction is presently

lacking. So far, other domains of LRRK2 such as the Roc and WD40 domains have been shown to mediate or regulate dimerization.[34,40,41,44] Similar to other protein kinases and G proteins, dimerization of LRRK2 appears to be required for enzymatic activity.[45,46] Mutations that impair dimerization were unable to hydrolyze GTP (i.e., K1347A or T1348N) whereas monomeric LRRK2 lacks kinase activity suggesting that dimerization is critically required for enzymatic activity.[35,42] It has been hypothesized that the GTPase activity of LRRK2 is regulated by homodimerization and LRRK2 activity could be regulated through a G protein activated by nucleotide-dependent dimerization (GAD) mechanism, similar to *C. tepidum* ROCO protein.[43,45] Dimerization via the COR domains induces conformational alterations that bring the Roc domains into close proximity in a guanine nucleotide-dependent manner, with GTP hydrolysis mediated by the adjacent Roc domains coupled to the interaction with an effector protein such as the kinase domain.[43,45] In this model the cycling between GTP-bound and GDP-bound conformations does not require accessory proteins. It is insightful to interpret the effects of pathogenic mutations within a putative GAD model for LRRK2. In the structural model of *C. tepidum* ROCO protein, the equivalent residues to R1441 (Y558 in *Ct* ROCO) and Y1699 (Y804 in *Ct* ROCO) in human LRRK2 are located at a major intramolecular hydrophobic interface between Roc and COR domains, that is thought to contribute to the flexible hinge region required for the juxtaposition of Roc domains upon dimerization.[43] Accordingly, the Y804C and Y558A mutations within *C. tepidum* ROCO protein exhibit normal dimerization but result in impaired GTP hydrolysis compared with WT protein,[43] similar to the Y1699C and R1441C mutations in human LRRK2,[28,33,34] potentially by limiting the flexibility of the Roc/COR interaction within a monomer. In human LRRK2, the R1441C/G/H and Y1699C mutations impair the interaction between isolated Roc and COR domains which recapitulates the expected intramolecular interaction interface if LRRK2 functions as a GAD.[34] Thus, familial mutations could act to disrupt intramolecular Roc/COR interactions within LRRK2 that are required for proper GTPase activity. Although a GAD-like function for LRRK2 could provide an attractive mechanism for the regulation of the GTPase domain, it is important to note that a crystal structure for the Roc-COR tandem domain is not yet available to confirm such a mechanism whereas dimerization of LRRK2 has not been shown to occur in a guanine nucleotide-dependent manner.[34,35,41] Furthermore, LRRK2 dimerization has not yet been demonstrated to occur solely through COR domain interactions whereas instead the Roc and WD40 domains are suggested to be implicated.[34,40,41,44] Furthermore, the crystal structure of the isolated Roc domain of human LRRK2 suggests the existence of a domain-swapped dimer although this has been challenged as a potential artifact of crystallization.[40,43] Overall, the dimerization of LRRK2 seems to be essential for GTPase activity however it is still not clarified if LRRK2 functions as a conventional GTPase or through a GAD mechanism. Structural analyses of the central catalytic domains of human LRRK2 bound to guanine nucleotides is essential to further understand the role of dimerization in regulating enzymatic activity.

