Modeling LRRK2 Pathobiology in Parkinson's Disease: From Yeast to Rodents

Guillaume Daniel and Darren J. Moore

Abstract Mutations in the *leucine-rich repeat kinase 2* (*LRRK2*, *PARK8*) gene represent the most common cause of familial Parkinson's disease (PD) with autosomal dominant inheritance, whereas common variation at the *LRRK2* genomic locus influences the risk of developing idiopathic PD. LRRK2 is a member of the ROCO protein family and contains multiple domains, including Ras-of-Complex (ROC) GTPase, kinase, and protein-protein interaction domains. In the last decade, the biochemical characterization of LRRK2 and the development of animal models have provided important insight into the pathobiology of LRRK2. In this review, we comprehensively describe the different models employed to understand *LRRK2*-associated PD, including yeast, invertebrates, transgenic and viral-based rodents, and patient-derived induced pluripotent stem cells. We discuss how these models have contributed to understanding LRRK2 pathobiology and the advantages and limitations of each model for exploring aspects of *LRRK2*-associated PD.

Keywords *LRRK2* · *PARK8* · Parkinson's disease · Parkinsonism · Animal model · Neurodegeneration · Dopaminergic

Contents

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1 *LRRK2* and Parkinson's Disease

Parkinson's disease (PD) is a chronic neurodegenerative movement disorder that affects 1–2 % of individuals above 65 years of age (Lang and Lozano 1998a, b). PD is classically defined by the cardinal motor symptoms of bradykinesia, muscular rigidity, resting tremor, and postural instability, although numerous non-motor symptoms can also manifest including myriad cognitive, psychiatric, and autonomic disturbances (Jankovic 2008). Underlying the motor symptoms of PD is the relatively selective degeneration of substantia nigra pars compacta dopaminergic neurons and their projections to the caudate-putamen that results in a marked reduction of the neurotransmitter dopamine (Lang and Lozano 1998a, b). Therapies aimed at restoring dopamine (i.e., L-dopa) or dopamine-related signaling (i.e., dopamine receptor agonists) form the basis of current treatments that are initially effective at improving motor symptoms, but are palliative rather than disease—modifying. Accompanying the degeneration of dopaminergic neurons is the formation of intracytoplasmic proteinaceous inclusions in surviving brainstem neurons, termed Lewy bodies, which are enriched with fibrillar forms of the presynaptic protein α-synuclein (Spillantini et al. 1997). PD generally occurs as an idiopathic disease, although 5–10 % of cases manifest in a familial manner and to date mutations in a number of genes have been identified to unambiguously cause rare Mendelian forms of PD (Gasser 2009; Bonifati 2014). Mutations are found in the genes encoding α-synuclein (*PARK1/4*) (Polymeropoulos et al. 1997; Singleton et al. 2003), parkin (*PARK2*) (Kitada et al. 1998), DJ-1 (*PARK7*) (Bonifati et al. 2003), PTEN-induced kinase 1 (PINK1; *PARK6*) (Valente et al. 2004), leucine-rich repeat kinase 2 (LRRK2; *PARK8*) (Paisan-Ruiz et al. 2004; Zimprich et al. 2004), ATP13A2 (*PARK9*) (Ramirez et al. 2006), FBX07 (*PARK15*) (Di Fonzo et al. 2009), VPS35 (*PARK17*) (Vilarino-Guell et al. 2011; Zimprich et al. 2011), EIF4G1 (*PARK18*) (Chartier-Harlin et al. 2011), synaptojanin 1 (*SYNJ1*; *PARK20*) (Krebs et al. 2013; Quadri et al. 2013) and DNAJC6 (Edvardson et al. 2012). The identification of genetic mutations causing familial PD has provided tremendous insight into the molecular mechanisms and cellular pathways underlying neuronal degeneration.

Mutations in the *LRRK2* gene cause late-onset, autosomal dominant PD and represent the most common cause of familial PD (Biskup and West 2009).The relatively frequent G2019S mutation in *LRRK2* has also been identified in 1–2 % of idiopathic PD cases and up to 40 % of patients with familial PD depending on ethnicity (Healy et al. 2008). *LRRK2* G2019S mutation penetrance is high and

