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LRRK2 in Parkinson's Disease: Function in Cells and Neurodegeneration

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Abstract

Detailed characterization of LRRK2 function may provide insight into the molecular basis of neurodegeneration in Parkinson's disease (PD) since mutations in LRRK2 cause a phenotype with strong overlap to typical late-onset disease and LRRK2 mutations are responsible for significant proportions of PD in some populations. The complexity of large multi-domain protein kinases like LRRK2 challenge traditional functional approaches, although initial studies have successfully defined the basic mechanisms of enzyme activity with putative effects of pathogenic mutations on kinase activity. The role of LRRK2 in cells remains elusive, with potential function in MAP kinase pathways, protein translation control, programmed cell death pathways, and activity in cytoskeleton dynamics. The initial focus on LRRK2-kinase dependent phenomena places emphasis on the discovery of LRRK2 kinase substrates, although candidate substrates are yet confined to *in vitro* assays. Herein, hypothetical mechanisms for LRRK2-mediated cell death and kinase activation are proposed. As a promising target for neuroprotection strategies in PD, *in vitro* and *in vivo* models that accurately demonstrate LRRK2 function relevant to neurodegeneration will aid in the identification of molecules with the highest chance for success in the clinic.

The discovery of mutations in the LRRK2 gene in high percentages of Parkinson's disease (PD) cases in some populations redefine the role of genetic susceptibilities in PD, whereby rare and penetrant missense mutations in a single gene are often sufficient to mimic the complex milieu of symptoms associated with typical late-onset disease (reviewed in [1]). PD-affected individuals with the most common LRRK2 mutations usually cannot be differentiated from LRRK2-negative PD in the clinic [2]. The importance of this cannot be overstated since the debate over the relevance of some familial-forms of parkinsonism (and genetic susceptibilities) with typical late-onset PD has raged for over half a century. Thus, the strong overlap with LRRK2 mutations and typical PD suggests common pathogenic mechanisms and the possibility that LRRK2 activity is a rate-limiting factor in disease progression even in cases without LRRK2 mutations [3]. Elucidating the normal function of LRRK2, and the disease inducing functions mediated by mutant LRRK2, promises the opportunity to unveil the molecular basis of PD and the discovery of novel therapeutic targets for intervention and neurorestoration strategies. As of yet, conclusive details regarding the biochemical pathways manipulated by LRRK2 remain elusive. This review dedicates towards summarizing current thinking of what LRRK2 protein might do in cells, in addition to postulating mechanisms of regulation important in neurodegeneration.

Where the mutations lie

Human genetic studies provided the identification of autosomal-dominant mutations that that segregate with disease in a multitude of families from diverse ethnic origins, leaving little doubt regarding the pathogenicity of a number of mutations that tend to cluster in the conserved encoded enzymatic domains (see Kumari and Tan in this issue). However, few hypotheses regarding pathogenicity can be safely discarded through genetics alone since the impact of dominant negative action, haploinsufficiency, or combinations thereof seem to permeate all aspects of complex human disease. Perhaps the most logical route towards understanding how mutant LRRK2 can cause PD first focuses on the difference between PD-associated mutant LRRK2 and wild-type LRRK2 activity. *In vitro* evidence strongly suggests abnormal kinase activity due to the most common (known) pathogenic variant G2019S localized to the activation loop and Mg²⁺ binding site of the kinase domain (see Anand and Braithwaite in this issue). Missense mutations occurring in analogous regions in the b-RAF protein kinase (*e.g.*, the kinase activation loop) that lead to cancer, likewise, cause increases in kinase output. Although not all pathogenic variants in b-RAF recapitulate increased kinase activity *in vitro* (some even show decreased activity), it is relatively clear that the kinase activity of b-RAF is the oncogenic activity associated with the protein [4]. Other pathogenic LRRK2 mutations localize to the COR and ROC domain, leaving the possibility of distinct but overlapping mechanisms of pathogenic activation of LRRK2 protein.

Like b-RAF, LRRK2 encodes a kinase domain with serine/threonine activity [5], but in concert with a number of conserved domains including a GTPase domain. Multi-domain proteins that encode functional kinase domains often utilize intrinsic protein kinase activity distinct from the canonical protein kinase/substrate interaction. For the same reason that the existence of a LRRK2 kinase substrate abnormally phosphorylated in LRRK2-linked PD cannot be excluded, the idea that LRRK2 protein simply utilizes autophosphorylation as an internal regulatory mechanism to modify another output cannot be excluded. The theme of GTPase control over protein kinase activity recapitulates in the case of LRRK2 and other ROCO proteins since an intact GTPase domain (otherwise known as ROC in ROCO proteins) is required for kinase activity [5–7]; however, it is exceedingly unusual in the mammalian proteome for GTPase domains to be encoded together with protein kinase domains within the same molecule and this arrangement presents a unique set of problems for isolating the two activities. Although GTPase control over kinase activity represents another opportunity for kinase regulation in a one way signal transduction, potential feed-forward and feed-back loops may be difficult to untangle with the limited set of assays yet described. Understanding the functional effects of LRRK2 autophosphorylation on enzymatic activity, as well as structure studies of GTP-locked and GTPase inactive LRRK2 will help uncover the mechanisms of LRRK2 enzyme function, and guide studies that seek to determine the role of pathogenic variation on enzyme activity.