An alternative possibility to a GAD mechanism is that the GTPase activity of LRRK2 is regulated through the interaction with regulatory proteins similar to other small GTPases. In this case, guanine nucleotide-exchange factors (GEFs) that catalyze the exchange of GDP for GTP and GTPase-activating proteins (GAPs) that increase the rate of GTP hydrolysis may exist for LRRK2. Indeed it has been postulated that the low rate of GTP hydrolysis by LRRK2 (10-fold lower when compared with Ras) may be due to experimental conditions during measurement that disrupt essential interactions with GAPs or other co-factors.[27,28] ARHGEF7 was reported to be a putative GEF for LRRK2 since the two proteins were found to interact and ARHGEF7 can enhance the GTPase activity of LRRK2 in vitro.[47] In addition, a recent genetic screen in yeast to identify modifiers of LRRK2-mediated toxicity revealed that GTPase-dependent toxicity can be suppressed by deletion of *GCS1*, which encodes the yeast homolog of mammalian ADP-ribosylation factor GAP 1 (ArfGAP1),[33] a GAP for the small GTPase Arf1 involved in Golgi membrane trafficking.[48] Subsequent studies in mammalian cells and neurons demonstrated that ArfGAP1 and LRRK2 co-localize and interact and that ArfGAP1 promotes the GTPase activity of LRRK2 indicating that it may serve as a GAP-like protein.[37,38] Intriguingly, ArfGAP1 also enhances the kinase activity of LRRK2 supporting the idea that GTP hydrolysis can drive kinase activation.[35,37]

At this juncture the major output signal of LRRK2 is considered to be its kinase activity. It has been demonstrated by multiple laboratories that full-length LRRK2 is an active kinase that can autophosphorylate and phosphorylate generic kinase substrates such as myelin basic protein as well as the pseudosubstrate peptides LRRKtide and Nictide in vitro.[22,31,32,49-52] Evidence for putative LRRK2 substrates is largely derived from in vitro studies with recombinant proteins and in some cases invertebrate models although there are a lack of studies reporting authentic kinase substrates in mammalian cells or tissues.[11] Autophosphorylation is reported to be the most robust phosphorylation event mediated by LRRK2 that occurs at multiple serine and threonine residues clustering within or near the GTPase domain.[53-56] The role of autophosphorylation within the LRRK2 GTPase domain is unclear since kinase-inactive mutants of LRRK2 that lack the capacity for autophosphorylation are able to bind and hydrolyze GTP to the same extent as WT LRRK2 in vitro indicating that autophosphorylation is not necessary for GTPase activity.[35] It is likely that autophosphorylation serves a more subtle role in regulating or fine tuning LRRK2 enzymatic activity or is required for the phosphorylation-dependent recruitment and interaction with substrates and/or accessory proteins. Overall, LRRK2 possesses a complex intrinsic regulatory mechanism whereby it is possible that GTP-binding and autophosphorylation events coordinately control kinase activity. Recently, Sheng and colleagues have reported that autophosphorylation at Ser1292 of LRRK2 that occurs in an intramolecular manner is a determinant of neuronal toxicity and this modification can be detected in the brains of G2019S LRRK2 transgenic mice.[57] These observations may have important implications for monitoring LRRK2 kinase activity in vivo especially for the validation of selective kinase inhibitors as potential therapeutics for PD. At present, in the absence of a bona

fide mammalian substrate for LRRK2 kinase activity, it remains an open question whether kinase activity serves as an intrinsic regulatory mechanism or whether there exist physiological substrates that modulate downstream molecular signaling pathways.

The G2019S and I2020T pathogenic mutations are located within the activation loop of the kinase domain. This predicted magnesium binding motif, D(F/Y)G, is essential for substrate access and consequently the G2019S mutation greatly enhances kinase activity in vitro.[22,31,49,50,52] The effects of the I2020T mutation on kinase activity are less clear with some studies reporting enhanced kinase activity compared with WT protein while others report no effect.[32,49-51,57] It is unclear whether these conflicting outcomes are due to differences in assay design, are related to the quantity of LRRK2 protein analyzed in these assays, or whether this mutation acts through a distinct mechanism leading to neuronal damage. If the latter is true then it would indicate that there exist pathogenic mechanisms that are kinase-independent, a mechanism that has also been postulated for the effects of familial mutations that cluster within the Roc-COR tandem domain of LRRK2.