age-dependent, but incomplete, suggesting that genetic and/or environmental factors may associate with *LRRK2* to trigger dopaminergic neurodegeneration (Hulihan et al. 2008). Genome-wide association studies further indicate that common variation in the *LRRK2* gene is a risk factor for idiopathic PD (Satake et al. 2009; Simon-Sanchez et al. 2009, International Parkinson Disease Genomics et al. (2011), Lill et al. (2012). *LRRK2* mutations give rise to a late-onset form of familial PD that is clinically and neurochemically indistinguishable from idiopathic PD. Similar to idiopathic PD, brains from *LRRK2* mutant PD subjects are typically characterized by profound substantia nigra dopaminergic neurodegeneration and gliosis together with the appearance of α-synuclein-positive Lewy body pathology (Giasson et al. 2006; Ross et al. 2006). While Lewy body pathology is predominantly associated with *LRRK2* mutant PD cases, some cases reveal instead tau-positive neurofibrillary pathology, ubiquitin-positive inclusions, or even the absence of obvious pathological aggregates or inclusions (Zimprich et al. 2004; Biskup and West 2009; Crosiers et al. 2011). Therefore, *LRRK2*-associated PD shares many clinical and pathological similarities with idiopathic PD, with some minor exceptions, whereas genetically *LRRK2* variation contributes to familial and idiopathic PD.

LRRK2 is a multi-domain protein of 2,527 amino acids that belongs to the ROCO family of proteins. ROCO proteins contain a characteristic Ras-of-Complex (ROC) GTPase domain adjacent to a C-terminal-of-ROC (COR) linker region. LRRK2 also contains a serine/threonine protein kinase domain and several putative protein-protein interaction domains flanking the central ROC-COR-kinase catalytic region (Tsika and Moore 2012). LRRK2 predominantly exists as a dimeric structure and dimerization is required for its kinase activity and for its localization to cellular membranes (Greggio et al. 2008; Sen et al. 2009; Berger et al. 2010; James et al. 2012). LRRK2 is expressed at high levels in lung, kidney, and lymph nodes (Biskup et al. 2007; Westerlund et al. 2008; Hakimi et al. 2011), but also in various brain regions, including the cortex, striatum, hippocampus, cerebellum, and in the dopaminergic neurons of the SNpc, albeit at low levels (Mandemakers et al. 2012). Within the brain, LRRK2 is abundantly expressed in neurons, but can also be detected at lower levels in astrocytes and microglia where its expression can be induced by inflammatory stimuli (Moehle et al. 2012; Giesert et al. 2013). Within neurons, LRRK2 localizes to several vesicular structures and intracellular membranes (Biskup et al. 2006; Hatano et al. 2007; Alegre-Abarrategui et al. 2009) (i.e., endosomes, lysosomes, multivesicular bodies, mitochondrial outer membrane, lipid rafts, microtubule-associated transport vesicles, synaptosomes, the Golgi complex, and the endoplasmic reticulum). The structural organization and molecular function of LRRK2 are beyond the scope of this review and have been covered in detail elsewhere (Cookson 2010; Tsika and Moore 2012).

Until now, most putative substrates of LRRK2 kinase activity have been identified and validated in vitro or in invertebrate model organisms. These substrates include LRRK2 itself (Greggio et al. 2009; Webber et al. 2011; Sheng et al. 2012), MAP kinase kinase family members (Gloeckner et al. 2009; Chen et al. 2012), the ezrin/radixin/moesin (ERM) protein family (Jaleel et al. 2007; Parisiadou et al. 2009), *β*-tubulin (Gillardon 2009), Akt1 (Ohta et al. 2011), *FoxO1* (Kanao et al. 2010),

Drosophila Futsch (Lee et al. 2010b), microtubule-associated protein tau (Kawakami et al. 2012; Bailey et al. 2013), 4E-BP1 (Gehrke et al. 2010; Trancikova et al. 2012), ArfGAP1 (Stafa et al. 2012; Xiong et al. 2012), α-synuclein (Qing et al. 2009), snapin (Yun et al. 2013) and EndophilinA (Matta et al. 2012). LRRK2 autophosphorylation may serve a regulatory function and occurs at residues within or adjacent to the ROC GTPase domain (Greggio et al. 2009; Webber et al. 2011; Kamikawaji et al. 2013). The GTPase domain of LRRK2 binds guanine nucleotides and is capable of hydrolyzing GTP at a slow rate, apparently independent of its oligomerization state (Ito et al. 2007; Lewis et al. 2007; Taymans et al. 2011; Biosa et al. 2013; Liao et al. 2014). Although there is evidence for a functional interplay between the two enzymatic domains, the biochemical mechanisms governing LRRK2 enzymatic functions remain unclear. Interestingly, GTP hydrolysis and GTP binding activities of LRRK2 are both required for LRRK2 kinase activity, whereas the contribution of LRRK2 autophosphorylation within the GTPase domain to GTP binding and GTP hydrolysis activities is incompletely understood (Ito et al. 2007; West et al. 2007; Taymans et al. 2011; Biosa et al. 2013). Kinase-inactive variants of LRRK2 exhibit normal GTP binding and GTP hydrolysis activities, although mutation of individual autophosphorylation sites within the GTPase domain (i.e., T1503) can alter kinase activity (Webber et al. 2011; Biosa et al. 2013).