The look of LRRK2

Initial insights into LRRK2 structure and function in cells have been elucidated through localization, solubility and separation studies. LRRK2 protein is not exclusive to the brain since expression distributes fairly ubiquitously, although expression increases with development since LRRK2 is relatively poorly expressed in embryonic tissue [8,9]. LRRK2 protein spreads throughout the cytoplasm with some affinity for membrane containing structures (vesicles, mitochondria, golgi, etc.), demonstrated by both biochemical separations and immunocytochemistry [10–13]. A portion of LRRK2 protein is soluble and readily resolved, whereas another portion resolves only with strong detergents and reduced conditions. At the risk of drawing analogies from relatively simple small protein kinases

with semblance to the LRRK2 kinase domain, kinase oligomerization and differential membrane associations are common themes in regulation (for example, see [14–16]), that are consistent with observations made thus far for LRRK2.

While some protein kinases are devoid of self-interaction, for example Src kinase family members, oligomerization is the norm for some kinase families, most famously the receptor tyrosine kinases [17]. The LRRK2 kinase domain encodes a tyrosine kinase-like family member of the larger non-receptor protein-serine/threonine kinase family where examples of oligomerization-based regulation are abundant for well characterized proteins. LRRK2 phylogenetic non-receptor protein-serine/threonine neighbors b-RAF and MLK3 require self-interaction and dimerization for proper regulation and activation [18,19]. LRRK2 likewise forms structures consistent with dimers and oligomers [20,21], although highly specialized technology is required to resolve the leviathan-sized complexes even *in vitro*, and these structures have not been formally solved through direct observation. Nevertheless, based on precedent from other kinases that transition from oligomeric structures to dimer structures, GTPase-induced conformational changes in protein structure, and evidence that LRRK2 self-interacts through multiple domains, we propose a mechanism of kinase activation whereby LRRK2 resides as kinase-inactive high-molecular weight oligomer that destabilizes upon GTP binding, mediated by a protein encoding a guanine-exchange factor (GEF), which then allows kinase activity, autophosphorylation, and stabilization of a kinase-active homodimer (Figure 1). Competing phosphatase activity and loss of GTP and the subsequent rearrangement that may ensue would destabilize LRRK2 homodimers backwards to oligomers in this model.

If it looks like a duck... LRRK2 and the MAPK pathway

Protein kinases can be subdivided efficiently into families based on sequence similarity of recognizable substructures within the kinase domains, with the expectation that similar kinases may be involved in similar roles in cells. LRRK1 and LRRK2 are awkwardly nestled within the tyrosine-kinase like family, assigned relative to other kinases primarily by sequence similarity with additional consideration for biological functions and domain structure [22]. LRRK2 is positioned near the kinases with the highest kinase domain sequence similarity (mixed-lineage kinases, MLKs), but closest to the multi-domain RIPK families and death-domain containing IRAK family. Many of these kinases have clear roles in the MAPK pathway, and MLK proteins serve as critical MAP-KKK proteins in signaling cell death upon a number of cytotoxic insults in neurons [23,24].

Recently, mitogen-activated protein kinase (MAPK) kinases (MKK) were documented as substrates of LRRK2 kinase activity through detailed *in vitro* analyses [25]. LRRK2 phosphorylates MKK4 and MKK7 within the activation loop where phosphorylation primes the kinase domain for activity, leading to downstream activation of c-Jun N-terminal kinase (JNK), consistent with the assignment of LRRK2 as a potential MAP-KKK. However, over-expression of LRRK2 protein in cells does not lead to an obvious up-regulation of phosphorylated JNK or c-Jun as might be anticipated [5], suggesting either a lack of necessary co-factors or that LRRK2 phosphorylation of MAP-KK proteins does not occur with high efficiency in cells. An emerging theme in the MAPK pathway suggests that scaffolding proteins play critical roles in mediating phosphorylation events that are otherwise unlikely to occur [26,27]. Given the number of predicted protein-interaction domains within LRRK2 and the similarity of the encoded kinase domain with MAP-KKK proteins, LRRK2 may serve as a protein scaffold for MAPK signaling, where identification of binding partners and necessary cofactors are required before definitive assignment of LRRK2 into the MAP-kinase pathway. While evidence of MAPK activation derived from post-mortem tissue in PD-cases is difficult to interpret, an initial study documents that leukocytes derived from patients with the G2019S-LRRK2 mutation versus controls found

decreases in phosphorylated JNK[28]. The hypothetical models where LRRK2 might function as a MAP-KKK protein and a potential scaffold would suggest that LRRK2 bearing artificial mutations that inactivate kinase function might show dominant negative activity in the MAPK pathway, although there is no evidence to suggest kinase-dead LRRK2 has neuroprotective properties. Thus, initial observations raise more questions than are answered, and the complex MAPK pathway is not likely to reserve an obvious place for LRRK2.