LRRK2-Induced Neurotoxicity

Pathogenic mutations in LRRK2 may culminate in neuronal toxicity either through kinase-dependent or putative GTPase-dependent events. Numerous reports from cultured neural cell lines and primary neurons have demonstrated that expression of G2019S LRRK2 induces neuronal toxicity as assessed by measures of inclusion formation, neurite process shortening and induction of apoptosis (assessed by TUNEL labeling, propidium iodide uptake or nuclear condensation).[11,31,32,50] These neurotoxic phenotypes induced by G2019S and R1441C LRRK2 are diminished or rescued by the simultaneous introduction of kinase-inactive mutations or by pharmacological inhibition of kinase activity.[31,32,50,58,59] Intriguingly, for the R1441C mutation which does not consistently exhibit altered kinase activity, neuronal toxicity is still apparently mediated through a kinase-dependent mechanism.[31,59] Viral-based gene transfer rodent models expressing human G2019S LRRK2 display degeneration of substantia nigra dopaminergic neurons, a hallmark pathological feature of PD, while genetic or pharmacological inhibition of kinase activity attenuates this neuronal loss.[58,60] The contribution of GTPase activity to the pathogenic effects of G2019S LRRK2 are difficult to interpret since genetic inhibition of guanine nucleotide binding via the K1347A or T1348N mutations also severely compromises LRRK2 dimerization and protein stability in cultured rodent neurons resulting in non-equivalent protein levels between LRRK2 variants.[32,35,37] However, modulating GTP hydrolysis activity via the R1398L and R1398L/T1343V mutations was not sufficient to robustly modify the neurite shortening phenotype induced by G2019S LRRK2 in primary neuronal cultures.[35] Thus, the impact of altering GTP binding and hydrolysis on the neurotoxic effects of familial LRRK2 mutations require further studies in neurons and animal models taking advantage of synthetic functional mutations that alter GTPase activity, putative accessory proteins (i.e., ArfGAP1) or in future pharmacological agents that target the GTPase domain of LRRK2.

Despite substantial evidence reinforcing kinase activity as the major pathogenic mediator of LRRK2-induced neurodegeneration, one should also consider the mechanism(s) underlying pathogenic mutations within the Roc-COR tandem domain (R1441C/G and Y1699C) that do not have consistent effects on kinase activity but which instead consistently impair GTPase activity.[33,61] As noted above, kinase activity appears to play a role in the neurotoxic actions of R1441C LRRK2 in cellular models[31,59] but whether this broadly applies to additional familial mutations is not yet known. Likewise, the contribution of GTPase activity to neurotoxicity induced by R1441C LRRK2 (or any Roc-COR mutation) has not been explored. Transgenic or knockin mice expressing human R1441G or R1441C LRRK2 develop motor impairments, defects in dopaminergic neurotransmission and abnormal autophagy suggesting that similar to the G2019S mutation they are capable of exerting pathological effects in vivo.[62-64] Recent studies in simpler models have highlighted the importance of the GTPase domain as a major determinant of LRRK2-induced toxicity in the baker's yeast, *Saccharomyces cerevisiae*, as well as in rodent primary neuronal models.[33,37] Studies in a yeast model demonstrate prominent cellular toxicity induced by expression of the central Roc-COR-kinase catalytic domain of human LRRK2 (since full-length LRRK2 is insoluble in yeast) which is dependent on GTPase activity and is related to deficits in endocytic vesicular trafficking and autophagy. LRRK2-induced cellular toxicity was potentiated by impairing GTP binding/hydrolysis (i.e., via K1347A or T1348N mutations) and attenuated by enhancing GTP hydrolysis (i.e., via R1398L or R1398Q/T1343G mutations).[33] Genome-wide genetic screening in this yeast model identified novel genetic modifiers of LRRK2-induced toxicity most notably *GCS1* encoding an ortholog of ArfGAP1 which suppress toxicity upon deletion in yeast[33] or upon gene silencing in rodent primary neurons.[37] Overall, these observations indicate that the GTPase domain could be central to LRRK2-induced cellular toxicity and may represent a promising molecular target for interfering with this toxicity.

GTPase as a Therapeutic Target?