Although a number of familial mutations in *LRRK2* have been reported, only a few are truly pathogenic and affect highly conserved residues in various functional domains of the protein, including the ROC GTPase domain (R1441C, R1441G, R1441H), the COR linker (Y1699C), and the kinase domain (G2019S, I2020T). The G2019S mutation in the kinase domain has been shown to consistently enhance *LRRK2* kinase activity (West et al. 2005; Greggio and Cookson 2009), whereas the effect of the I2020T mutation on kinase activity is ambiguous (Gloeckner et al. 2006; Jaleel et al. 2007). Recent in vitro findings suggest that the R1441H mutation prolongs the GTP-bound state of the LRRK2 ROC domain by compromising GTPase activity and increasing GDP-GTP exchange (Liao et al. 2014). Overall, mutations in the ROC-COR domain diminish GTPase activity with little if any consistent effect on kinase activity (Lewis et al. 2007; Greggio and Cookson 2009; Xiong et al. 2010; Daniels et al. 2011; Liao et al. 2014).

In summary, pathogenic mutations of *LRRK2* are located in functional domains and alter the enzymatic activity of LRRK2, which suggests that both GTPase and kinase activity are likely to be important for LRRK2-dependent neurodegeneration in PD. Therefore, pharmacological modulation of LRRK2 enzymatic activity may comprise a promising therapeutic approach for the treatment of familial and idiopathic PD.

2 Models of *LRRK2*-Associated Parkinson's Disease

Animal models and model organisms have proven to be fundamental tools to identify and validate the molecular and cellular mechanisms underlying genetically linked disease. LRRK2 is an attractive therapeutic target for PD and insights

gained from LRRK2 animal models should help to develop and validate new therapeutic approaches for both familial and idiopathic PD. Accordingly, intense efforts have focused on the generation of LRRK2 cellular and animal models, which include simple eukaryotic organisms, invertebrates, rodents, and patient-derived neurons. These LRRK2 models and the major insights derived from them so far will be described herein.

2.1 Simple Eukaryotic LRRK2 Models: Saccharomyces cerevisiae

The baker's yeast, *Saccharomyces cerevisiae*, is commonly used in different areas of biology to unravel the molecular function(s) of proteins and the fundamental cellular processes and pathways in which they are implicated. Although lacking the physiological and genetic complexity of mammalian neurons, yeast exhibit a high degree of conservation of basic protein function and cellular pathways with mammalian cells that are implicated in neurodegenerative processes (i.e., vesicular trafficking, protein folding and aggregation, protein catabolism, mitochondrial function, etc.). In addition, the accessibility of yeast cells to genetic manipulation and genome-wide screening approaches enables the rapid identification of molecular pathways and biological processes associated with or regulated by a given protein. In the context of PD, studies in yeast have provided unique insight into the pathobiology of the α-synuclein protein and the identification of key cellular processes mediating human α-synuclein-dependent toxicity (Outeiro and Lindquist 2003; Willingham et al. 2003; Cooper et al. 2006; Gitler et al. 2008, 2009; Yeger-Lotem et al. 2009).

Yeast has been employed to investigate the mechanisms underlying the pathobiology of LRRK2, which has revealed a key role for the GTPase domain of LRRK2 in mediating cellular toxicity (Xiong et al. 2010). Overexpression of full-length human LRRK2 under the control of a galactose-inducible promoter failed to elicit cellular toxicity owing to the sequestration of LRRK2 into highly insoluble cytoplasmic inclusions (Xiong et al. 2010; Pereira et al. 2014). Xiong and coworkers focused on the effects of domain fragments of *LRRK2* on yeast viability and observed that overexpression of a large fragment containing the central ROC GTPase, COR, and kinase domains was highly cytotoxic, and that the expression of the GTPase domain alone was also sufficient to markedly reduce yeast viability. Furthermore, the introduction of GTP binding-deficient (K1347A or T1348N) variants exacerbated toxicity compared to wild-type (WT) LRRK2, whereas GTPase-hyperactive variants (R1398L or Ras-like R1398Q/T1343G) partially improved yeast viability. Importantly, however, pathogenic mutations associated with familial PD (i.e., R1441C and G2019S) do not influence the toxicity induced by human LRRK2 in yeast, which perhaps suggests that these mutations may only exert their deleterious effects in the context of full-length LRRK2 and/or in mammalian cells (Xiong et al. 2010; Pereira et al. 2014). Together, studies in yeast