The Hunt for LRRK2 kinase substrates

The human genome encodes more than five-hundred protein kinases coupled with thousands to tens of thousands of peptides in the proteome that become phosphorylated [22,29]; needless to say, only a very small fraction of phosphorylation events are yet linked to a particular kinase. Of those events identified through *in vitro* approaches, perhaps only a fraction would be expected to have relevance *in vivo*, since *in vitro* reactions do not necessarily recapitulate correct protein localization and interaction, activity, and structure. Nevertheless, *in vitro* approaches provide a clear path forward but have, unfortunately, provided lackluster results thus far for LRRK2 substrate identification. An initial screen utilizing truncated LRRK2 protein with reasonable autophosphorylation and kinase activity suggested that moesin and related proteins might serve as LRRK2 substrates [30]. LRRK2 can only phosphorylate denatured moesin, a curious arrangement since the proposed LRRK2 phosphorylation site on moesin can be efficiently phosphorylated by other kinases without the requirement for denaturation [31]. Moesin and other potential substrates derived from *in vitro* screens and arrays require further evaluation for LRRK2-dependent phosphorylation *in vivo*.

An ideal LRRK2 substrate would show diminished phosphorylation concurrent with a reduction of LRRK2 levels, and enhanced phosphorylation with LRRK2 over-expression or overactivity. One issue with the published set of *in vitro* LRRK2 substrates that include moesin, MAP-KK proteins and 4EBP1 is that the proposed phospho-residue also serves as a site of phosphorylation for other potentially more abundant and more active kinases [25,30,32]. Since PD is relatively selective in terms of cell degeneration and loss, without accurate model systems of disease (that may not yet exist in PD research), it is relatively easy to propose a substrate but difficult to rule out the potential impact of a proposed substrate in future studies, where an effect could be important or even present only in select cell types.

Extending LRRK2 function in cells

As mediators of many critical and diverse pathways, it is no surprise that protein kinases can be involved, both directly and indirectly, in the regulation of process outgrowth and retraction in cells. Phosphorylation of many components of the cytoskeleton can have immediate impact on cell architecture. An RNA-interference screen in neuroblastoma-derived cell lines demonstrates that nearly one in ten protein kinases, targeted with siRNAs, demonstrated significant roles in neurite retraction, whereas another one in ten shows involvement in neurite extension [33]. In the described screen, the MAP-KKK proteins and other tyrosine-kinase like family members heavily populate the pool of kinases that inhibit neurite outgrowth, while no tyrosine-kinase like family members were identified that enhance neurite outgrowth. Specific RNA-interference targeting of LRRK2 results in changes of expression for several transcripts involved in cell projection morphogenesis, cell motility and anatomical morphogenesis, with the caveat that successful LRRK2 knockdown and even verification of endogenous expression is difficult to assess in most cell lines due to presumed low levels of protein and lack of potent antibodies [34].

On a subcellular level in the brain, LRRK2 protein distributes within neuronal perikarya but also dendrites and axons [10,35]. Over-expression of kinase dead LRRK2 and RNA interference approaches in cortical neurons results in increased neurite length, and this effect may be rescued by over-expression of the LRRK2 kinase domain [36]. LRRK2-knockout mice have not yet been described with changes in neuronal outgrowth, and RNA interference approaches in other cell types or with complementary techniques have yet to confirm the putative effects of LRRK2 mediated process extension *in vivo*. Given the number of protein kinases that may have effects on cell morphology, the challenge lies with deciphering the mechanism of LRRK2 function since a multitude of diverse cell processes may ultimately impact the cytoskeleton.

As a presumed consequence of toxicity and neurodegeneration, pathogenic mutations in LRRK2 associate with the presence of dystrophic processes in post mortem brain tissue and decreased neurite lengths in differentiated SH-SY5Y cultures and primary cortical neurons derived from rodents [36–38]. However, over-expression of kinase dead LRRK2 in neuroblastoma-derived cell lines does not induce a significant change in neurite length [38]. Over-expression of LRRK2 with PD-associated mutations also increases swollen lysosome content and expression of autophagy, potentially important in regards to neurite length since autophagy may play a critical role in process length regulation. Inhibition of the autophagy response by knockdown of necessary autophagy components and inhibition of MAPK/ERK kinase (MEK) by U0126 prevented neurite shortening caused by over-expressed LRRK2. Thus, at least in some model systems, LRRK2 neurite shortening appears to be a kinase-dependent phenomenon that is linked to toxicity rather than a specific remodeling of the cell cytoskeleton.