Mutations in the *LRRK2* locus that unambiguously cause PD are clustered within the central Roc-COR-kinase region of the protein and to date there is no clear unifying mechanistic hypothesis for explaining the pathogenic effects of mutations located in domains with distinct function, which would be critical for the development of broadly-applicable therapeutic agents. The observation that increased kinase activity of LRRK2 is associated with increased neuronal toxicity, at least for the common G2019S mutation, has shifted the primary focus of research toward the identification of kinase inhibitors as potential disease-modifying agents. Accordingly, pharmacological and genetic inhibition of LRRK2 kinase activity has been proven to provide neuroprotective effects in cellular models and in a single rodent model study.[31,32,50,58,59] While a potentially promising approach, further genetic and pharmacological validation in independent LRRK2 animal models is now required, especially using highly selective and brain-penetrable inhibitors

of LRRK2 kinase activity. However, current evidence suggests that the G2019S mutation limits its effects to the kinase domain and does not reciprocally influence GTPase function, whereas familial mutations within the Roc-COR tandem domain impair GTPase activity but without consistent effects on kinase activity. Since GTP binding and hydrolysis both appear to be important for kinase activity, a potential therapeutic strategy that incorporates all familial mutations would be to target the GTPase domain. Potential strategies beyond inhibition of kinase activity might include enhancing GTP hydrolysis, inhibition of GTP binding or disruption of Roc-COR-mediated dimerization to attenuate the functional output of LRRK2 (including kinase activity) and thereby elicit neuroprotection.[65] Many of these strategies have not yet been tested in neuronal culture or rodent models but evaluating such approaches is of particular importance for the development of effective and selective inhibitors of LRRK2. For example, the adenoviral-mediated expression of G2019S LRRK2 in the rat nigrostriatal pathway confers selective and progressive dopaminergic neuronal degeneration within a relatively short time frame that could be employed as a powerful model to rapidly evaluate the potential beneficial effects of genetically inhibiting GTP binding (i.e., T1348N) or modulating GTP hydrolysis (i.e., R1398L or R1398L/T1343V) of LRRK2.[60] Alternatively, strategies to disrupt LRRK2 dimerization via interfering with COR/COR or Roc/COR hydrophobic interaction interfaces would be expected to reduce kinase activity and limit neuronal toxicity. Based on the unique insights we have gained from studying the biochemical and cellular properties of LRRK2 such therapeutic approaches are expected to attenuate kinase activity and subsequent neuronal toxicity that is due to the pathogenic G2019S mutation but would also be beneficial for the R1441C/G/H and Y1699C mutations indicating that targeting enzymatic or structural features of the GTPase domain offers a promising therapeutic approach to treat many cases of *LRRK2*-associated PD.

References

1. Gasser T. Mendelian forms of Parkinson's disease. Biochim Biophys Acta 2009; 1792:587-96; PMID:19168133; http://dx.doi.org/10.1016/j.bbadis.2008.12.007

2. Thomas B, Beal MF. Parkinson's disease. Hum Mol Genet 2007; 16 Spec No. 2:R183-94; PMID:17911161; http://dx.doi.org/10.1093/hmg/ddm159

3. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature 1997; 388:839-40; PMID:9278044; http://dx.doi.org/10.1038/42166

4. Hardy J. Genetic analysis of pathways to Parkinson disease. Neuron 2010; 68:201-6; PMID:20955928; http://dx.doi.org/10.1016/j.neuron.2010.10.014

5. Funayama M, Hasegawa K, Kowa H, Saito M, Tsuji S, Obata F. A new locus for Parkinson's disease (PARK8) maps to chromosome 12p11.2-q13.1. Ann Neurol 2002; 51:296-301; PMID:11891824; http://dx.doi.org/10.1002/ana.10113

6. Paisán-Ruíz C, Jain S, Evans EW, Gilks WP, Simón J, van der Brug M, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 2004; 44:595-600; PMID:15541308; http://dx.doi.org/10.1016/j.neuron.2004.10.023

7. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 2004; 44:601-7; PMID:15541309; http://dx.doi.org/10.1016/j.neuron.2004.11.005

8. Simón-Sánchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet 2009; 41:1308-12; PMID:19915575; http://dx.doi.org/10.1038/ng.487

9. Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, et al. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. Nat Genet 2009; 41:1303-7; PMID:19915576; http://dx.doi.org/10.1038/ng.485

10. Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, et al.; International LRRK2 Consortium. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. Lancet Neurol 2008; 7:583-90; PMID:18539534; http://dx.doi.org/10.1016/S1474-4422(08)70117-0

11. Tsika E, Moore DJ. Mechanisms of LRRK2-mediated neurodegeneration. Curr Neurol Neurosci Rep 2012; 12:251-60; PMID:22441981; http://dx.doi.org/10.1007/s11910-012-0265-8

12. Rajput A, Dickson DW, Robinson CA, Ross OA, Dächsel JC, Lincoln SJ, et al. Parkinsonism, Lrrk2 G2019S, and tau neuropathology. Neurology 2006; 67:1506-8; PMID:17060589; http://dx.doi.org/10.1212/01.wnl.0000240220.33950.0c

13. Wszolek ZK, Pfeiffer RF, Tsuboi Y, Uitti RJ, McComb RD, Stoessl AJ, et al. Autosomal dominant parkinsonism associated with variable synuclein and tau pathology. Neurology 2004; 62:1619-22; PMID:15136696; http://dx.doi.org/10.1212/01.WNL.0000125015.06989.DB

14. Ross OA, Toft M, Whittle AJ, Johnson JL, Papapetropoulos S, Mash DC, et al. Lrrk2 and Lewy body disease. Ann Neurol 2006; 59:388-93; PMID:16437559; http://dx.doi.org/10.1002/ana.20731

15. Bosgraaf L, Van Haastert PJM. Roc, a Ras/GTPase domain in complex proteins. Biochim Biophys Acta 2003; 1643:5-10; PMID:14654223; http://dx.doi.org/10.1016/j.bbamcr.2003.08.008

16. Marín I. The Parkinson disease gene LRRK2: evolutionary and structural insights. Mol Biol Evol 2006; 23:2423-33; PMID:16966681; http://dx.doi.org/10.1093/molbev/msl114

17. Goldberg JM, Bosgraaf L, Van Haastert PJM, Smith JL. Identification of four candidate cGMP targets in Dictyostelium. Proc Natl Acad Sci U S A 2002; 99:6749-54; PMID:12011437; http://dx.doi.org/10.1073/pnas.102167299

18. Marín I, van Egmond WN, van Haastert PJM. The Roco protein family: a functional perspective. FASEB J 2008; 22:3103-10; PMID:18523161; http://dx.doi.org/10.1096/fj.08-111310

19. Taymans JM. The GTPase function of LRRK2. Biochem Soc Trans 2012; 40:1063-9; PMID:22988866; http://dx.doi.org/10.1042/BST20120133

20. Alegre-Abarrategui J, Christian H, Lufino MM, Mutihac R, Venda LL, Ansorge O, et al. LRRK2 regulates autophagic activity and localizes to specific membrane microdomains in a novel human genomic reporter cellular model. Hum Mol Genet 2009; 18:4022-34; PMID:19640926; http://dx.doi.org/10.1093/hmg/ddp346

21. Biskup S, Moore DJ, Celsi F, Higashi S, West AB, Andrabi SA, et al. Localization of LRRK2 to membranous and vesicular structures in mammalian brain. Ann Neurol 2006; 60:557-69; PMID:17120249; http://dx.doi.org/10.1002/ana.21019

22. MacLeod D, Dowman J, Hammond R, Leete T, Inoue K, Abeliovich A. The familial Parkinsonism gene LRRK2 regulates neurite process morphology. Neuron 2006; 52:587-93; PMID:17114044; http://dx.doi.org/10.1016/j.neuron.2006.10.008