support a critical role for GTPase activity in LRRK2-mediated toxicity. Defects in endocytic vesicular trafficking to the vacuole (equivalent to the mammalian lysosome) and the accumulation of autophagic vacuoles coincided with *LRRK2*-induced toxicity in yeast. Furthermore, LRRK2-induced toxicity in yeast appears to act through a mechanism distinct from toxicity induced by human α-synuclein since overexpression of the yeast proteins Ypt1 (an ortholog of mammalian Rab1) and Ykt6, which are potent suppressors of α-synuclein-induced toxicity (Cooper et al. 2006), failed to similarly alter LRRK2-mediated toxicity. A genome-wide genetic screen in yeast identified nine modifiers of LRRK2-induced toxicity, including *SLT2* and *GCS1*, which are orthologous to human MAP kinases (MAPK1, 3, 11, and 14) and ADP-ribosylation factor GTPase-activating protein 1 (ArfGAP1), respectively. ArfGAP1 is a GTPase-activating protein that plays a role in vesicular trafficking at the Golgi complex, and is critical for maintaining Golgi integrity by promoting the GTP hydrolysis of the small GTPase Arf1 (Shiba and Randazzo 2012). Subsequent studies have demonstrated a conserved interaction between LRRK2 and GCS1/ArfGAP1 in *Drosophila* (Xiong et al. 2012), as discussed later, and in mammalian cells and rodent neurons (Stafa et al. 2012). ArfGAP1 and LRRK2 proteins biochemically interact in vitro and in vivo, and ArfGAP1 serves as a GAP-like protein for LRRK2 to enhance its GTPase activity (Stafa et al. 2012; Xiong et al. 2012). Gene silencing of ArfGAP1 expression in rat primary cortical neurons rescues the impaired neurite outgrowth induced by G2019S LRRK2 (Stafa et al. 2012), similar to the suppressor effect of *GCS1* deletion in yeast (Xiong et al. 2010). LRRK2 also reciprocally phosphorylates ArfGAP1 and is required for ArfGAP1-induced neuronal toxicity (Stafa et al. 2012; Xiong et al. 2012). Therefore, the functional interaction of LRRK2 with GCS1/ArfGAP1 is conserved from yeast to mammals and may comprise an important molecular pathway for mediating LRRK2-induced neuronal toxicity.

Pereira and coworkers recently observed that low-level overexpression of full-length human WT LRRK2 in yeast confers resistance against hydrogen peroxide (H_2O_2) exposure potentially through a mechanism involving mitochondrial function and endocytosis (Pereira et al. 2014). The pathogenic G2019S and R1441C mutations, or the absence of kinase activity and the WD40 domain, abolished this resistance. Furthermore, the overexpression of WT LRRK2 modestly decreased the H_2O_2-induced production of reactive oxygen species (ROS), whereas expression of G2019S or R1441C LRRK2 oppositely stimulated ROS production, increased mitochondrial membrane potential, and induced endocytic defects in the context of H_2O_2 exposure (Pereira et al. 2014). Collectively, these observations support a role for LRRK2 in mediating protection against oxidative stress through a pathway involving mitochondrial function and endocytosis.

The baker's yeast, *Saccharomyces cerevisiae*, provides a powerful genetic and cell biological tool to dissect the fundamental biology and pathobiology of human *LRRK2*, and for the rapid identification of novel pathways that are important for *LRRK2*-mediated neuronal degeneration. Furthermore, yeast can be employed as an initial cellular model to broadly screen for chemical or genetic modifiers of LRRK2-induced toxicity prior to further validation in disease-relevant mammalian models.

2.2 *Invertebrate LRRK2 Models: Drosophila melanogaster and Caenorhabditis elegans*

2.2.1 *Drosophila melanogaster*

The fruit fly, *Drosophila melanogaster*, has proven to be a powerful model for the study of neurodegenerative diseases and in particular for investigating the function of genes associated with familial PD (i.e., α-synuclein, parkin, *PINK1*, *DJ-1*) (Chen and Feany 2005; Meulener et al. 2005; Clark et al. 2006; Park et al. 2006; Tain et al. 2009). The *LRRK2* gene is highly conserved across species. *C. elegans* and *Drosophila* each have a single paralog of human *LRRK2* and *LRRK1* (Marin 2008). Since the key residues mutated in *LRRK2*-associated familial PD are conserved between human *LRRK2* and *Drosophila LRRK* (*dLRRK*), several studies have described the generation of transgenic or mutant *Drosophila* as a model to investigate the molecular and cellular pathobiology of *LRRK2*-related PD.

Loss-of-function studies reveal that *dLRRK* is dispensable for the development and maintenance of dopaminergic neurons, but appears important for maintaining their integrity (Lee et al. 2007; Imai et al. 2008; Wang et al. 2008a). Moreover, disruption of *dLRRK* influences the sensitivity of flies to hydrogen peroxide, although it remains unclear whether dLRRK protects or sensitizes dopaminergic neurons to oxidative insult (Imai et al. 2008; Wang et al. 2008a). Homozygous *dLRRK* deletion mutants are viable into adulthood and exhibit a normal life span although while male mutants are fertile, female mutants exhibit reduced fecundity (Lee et al. 2007; Imai et al. 2008).