LRRK2 induced death

Over-expression of LRRK2 protein harboring PD-associated mutations may elicit a certain degree of toxicity in some cell types in a kinase dependent manner [5,39–42]. These experiments achieve some level of specificity since PD-associated mutations exacerbate toxicity relative to wild-type LRRK2, and specific alterations of the kinase domain that inactive kinase activity likewise reduces toxicity. In one cell model, LRRK2 expression may cause increases in caspase-8 activation due to a kinase-sensitive association between LRRK2 and Fas-associated protein with death domain (FADD) [42]. The interaction between LRRK2 and FADD is enhanced by pathogenic LRRK2 mutations, although the enhancement due to the G2019S mutation is markedly less than other pathogenic mutations. FADD associates with the transmembrane receptor Fas upon ligand dependent activation to form the death inducing signaling complex (DISC) which recruits and activates caspase-8 [43]. Other kinases interact with FADD and phosphorylation of FADD does affect function, where a carboxyl terminal serine phosphorylation may play a role in FADD-mediated cell proliferation [44], although it is not known whether LRRK2 phosphorylates FADD.

LRRK2 also interacts, either directly or indirectly, with TRADD and RIP1, proteins that also interact with FADD and activate caspase-8 [42,45]. Although speculative, LRRK2 may serve as a scaffold for the recruitment of FADD together with TRADD, Traff2 and RIP1 in the formation of complex II. The specific LRRK2 domain that interacts with FADD is not known, and the sensitivity of the interaction with intrinsic LRRK2 kinase activity is difficult to rationalize unless autophosphorylation or other LRRK2-kinase dependent structural changes alter affinity for FADD. Opposing FADD action towards caspase-8 activation, DISC and complex II are inhibited by FLIP, which competes for death-domain binding and FADD association [46,47]. Where LRRK2 enhances complex II formation, FLIP association with complex II should be diminished and can be measured in the LRRK2 over-expression paradigm.

Some evidence suggests mutant LRRK2 over-expression in SH-SY5Y neuroblastoma cell lines causes enhanced caspase-3 activation, and LRRK2-induced caspase-3 activation is dependent on Apaf1 expression in embryonic-derived cell lines [41]. Apaf1, caspase-9 and cytochrome *c* form the apoptosome where caspase-9 undergoes a conformational change, rather than cleavage, allowing for proteolytic cleavage of substrates that can include caspase-3 [48]. Caspase-8 activation of caspase-3 is sufficient to initiate death in some but not all cells [49]. Caspase-8 is capable of BID activation leading to translocation to the mitochondria and possible release of cytochrome *c*, which also can lead to apoptosome formation and caspase-9 activation [43]. Over-expressed LRRK2 can therefore enhance caspase 3 cleavage in an apparent kinase-dependent manner through both mitochondrial dependent pathways in addition to mitochondria independent pathways (Figure 2).

Trashing LRRK2

If LRRK2 over-activity is associated with disease, regardless of the specifics of that activity, a straightforward way to modify disease-associated output would be through direct reduction of LRRK2 protein levels. Data from transiently transfected HEK 293T cells indicate that LRRK2 and carboxyl terminus of HSP-70-interacting protein (CHIP) interact via the ROC domain and tetratricopeptide (TPR) domain, respectively [50]. CHIP counters the DnaJ-dependent ATPase activity of HSP-70 required for substrate affinity and protein refolding through E3 ligase activity mediated by a U-box domain and the ubiquitination of substrate proteins [51,52]. Many CHIP substrates are shunted to the ubiquitin-proteasome degradation pathway as opposed to ATP-dependent HSP-70 mediated protein refolding spurred by DnaJ proteins, although some substrates are possibly functionally modified by CHIP mediated ubiquitination events outside of protein degradation. Transiently over-expressed LRRK2 is ubiquitinated by CHIP in a kinase independent manner leading to enhanced degradation, and thus LRRK2 toxicity is rescued by co-expression with CHIP in culture [50].

LRRK2 levels are maintained by HSP-90, a chaperone which commonly stabilizes over-expressed proteins including notable aberrant kinases responsible for some types of cancer [53]. Blockage of the ATP-binding pocket of HSP-90 with the small molecule inhibitor PU-H71 or geldanamycin prevents chaperone activity and reduces steady state levels of LRRK2, and therefore rescues mutant-LRRK2 toxicity *in vitro* [54]. HSP-90 may preferentially stabilize aberrant kinases potentially due to the complex and oligomeric structures kinases often adopt, and HSP-90 inhibitors serve as potent anti-tumor agents due in part to destabilization of kinases critical in cell survival [55]. Further, oncogenic variation in some protein kinases such as b-RAF become more dependent on HSP-90 mediated stabilization compared to wild-type counterparts [56,57]. Likewise, LRRK2 protein harboring the pathogenic G2019S mutations may depend on HSP-90 for stability more so than wild-type protein, offering a potential point of intervention, at least in simple model systems [54]. Taken together, LRRK2 steady state levels, as with many proteins and especially complex protein kinases, are held in balance by the CHIP-HSP-70 and HSP-90 chaperone system. However, the heat-shock chaperone pathway is entirely unselective in nature, and a potentially problematic target for a continuous neuroprotection strategy in PD.