23. Matta S, Van Kolen K, da Cunha R, van den Bogaart G, Mandemakers W, Miskiewicz K, et al. LRRK2 controls an EndoA phosphorylation cycle in synaptic endocytosis. Neuron 2012; 75:1008-21; PMID:22998870; http://dx.doi.org/10.1016/j.neuron.2012.08.022

24. Berwick DC, Harvey K. LRRK2 functions as a Wnt signaling scaffold, bridging cytosolic proteins and membrane-localized LRP6. Hum Mol Genet 2012; 21:4966-79; PMID:22899650; http://dx.doi.org/10.1093/hmg/dds342

25. Gehrke S, Imai Y, Sokol N, Lu B. Pathogenic LRRK2 negatively regulates microRNA-mediated translational repression. Nature 2010; 466:637-41; PMID:20671708; http://dx.doi.org/10.1038/nature09191

26. Aasly JO, Vilariño-Güell C, Dachsel JC, Webber PJ, West AB, Haugarvoll K, et al. Novel pathogenic LRRK2 p.Asn1437His substitution in familial Parkinson's disease. Mov Disord 2010; 25:2156-63; PMID:20669305; http://dx.doi.org/10.1002/mds.23265

27. Ito G, Okai T, Fujino G, Takeda K, Ichijo H, Katada T, et al. GTP binding is essential to the protein kinase activity of LRRK2, a causative gene product for familial Parkinson's disease. Biochemistry 2007; 46:1380-8; PMID:17260967; http://dx.doi.org/10.1021/bi061960m

28. 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; 357:668-71; PMID:17442267; http://dx.doi.org/10.1016/j.bbrc.2007.04.006

29. Li X, Tan YC, Poulose S, Olanow CW, Huang XY, 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; 103:238-47; PMID:17623048

30. Liu M, Dobson B, Glicksman MA, Yue Z, Stein RL. Kinetic mechanistic studies of wild-type leucine-rich repeat kinase 2: characterization of the kinase and GTPase activities. Biochemistry 2010; 49:2008-17; PMID:20146535; http://dx.doi.org/10.1021/bi901851y

31. Smith WW, Pei Z, Jiang H, Dawson VL, Dawson TM, Ross CA. Kinase activity of mutant LRRK2 mediates neuronal toxicity. Nat Neurosci 2006; 9:1231-3; PMID:16980962; http://dx.doi.org/10.1038/nn1776

32. West AB, Moore DJ, Choi C, Andrabi SA, Li X, Dikeman D, et al. Parkinson's disease-associated mutations in LRRK2 link enhanced GTP-binding and kinase activities to neuronal toxicity. Hum Mol Genet 2007; 16:223-32; PMID:17200152; http://dx.doi.org/10.1093/hmg/ddl471

33. Xiong Y, Coombes CE, Kilaru A, Li X, Gitler AD, Bowers WJ, et al. GTPase activity plays a key role in the pathobiology of LRRK2. PLoS Genet 2010; 6:e1000902; PMID:20386743; http://dx.doi.org/10.1371/journal.pgen.1000902

34. Daniëls V, Vancraenenbroeck R, Law BMH, Greggio E, Lobbestael E, Gao F, et al. Insight into the mode of action of the LRRK2 Y1699C pathogenic mutant. J Neurochem 2011; 116:304-15; PMID:21073465; http://dx.doi.org/10.1111/j.1471-4159.2010.07105.x

35. Biosa A, Trancikova A, Civiero L, Glauser L, Bubacco L, Greggio E, et al. GTPase activity regulates kinase activity and cellular phenotypes of Parkinson's disease-associated LRRK2. Hum Mol Genet 2013; 22:1140-56; PMID:23241358; http://dx.doi.org/10.1093/hmg/dds522