Although loss-of-function approaches help to clarify certain functions of *LRRK2*, they do not represent an appropriate model to investigate the pathological effects of PD-related mutations in human *LRRK2* which appear to act through a gain-of-function mechanism. Accordingly, transgenic fly models have been developed to study the impact of these familial mutations on dopaminergic neurons. Most of these studies employ the *GAL4/UAS* gene system, which relies on the transcriptional activation of an upstream-activating sequence (*UAS*) by the yeast transcriptional activator *GAL4* to express a transgene in distinct neuronal populations. Briefly, this is achieved by crossing a first transgenic responder strain where the gene of interest (dLRRK or human *LRRK2*) is inserted downstream of a genomic sequence containing multiple *GAL4* binding sites (*UAS*), with a second driver strain where *GAL4* is expressed under the control of a cell- or tissue-specific promoter (Brand and Perrimon 1993). Hence, the use of different neuronal *GAL4* drivers (i.e., *gmr*, *elav*, *TH,* or *ddc*) to drive the expression of a native or codon-optimized human *LRRK2* transgene, combined with a unique genomic integration site and variable copy number for each transgene cassette, may result in important experimental variations that can potentially explain the broad array of phenotypes observed in the different *Drosophila* LRRK2 transgenic models described to date.

Pathogenic effects of LRRK2: Transgenic flies overexpressing pathogenic forms of dLRRK or human LRRK2 recapitulate certain features of *LRRK2*-linked

PD. Collectively, the overexpression of pathogenic dLRRK, human WT LRRK2, and to a more severe extent, G2019S, Y1699C, or G2385R mutant human LRRK2, induces an adult-onset and progressive loss of dopaminergic neurons, early mortality and impaired motor function that could be attenuated by treatment with L-DOPA, and exacerbated by the mitochondrial toxin rotenone (Imai et al. 2008; Liu et al. 2008; Ng et al. 2009; Venderova et al. 2009; Liu et al. 2011; MacLeod et al. 2013). Intriguingly, a recent study demonstrated that G2019S LRRK2 expression confined to dopaminergic neurons resulted in neurodegeneration throughout the fly visual system, including within brain regions lacking obvious dopaminergic innervation (Hindle et al. 2013). G2019S LRRK2 expression in dopaminergic neurons caused a non-cell autonomous progressive loss of photoreceptor function and retinal neurodegeneration accompanied by mitochondrial dysfunction, autophagic vacuole accumulation, and apoptosis in the vicinity of photoreceptors. Expression of additional PD-associated LRRK2 mutants (I1122V, R1441C, Y1387C, Y1699C, I1915T, I2020T, and G2385R) failed to similarly impair photoreceptor function. Furthermore, the visual deficits induced by G2019S LRRK2 expression were kinase-dependent as revealed by simultaneous introduction of the kinase-inactive variant, K1906M, which disrupts the ATP-binding pocket within the kinase domain (Hindle et al. 2013). Interestingly, increasing the demands on the visual system to adapt, or increasing the activity of dopaminergic neurons, accelerates the decline in visual function induced by G2019S LRRK2 expression. These observations suggest that increased neuronal energy demand might contribute to G2019S LRRK2-mediated neurodegeneration in this fly model.

Kinase and GTPase-dependent toxic effects of LRRK2: The eukaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP) was previously identified as a substrate of dLRRK and human *LRRK2* kinase activity that could mediate the toxic effects of LRRK2 (Imai et al. 2008). 4E-BP serves an important function for survival under starvation stress, oxidative stress, and unfolded protein stress in vivo whereby it inhibits eIF4E-mediated protein translation. Phosphorylation of 4E-BP relieves its inhibitory effect on protein translation. Familial PD mutations in dLRRK and human LRRK2 mediate the hyperphosphorylation and inactivation of 4E-BP in transgenic *Drosophila*, resulting in decreased resistance to oxidative stress, dopaminergic neuronal degeneration, and diminished climbing activity. Conversely, the overexpression of 4E-BP prevents the pathogenic effects of mutant dLRRK and attenuates neurodegeneration. This study potentially links the oxidative stress response and LRRK2 kinase activity in the context of PD. Additionally, loss of dLRRK causes the hypophosphorylation of 4E-BP and rescues dopaminergic neuronal loss in *PINK1* and *parkin* mutant fly models (Tain et al. 2009), whereas inhibitors of LRRK2 kinase activity attenuate dLRRK-mediated neurodegeneration (Liu et al. 2011). Taken together, dLRRK, through its kinase activity, is a negative regulator of dopaminergic neuronal survival.