Concluding Remarks

Evidence of pathogenic LRRK2 variants conclusively derives from genetic studies where the variants segregate with disease in large families. The most common of the known LRRK2 mutations (G2019S) increases *in vitro* kinase activity, analogous to mutations in the same kinase subdomain in the b-RAF protein that up-regulates kinase activity and causes various forms of cancer. However, the complex nature of LRRK2 leaves an uncomfortable opportunity for many possible functional effects that pathogenic variants may impart on

LRRK2 protein activity. As LRRK2 is a multi-domain protein, kinase activity may simply represent an intrinsic mechanism that modifies critical internal residues allowing additional activities, rather than phosphorylating substrate proteins. On the other hand, *in vitro* evidence thus far suggests that LRRK2 displays the normal capability to phosphorylate substrate proteins that usually associate with typical non-receptor serine/threonine kinases. Although the proportion of the known human kinome and phosphoproteome where particular kinases critically mediate the phosphorylation of particular peptides is exceedingly small, intensified efforts in future studies may reveal relevant LRRK2 kinase substrates that shed light on the pathogenic mechanisms occurring in PD.

Protein kinases similar to LRRK2 with respect to encoded kinase domains may provide insight into LRRK2 functionality in cells. In fact, early comparisons to MLK proteins further implicate the importance of the MAP-K pathway in neurodegeneration relevant to PD. While provocative *in vitro* data suggests LRRK2 as a MAP-KKK, data from cells and various toxicity studies do not yet support a strong role for LRRK2 as a critical MAP-KKK protein. LRRK2 PD mutants show little effect on activation in the MAP-K pathway and kinase-dead LRRK2 mutants fail to provide protection against insults that activate the MAP-K pathway. Nevertheless, over-expression of LRRK2 protein causes cell toxicity in a kinase dependent manner, perhaps through direct interaction with components of programmed cell death pathways. LRRK2 may serve to bridge together components as a scaffold that ultimately increases the likelihood of association of caspase inducing factors. The canonical heat-shock protein chaperones likely mediate LRRK2 stability, typical for protein kinase turnover and regulation; moreover, alteration of the heat-shock chaperone system may change LRRK2 structure and activity.

In summary, despite the shortcomings in understanding LRRK2 biology, the discovery of potential gain-of-function mutations in a protein considered modifiable by small molecules (e.g., protein kinases) may be the most important advance yet made in the eventual development of rationally derived neuroprotective therapies for PD.

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References

1. Biskup S, West AB. Zeroing in on LRRK2-linked pathogenic mechanisms in Parkinson's disease. *Biochim Biophys Acta*. 2008
2. Healy DG, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. *Lancet Neurol*. 2008; 7:583–90. [PubMed: 18539534]
3. Sen S, West AB. The Therapeutic Potential of LRRK2 and Alpha-synuclein in Parkinson's Disease. *Antioxid Redox Signal*. 2009
4. Wan PT, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell*. 2004; 116:855–67. [PubMed: 15035987]
5. West AB, 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. [PubMed: 17200152]
6. Korr D, Toschi L, Donner P, Pohlenz HD, Kreft B, Weiss B. LRRK1 protein kinase activity is stimulated upon binding of GTP to its Roc domain. *Cell Signal*. 2006; 18:910–20. [PubMed: 16243488]
7. van Egmond WN, Kortholt A, Plak K, Bosgraaf L, Bosgraaf S, Keizer-Gunnink I, van Haastert PJ. Intramolecular activation mechanism of the Dictyostelium LRRK2 homolog Roco protein GbpC. *J Biol Chem*. 2008; 283:30412–20. [PubMed: 18703517]