36. Taymans JM, Vancraenenbroeck R, Ollikainen P, Beilina A, Lobbestael E, De Maeyer M, et al. LRRK2 kinase activity is dependent on LRRK2 GTP binding capacity but independent of LRRK2 GTP binding. PLoS One 2011; 6:e23207; PMID:21858031; http://dx.doi.org/10.1371/journal.pone.0023207

37. 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:e1002526; PMID:22363216; http://dx.doi.org/10.1371/journal.pgen.1002526

38. 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; 32:3877-86; PMID:22423108; http://dx.doi.org/10.1523/JNEUROSCI.4566-11.2012

39. 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; 49:5511-23; PMID:20515039; http://dx.doi.org/10.1021/bi100157u

40. 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; 105:1499-504; PMID:18230735; http://dx.doi.org/10.1073/pnas.0709098105

41. Greggio E, Zambrano I, Kaganovich A, Beilina A, Taymans JM, Daniëls V, et al. The Parkinson disease-associated leucine-rich repeat kinase 2 (LRRK2) is a dimer that undergoes intramolecular autophosphorylation. J Biol Chem 2008; 283:16906-14; PMID:18397888; http://dx.doi.org/10.1074/jbc.M708718200

42. Sen S, Webber PJ, West AB. Dependence of leucine-rich repeat kinase 2 (LRRK2) kinase activity on dimerization. J Biol Chem 2009; 284:36346-56; PMID:19826009; http://dx.doi.org/10.1074/jbc.M109.025437

43. 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; 27:2239-49; PMID:18650931; http://dx.doi.org/10.1038/emboj.2008.150

44. Jorgensen ND, Peng Y, Ho CCY, Rideout HJ, Petrey D, Liu P, et al. The WD40 domain is required for LRRK2 neurotoxicity. PLoS One 2009; 4:e8463; PMID:20041156; http://dx.doi.org/10.1371/journal.pone.0008463

45. 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; 10:423-9; PMID:19424291; http://dx.doi.org/10.1038/nrm2689

46. Leung IWL, Lassam N. Dimerization via tandem leucine zippers is essential for the activation of the mitogen-activated protein kinase kinase kinase, MLK-3. J Biol Chem 1998; 273:32408-15; PMID:9829970; http://dx.doi.org/10.1074/jbc.273.49.32408

47. Haebig K, Gloeckner CJ, Miralles MG, Gillardon F, Schulte C, Riess O, et al. ARHGEF7 (Beta-PIX) acts as guanine nucleotide exchange factor for leucine-rich repeat kinase 2. PLoS One 2010; 5:e13762; PMID:21048939; http://dx.doi.org/10.1371/journal.pone.0013762

48. Shiba Y, Randazzo PA. ArfGAP1 function in COPI mediated membrane traffic: currently debated models and comparison to other coat-binding ArfGAPs. Histol Histopathol 2012; 27:1143-53; PMID:22806901

49. Gloeckner CJ, Kinkl N, Schumacher A, Braun RJ, O'Neill E, Meitinger T, et al. The Parkinson disease causing LRRK2 mutation I2020T is associated with increased kinase activity. Hum Mol Genet 2006; 15:223-32; PMID:16321986; http://dx.doi.org/10.1093/hmg/ddi439

50. Greggio E, Jain S, Kingsbury A, Bandopadhyay R, Lewis P, Kaganovich A, et al. Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. Neurobiol Dis 2006; 23:329-41; PMID:16750377; http://dx.doi.org/10.1016/j.nbd.2006.04.001

51. Jaleel M, Nichols RJ, Deak M, Campbell DG, Gillardon F, Knebel A, et al. LRRK2 phosphorylates moesin at threonine-558: characterization of how Parkinson's disease mutants affect kinase activity. Biochem J 2007; 405:307-17; PMID:17447891; http://dx.doi.org/10.1042/BJ20070209

52. West AB, Moore DJ, Biskup S, Bugayenko A, Smith WW, Ross CA, et al. Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. Proc Natl Acad Sci U S A 2005; 102:16842-7; PMID:16269541; http://dx.doi.org/10.1073/pnas.0507360102