LRRK2 has also been shown to functionally interact with the microRNA pathway to regulate protein translation. Mutant LRRK2 regulates the translation of E2F1 and DP mRNAs resulting in the overproduction of these two gene products,

which are implicated in cell cycle control and survival (Gehrke et al. 2010). LRRK2 inhibits the miRNAs let-7 and miR-184, which regulate E2F1 and DP mRNAs, respectively. Direct inhibition of the repressive activity of let-7 and miR-184 on E2F1 and DP protein synthesis could recapitulate the neurotoxic effects induced by LRRK2 in flies. Conversely, increasing the expression of let-7 or miR-184 attenuated the pathogenic effects of mutant LRRK2, suggesting that the microRNA-mediated regulation of E2F1 and DP is critical for mediating *LRRK2*-associated neurodegeneration. G2019S LRRK2 inhibits let-7 and miR-184 by interacting with and impairing the stability of *Drosophila* Argonaute-1 (dAgo1) of the RNA-induced silencing complex (RISC) in aged flies. Furthermore, G2019S LRRK2 promotes the interaction of phospho-4E-BP with dAgo1, relieving miRNA-mediated translational repression. Therefore, LRRK2-related neuronal damage could be mediated through the miRNA processing pathway, especially by regulating the translation of mRNAs.

A prior study found that dLRRK compromises dopaminergic neuronal survival in *Drosophila* through phosphorylation of *FoxO1*, a key regulator of myriad cellular processes including the cell cycle, cell death pathways, metabolism and oxidative stress, and the regulation of 4E-BP transcription. The phosphorylation of *FoxO1* by dLRRK caused an increase in expression of the pro-apoptotic proteins Bid and Him leading to activation of cell death pathways (Kanao et al. 2010). It was also shown that the overexpression of dLRRK, human WT LRRK2, and more potently, human G2019S LRRK2 induced defects in dendritic arborization (Lin et al. 2010). G2019S LRRK2 induced the redistribution and abnormal accumulation of tau in dendritic processes resulting in neurodegeneration. G2019S LRRK2 indirectly promotes the phosphorylation of tau at threonine-212 mediated by the *Drosophila* glycogen synthase kinase 3 homolog Shaggy (Sgg), which facilitates tau-dependent pathology, including microtubule fragmentation, inclusion body formation, and neuronal loss. Surprisingly, the RNAi-mediated silencing of endogenous tau expression rescued defects in dendritic arborization induced by G2019S LRRK2, whereas reduced *dLRRK* gene dosage alleviated pathogenic phenotypes induced by tau overexpression, including dendritic process degeneration, inclusion formation, and microtubule fragmentation. Collectively, these observations suggest that the functional interaction between LRRK2 and tau is rather complex and is unlikely to be mediated via a single linear pathway.

Xiong and coworkers showed that the GTPase domain of LRRK2 contributes to *LRRK2*-mediated neurodegeneration. They and others identified ArfGAP1 as a novel GAP protein that regulates the GTPase activity of *LRRK2* in vitro (Stafa et al. 2012; Xiong et al. 2012). Interestingly, although the overexpression of ArfGAP1 alone in flies induced substantial loss of dopaminergic neurons in the dorsomedial PPM1/2 cluster (equivalent to the mammalian substantia nigra), its co-expression with human WT or G2019S LRRK2 conferred protection against human *LRRK2*-induced neurotoxicity (Xiong et al. 2012). The genetic interaction between LRRK2 and ArfGAP1 appears complex, as ArfGAP1 enhances the GTP hydrolysis activity of LRRK2 in vitro and protects against LRRK2-induced dopaminergic neuronal degeneration in flies, whereas reciprocally LRRK2

phosphorylates ArfGAP1 which reduces its GAP activity in vitro and protects against ArfGAP1-mediated retinal degeneration in flies (Xiong et al. 2012). Furthermore, silencing of ArfGAP1 expression protects against LRRK2-induced neuronal toxicity in primary cultures (Stafa et al. 2012; Xiong et al. 2012), which suggests a direct role for ArfGAP1 in LRRK2-mediated neurodegeneration. However, whether ArfGAP1 is required for LRRK2-mediated neuronal toxicity in vivo in mammalian models is not yet known.