8. Westerlund M, Belin AC, Anvret A, Bickford P, Olson L, Galter D. Developmental regulation of leucine-rich repeat kinase 1 and 2 expression in the brain and other rodent and human organs: Implications for Parkinson's disease. *Neuroscience*. 2008; 152:429–36. [PubMed: 18272292]
9. Biskup S, Moore DJ, Rea A, Lorenz-Deperieux B, Coombes CE, Dawson VL, Dawson TM, West AB. Dynamic and redundant regulation of LRRK2 and LRRK1 expression. *BMC Neurosci*. 2007; 8:102. [PubMed: 18045479]
10. Biskup S, et al. Localization of LRRK2 to membranous and vesicular structures in mammalian brain. *Ann Neurol*. 2006; 60:557–69. [PubMed: 17120249]
11. Gloeckner CJ, et al. The Parkinson disease causing LRRK2 mutation I2020T is associated with increased kinase activity. *Hum Mol Genet*. 2006; 15:223–32. [PubMed: 16321986]
12. Hatano T, Kubo S, Imai S, Maeda M, Ishikawa K, Mizuno Y, Hattori N. Leucine-rich repeat kinase 2 associates with lipid rafts. *Hum Mol Genet*. 2007; 16:678–90. [PubMed: 17341485]
13. 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; 102:16842–7. [PubMed: 16269541]
14. Li YH, et al. Transmembrane helix of novel oncogene with kinase-domain (NOK) influences its oligomerization and limits the activation of RAS/MAPK signaling. *Mol Cells*. 2009; 27:39–45. [PubMed: 19214432]
15. Rosenberg OS, Deindl S, Comolli LR, Hoelz A, Downing KH, Nairn AC, Kuriyan J. Oligomerization states of the association domain and the holoenzyme of Ca²⁺/CaM kinase II. *FEBS J*. 2006; 273:682–94. [PubMed: 16441656]
16. Sheth PR, Watowich SJ. Oligomerization-induced differential dephosphorylation of c-Met receptor tyrosine kinase. *Biochemistry*. 2005; 44:10984–93. [PubMed: 16101282]
17. Pelech S. Dimerization in protein kinase signaling. *J Biol*. 2006; 5:12. [PubMed: 16879720]
18. Leung IW, 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. [PubMed: 9829970]
19. Terai K, Matsuda M. The amino-terminal B-Raf-specific region mediates calcium-dependent homo- and hetero-dimerization of Raf. *Embo J*. 2006; 25:3556–64. [PubMed: 16858395]
20. 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. [PubMed: 18230735]
21. Greggio E, 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. [PubMed: 18397888]
22. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. *Science*. 2002; 298:1912–34. [PubMed: 12471243]
23. Maroney AC, et al. Cep-1347 (KT7515), a semisynthetic inhibitor of the mixed lineage kinase family. *J Biol Chem*. 2001; 276:25302–8. [PubMed: 11325962]
24. Rana A, Gallo K, Godowski P, Hirai S, Ohno S, Zon L, Kyriakis JM, Avruch J. The mixed lineage kinase SPRK phosphorylates and activates the stress-activated protein kinase activator, SEK-1. *J Biol Chem*. 1996; 271:19025–8. [PubMed: 8702571]
25. Gloeckner CJ, Schumacher A, Boldt K, Ueffing M. The Parkinson disease-associated protein kinase LRRK2 exhibits MAPKKK activity and phosphorylates MKK3/6 and MKK4/7, in vitro. *J Neurochem*. 2009
26. Kolch W. Coordinating ERK/MAPK signalling through scaffolds and inhibitors. *Nat Rev Mol Cell Biol*. 2005; 6:827–37. [PubMed: 16227978]
27. Good M, Tang G, Singleton J, Remenyi A, Lim WA. The Ste5 scaffold directs mating signaling by catalytically unlocking the Fus3 MAP kinase for activation. *Cell*. 2009; 136:1085–97. [PubMed: 19303851]
28. White LR, Toft M, Kvam SN, Farrer MJ, Aasly JO. MAPK-pathway activity, Lrrk2 G2019S, and Parkinson's disease. *J Neurosci Res*. 2007; 85:1288–94. [PubMed: 17385669]