53. Gloeckner CJ, Boldt K, von Zweydorf F, Helm S, Wiesent L, Sarioglu H, et al. 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; 9:1738-45; PMID:20108944; http://dx.doi.org/10.1021/pr9008578

54. Greggio E, Taymans JM, Zhen EY, Ryder J, Vancraenenbroeck R, Beilina A, et al. The Parkinson's disease kinase LRRK2 autophosphorylates its GTPase domain at multiple sites. Biochem Biophys Res Commun 2009; 389:449-54; PMID:19733152; http://dx.doi.org/10.1016/j.bbrc.2009.08.163

55. Kamikawaji S, Ito G, Iwatsubo T. Identification of the autophosphorylation sites of LRRK2. Biochemistry 2009; 48:10963-75; PMID:19824698; http://dx.doi.org/10.1021/bi9011379

56. 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; 412:94-110; PMID:21806997; http://dx.doi.org/10.1016/j.jmb.2011.07.033

57. Sheng Z, Zhang S, Bustos D, Kleinheinz T, Le Pichon CE, Dominguez SL, et al. Ser1292 autophosphorylation is an indicator of LRRK2 kinase activity and contributes to the cellular effects of PD mutations. Sci Trans Med 2012; 4:164ra161.

58. Lee BD, Shin JH, VanKampen J, Petrucelli L, West AB, Ko HS, et al. Inhibitors of leucine-rich repeat kinase-2 protect against models of Parkinson's disease. Nat Med 2010; 16:998-1000; PMID:20729864; http://dx.doi.org/10.1038/nm.2199

59. Ramsden N, Perrin J, Ren Z, Lee BD, Zinn N, Dawson VL, et al. Chemoproteomics-based design of potent LRRK2-selective lead compounds that attenuate Parkinson's disease-related toxicity in human neurons. ACS Chem Biol 2011; 6:1021-8; PMID:21812418; http://dx.doi.org/10.1021/cb2002413

60. Dusonchet J, Kochubey O, Stafa K, Young SM Jr., Zufferey R, Moore DJ, et al. A rat model of progressive nigral neurodegeneration induced by the Parkinson's disease-associated G2019S mutation in LRRK2. J Neurosci 2011; 31:907-12; PMID:21248115; http://dx.doi.org/10.1523/JNEUROSCI.5092-10.2011

61. Greggio E, Cookson MR. Leucine-rich repeat kinase 2 mutations and Parkinson's disease: three questions. ASN Neuro 2009; 1:e00002; PMID:19570025; http://dx.doi.org/10.1042/AN20090007

62. Li Y, Liu W, Oo TF, Wang L, Tang Y, Jackson-Lewis V, et al. Mutant LRRK2(R1441G) BAC transgenic mice recapitulate cardinal features of Parkinson's disease. Nat Neurosci 2009; 12:826-8; PMID:19503083; http://dx.doi.org/10.1038/nn.2349

63. Ramonet D, Daher JP, Lin BM, Stafa K, Kim J, Banerjee R, et al. Dopaminergic neuronal loss, reduced neurite complexity and autophagic abnormalities in transgenic mice expressing G2019S mutant LRRK2. PLoS One 2011; 6:e18568; PMID:21494637; http://dx.doi.org/10.1371/journal.pone.0018568

64. Tong Y, Pisani A, Martella G, Karouani M, Yamaguchi H, Pothos EN, et al. R1441C mutation in LRRK2 impairs dopaminergic neurotransmission in mice. Proc Natl Acad Sci U S A 2009; 106:14622-7; PMID:19667187; http://dx.doi.org/10.1073/pnas.0906334106

65. Rudenko IN, Chia R, Cookson MR. Is inhibition of kinase activity the only therapeutic strategy for LRRK2-associated Parkinson's disease? BMC Med 2012; 10:20; PMID:22361010; http://dx.doi.org/10.1186/1741-7015-10-20