LRRK2 contributes to the homeostasis of different cellular compartments: LRRK2-induced pathology might be mediated through deregulated mitochondrial dynamics and quality control. Overexpression of G2019S LRRK2 in flight muscles and dopaminergic neurons induces marked mitochondrial pathology, and impairs locomotor activity, that can be rescued by co-expression of the PD-associated protein parkin (Ng et al. 2009). Activation of AMPK by pharmacological treatment or genetic activation could rescue dopaminergic neuronal loss and locomotor deficits, and mitigates the mitochondrial pathology induced by G2019S LRRK2 overexpression or *parkin* deletion in flies (Ng et al. 2012). Furthermore, the increased sensitivity to rotenone of LRRK2 transgenic fly models (Ng et al. 2009; Venderova et al. 2009), and the genetic interaction between *LRRK2* and other PD-associated genes, *DJ-1* and *PINK1* (Venderova et al. 2009) involved in mitochondrial homeostasis suggest that mitochondrial dysfunction could be important for LRRK2-mediated pathology.

A role for LRRK2 in dopaminergic neuronal survival could also potentially be related to its function in synaptic transmission and synaptic morphogenesis. LRRK2 controls synapse morphogenesis at the *Drosophila* neuromuscular junction through complex formation with tubulin and the microtubule (MT)-binding protein Futsch in the presynaptic compartment, and interaction with 4E-BP at the postsynaptic level (Lee et al. 2010b). Thus, *LRRK2* pathogenic mutations may impede synaptic function through deregulation of protein synthesis and MT dynamics. Additionally, EndophilinA, a presynaptic membrane-binding protein that participates in clathrin-dependent endocytosis of synaptic vesicle membranes, was recently identified as a substrate of LRRK2 kinase activity (Matta et al. 2012). G2019S LRRK2 induced the hyperphosphorylation of EndophilinA in cells and fly brain with reduced phosphorylation in *dLRRK* null flies. LRRK2-mediated phosphorylation of EndophilinA inhibits membrane tubulation and membrane association, and controls a phosphorylation cycle that regulates synaptic vesicle endocytosis (Matta et al. 2012). These observations suggest that LRRK2 regulates neurotransmission and that a tight control of LRRK2 kinase activity is required for regulating synaptic vesicle formation and endocytic function.

Finally, recent studies support a role for LRRK2/dLRRK in late endosomes, lysosomes, and the retromer complex that guide protein sorting from the endosome-lysosome pathway to the Golgi complex (Dodson et al. 2012; MacLeod et al. 2013). Dodson et al. showed that in follicle cells, dLRRK is localized to late endosomal and lysosomal membranes, where it interacts with Rab7, a key regulator of late endosomal transport. dLRRK negatively regulates the Rab7-mediated perinuclear clustering of lysosomes, whereas a mutant form of dLRRK, analogous

to the pathogenic G2019S variant, promotes lysosomal tethering and perinuclear positioning of lysosomes in a process requiring dynein and microtubules (Dodson et al. 2012). Furthermore, LRRK2 interacts with Rab7L1, a small GTPase involved in vesicular trafficking to lysosomal-related organelles and in regulating neurite process length (MacLeod et al. 2013; Beilina et al. 2014). G2019S LRRK2 overexpression altered the morphology of lysosomes and the Golgi complex that may result from defects in retromer-associated protein sorting. Co-expressing Rab7L1 or restoring retromer function by co-expressing the PD-associated protein VPS35 (Vilarino-Guell et al. 2011), a key component of the retromer complex, rescued G2019S LRRK2-mediated dopaminergic neurodegeneration in flies (MacLeod et al. 2013). Interestingly, overexpression of Rab7, the only Rab with a described role in regulation of the retromer complex, partially attenuated early lethality in G2019S LRRK2 flies relative to the more robust effects of Rab7L1 (MacLeod et al. 2013). Together, these observations suggest that impaired lysosomal activity and defective protein sorting in endosomal and lysosomal vesicular compartments may play a role in LRRK2-associated neuronal damage.

2.2.2 *Caenorhabditis elegans*

The major advantage of the nematode worm, *C. elegans*, as a model organism is the ease of conducting genetic screens and evaluating compounds or toxicants with neuroprotective or neurotoxic effects. Hence, most studies have focused on the role of LRRK2 in the response to oxidative stress, a key process implicated in PD (Wolozin et al. 2008; Saha et al. 2009; Samann et al. 2009). *LRK-1* is the *C. elegans* paralog of human *LRRK2* and *LRRK1*. LRK-1 localizes to the Golgi complex and is expressed in dopaminergic neurons of worms (Sakaguchi-Nakashima et al. 2007; Samann et al. 2009). Similar to *Drosophila* models, the effects of *LRK-1* deletion are subtle and phenotypes observed in different models are often variable.