29. Olsen JV, Blagoev B, Gnäd F, Macek B, Kumar C, Mortensen P, Mann M. Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. *Cell*. 2006; 127:635–48. [PubMed: 17081983]
30. 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; 405:307–17. [PubMed: 17447891]
31. Oshiro N, Fukata Y, Kaibuchi K. Phosphorylation of moesin by rho-associated kinase (Rho-kinase) plays a crucial role in the formation of microvilli-like structures. *J Biol Chem*. 1998; 273:34663–6. [PubMed: 9856983]
32. Imai Y, Gehrke S, Wang HQ, Takahashi R, Hasegawa K, Oota E, Lu B. Phosphorylation of 4E-BP by LRRK2 affects the maintenance of dopaminergic neurons in *Drosophila*. *Embo J*. 2008; 27:2432–43. [PubMed: 18701920]
33. Loh SH, Francescut L, Lingor P, Bahr M, Nicotera P. Identification of new kinase clusters required for neurite outgrowth and retraction by a loss-of-function RNA interference screen. *Cell Death Differ*. 2008; 15:283–98. [PubMed: 18007665]
34. Habig K, Walter M, Poths S, Riess O, Bonin M. RNA interference of LRRK2-microarray expression analysis of a Parkinson's disease key player. *Neurogenetics*. 2008; 9:83–94. [PubMed: 18097693]
35. Higashi S, et al. Localization of Parkinson's disease-associated LRRK2 in normal and pathological human brain. *Brain Res*. 2007; 1155:208–19. [PubMed: 17512502]
36. 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. [PubMed: 17114044]
37. Giasson BI, Covy JP, Bonini NM, Hurtig HI, Farrer MJ, Trojanowski JQ, Van Deerlin VM. Biochemical and pathological characterization of Lrrk2. *Ann Neurol*. 2006; 59:315–22. [PubMed: 16437584]
38. 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; 105:1048–56. [PubMed: 18182054]
39. Greggio E, et al. Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. *Neurobiol Dis*. 2006; 23:329–41. [PubMed: 16750377]
40. 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. [PubMed: 16980962]
41. Iaccarino C, Crosio C, Vitale C, Sanna G, Carri MT, Barone P. Apoptotic mechanisms in mutant LRRK2-mediated cell death. *Hum Mol Genet*. 2007; 16:1319–26. [PubMed: 17409193]
42. Ho CC, Rideout HJ, Ribe E, Troy CM, Dauer WT. The Parkinson disease protein leucine-rich repeat kinase 2 transduces death signals via Fas-associated protein with death domain and caspase-8 in a cellular model of neurodegeneration. *J Neurosci*. 2009; 29:1011–6. [PubMed: 19176810]
43. Li J, Yuan J. Caspases in apoptosis and beyond. *Oncogene*. 2008; 27:6194–206. [PubMed: 18931687]
44. Imtiyaz HZ, Zhou X, Zhang H, Chen D, Hu T, Zhang J. The death domain of FADD is essential for embryogenesis, lymphocyte development, and proliferation. *J Biol Chem*. 2009; 284:9917–26. [PubMed: 19203997]
45. Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell*. 2003; 114:181–90. [PubMed: 12887920]
46. Li FY, Jeffrey PD, Yu JW, Shi Y. Crystal structure of a viral FLIP: insights into FLIP-mediated inhibition of death receptor signaling. *J Biol Chem*. 2006; 281:2960–8. [PubMed: 16317000]
47. Yu JW, Shi Y. FLIP and the death effector domain family. *Oncogene*. 2008; 27:6216–27. [PubMed: 18931689]
48. Shi Y. Apoptosome: the cellular engine for the activation of caspase-9. *Structure*. 2002; 10:285–8. [PubMed: 12005427]
49. Scaffidi C, et al. Two CD95 (APO-1/Fas) signaling pathways. *Embo J*. 1998; 17:1675–87. [PubMed: 9501089]

50. Ko HS, et al. CHIP regulates leucine-rich repeat kinase-2 ubiquitination, degradation, and toxicity. *Proc Natl Acad Sci U S A*. 2009; 106:2897–902. [PubMed: 19196961]
51. Jiang J, Ballinger CA, Wu Y, Dai Q, Cyr DM, Hohfeld J, Patterson C. CHIP is a U-box-dependent E3 ubiquitin ligase: identification of Hsc70 as a target for ubiquitylation. *J Biol Chem*. 2001; 276:42938–44. [PubMed: 11557750]
52. Murata S, Minami Y, Minami M, Chiba T, Tanaka K. CHIP is a chaperone-dependent E3 ligase that ubiquitylates unfolded protein. *EMBO Rep*. 2001; 2:1133–8. [PubMed: 11743028]
53. Caplan AJ, Mandal AK, Theodoraki MA. Molecular chaperones and protein kinase quality control. *Trends Cell Biol*. 2007; 17:87–92. [PubMed: 17184992]
54. Wang L, et al. The chaperone activity of heat shock protein 90 is critical for maintaining the stability of leucine-rich repeat kinase 2. *J Neurosci*. 2008; 28:3384–91. [PubMed: 18367605]
55. Banerji U. Heat shock protein 90 as a drug target: some like it hot. *Clin Cancer Res*. 2009; 15:9–14. [PubMed: 19118027]
56. da Rocha Dias S, Friedlos F, Light Y, Springer C, Workman P, Marais R. Activated B-RAF is an Hsp90 client protein that is targeted by the anticancer drug 17-allylamino-17-demethoxygeldanamycin. *Cancer Res*. 2005; 65:10686–91. [PubMed: 16322212]
57. Grbovic OM, Basso AD, Sawai A, Ye Q, Friedlander P, Solit D, Rosen N. V600E B-Raf requires the Hsp90 chaperone for stability and is degraded in response to Hsp90 inhibitors. *Proc Natl Acad Sci U S A*. 2006; 103:57–62. [PubMed: 16371460]

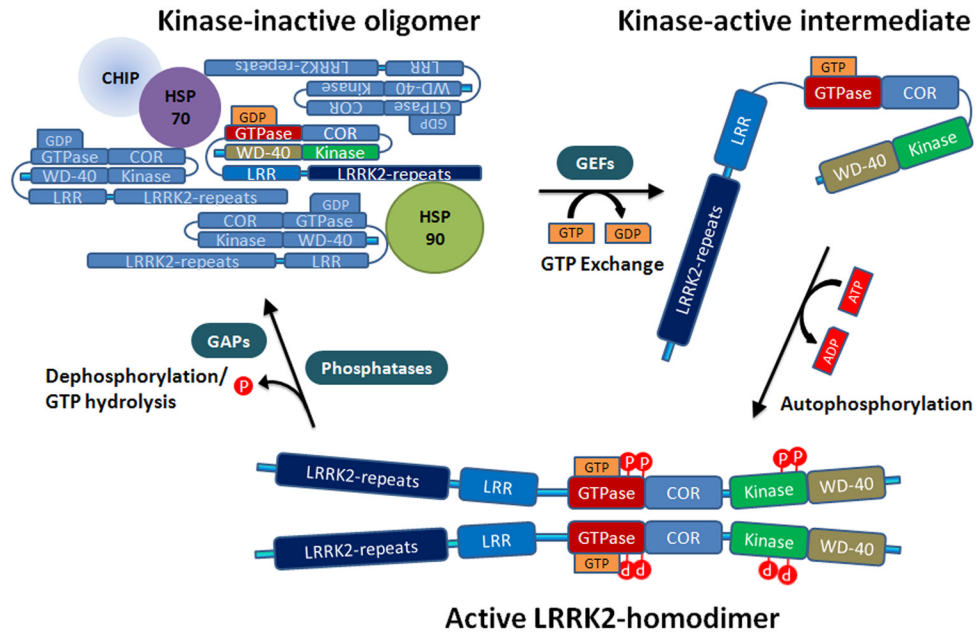


Figure 1. Hypothetical model of LRRK2 kinase activation

LRRK2 forms large oligomeric complexes that may be stabilized by HSP-90 and polyubiquitinated by CHIP, and the oligomer may have limited or no kinase activity. GDP/GTP exchange mediated by cofactors and GEF proteins causes a conformation change that releases LRRK2 from possible N-terminal domain (LRRK2 repeats) mediated steric inhibition of the kinase domain. In a GTP-bound form, the LRRK2 kinase domain may access autophosphorylation sites that serve to stabilize a kinase-active form such as a homodimer able to interact with and phosphorylate substrate proteins. Reversion of the kinase-active structure back to oligomeric form may be facilitated through GTPase activity stimulated by GAP proteins or phosphatases that remove stabilizing phosphorylated residues.

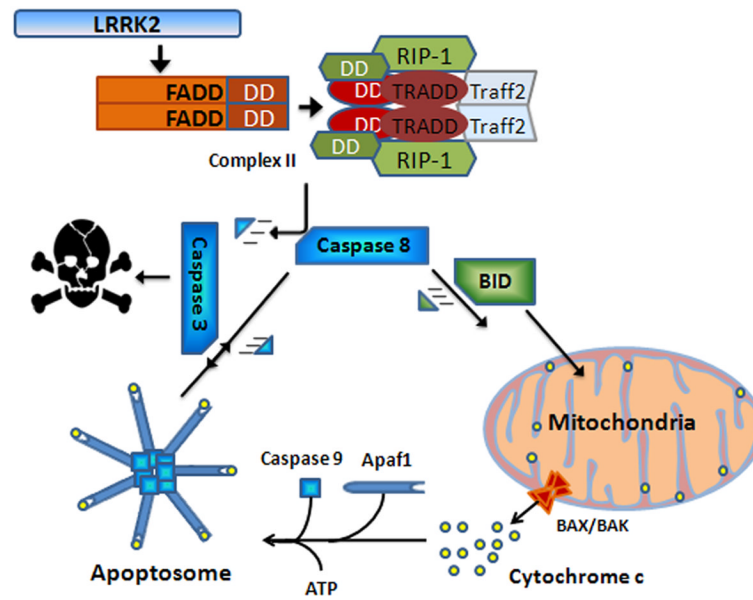


Figure 2. LRRK2 activates caspase mediated cell death

Hypothetical model predicts a means for LRRK2 toxicity. LRRK2 associates with components of complex II (RIP, TRAFF, TRADD) in a kinase-sensitive manner through interaction with Fas-associated protein with death domain (FADD). Initiator caspase-8 is activated by cleavage leading to subsequent cleavage of caspase-3. In some cells this is sufficient to induce cell death; other cells require signal amplification caused by cleavage of Bid, a Bcl-2 pro-apoptotic protein. Activated Bid translocates to the mitochondria where it signals the formation of BAX-BAK oligomers into a proteolipid pore. Cytochrome c and other factors are released from the intramembrane space into the cytosol, where cytochrome c activates apoptosome formation. Initiator caspase-9 undergoes a conformational change, activating photolytic activity. The apoptosome cleaves effector caspase-3 that may result in cell death.