A few studies have reported the effects of *LRK-1* deletion in worms. Sakaguchi et al. showed that deletion of *LRK-1* impaired the sorting and localization of synaptic vesicles (SV) and SV-associated proteins (Sakaguchi-Nakashima et al. 2007). LRK-1 normally excludes SV proteins from a dendritic-specific transport pathway mediated by the AP-1 clathrin adaptor, which supports a role for LRK-1 in SV-associated protein sorting to the pre-synaptic compartment of axons. Although the effects of *LRK-1* deletion on dopaminergic neuronal function or survival were not explored in this study, *LRK-1* mutants exhibit subtle defects in movement and were partially defective in chemotaxis. The loss of *LRK-1* sensitizes worms to toxicity induced by the mitochondrial Complex-I inhibitor rotenone (Wolozin et al. 2008; Saha et al. 2009) and to induction of endoplasmic reticulum stress induced by tunicamycin (Samann et al. 2009), suggesting a role for LRK-1 in cellular stress responses. The sensitivity to tunicamycin in *LRK-1* mutant worms appears to be mediated via PINK1 since its deletion suppressed the vulnerability of *LRK-1* mutants to the toxin. Oppositely, *LRK-1* deletion suppressed the enhanced sensitivity of *PINK1* mutant worms to paraquat and rescued defects in

mitochondrial cristae and impaired axonal guidance (Samann et al. 2009). LRK-1 and PINK1 act antagonistically in *C. elegans* to regulate the response to stress and neurite outgrowth. Recently, Yuan et al. showed that *LRK-1* loss-of-function in worms results in increased sensitivity of dopaminergic neurons to toxicity induced by 6-OHDA and human α-synuclein overexpression (Yuan et al. 2011) suggesting a neuroprotective effect of LRK-1. Furthermore, human LRRK2 functionally substitutes for LRK-1 to protect from human α-synuclein-induced dopaminergic neuron degeneration and LRRK2 kinase activity contributes to this neuroprotective effect. The protective effects of LRRK2 in worms require kinase activity and are mediated in part through p38 MAP kinase signaling.

Worms expressing human WT or G2019S LRRK2 exhibit reduced vulnerability to rotenone (Wolozin et al. 2008) and paraquat (Saha et al. 2009) toxicity, suggesting a protective role for LRRK2 in mitochondria and/or the oxidative stress response. In addition, overexpression of LRRK2 extended the lifespan of worms, suggesting a potentially beneficial role for LRRK2 in the aging process (Wolozin et al. 2008). Yao et al. demonstrated that overexpression of human WT, R1441C and G2019S LRRK2 in dopaminergic neurons caused age-dependent neurodegeneration, dopamine deficiency, and locomotor deficits in worms (Yao et al. 2010). In comparison to WT LRRK2, the R1441C and G2019S pathogenic variants induced more severe neurodegeneration and behavioral deficits that could be rescued by dopamine replacement. Furthermore, overexpression of K1347A LRRK2, a GTP binding-deficient mutant with impaired kinase activity, did not induce dopaminergic neurodegeneration or behavioral deficits. Yao and coworkers have also demonstrated that pharmacological inhibition of LRRK2 kinase activity rescued dopaminergic neurodegeneration and dopamine-mediated behavioral deficits in worms overexpressing R1441C or G2019S LRRK2 (Yao et al. 2013). These observations support a role for kinase activity as a critical mediator of neurotoxicity induced by R1441C and G2019S mutant LRRK2 in worm models.

2.3 Vertebrate LRRK2 Models

2.3.1 Zebrafish

Zebrafish LRRK2 (zLRRK2) protein contains all of the functional domains of human LRRK2 and displays a high degree of conservation of amino acid sequence with human LRRK2 particularly within the kinase domain (Sheng et al. 2010). zLRRK2 is strongly expressed in the brain during development and larval stages, and is expressed in muscle, ovary, gut, and most prominently in the brain of adult fish (Sheng et al. 2010). Silencing of z*LRRK2* expression using morpholinos led to severe embryonic defects and lethality. Surviving morphants displayed reduced brain size and heart edema, which could be partially rescued by human LRRK2 overexpression. Interestingly, deletion of the WD40 domain from zLRRK2 did not cause developmental defects, but instead produced PD-related phenotypes

including loss of dopaminergic neurons and locomotor defects, which could be rescued by L-DOPA treatment (Sheng et al. 2010). Furthermore, deletion of the WD40 domain led to reduced and disorganized axon tracts in the midbrain, which implicates the WD40 domain in neural development and/or neuronal maintenance. Additionally, overexpression of human LRRK2 could rescue the dopaminergic neurodegeneration in zLRRK2 ΔWD40 morphants, indicating that zLRRK2 and human LRRK2 are orthologs and that zebrafish could be considered as a relevant model to investigate the role of LRRK2 in PD and to identify therapeutic agents directed at LRRK2 (Sheng et al. 2010). Despite these promising observations, a similar study reports that deletion of the WD40 domain of zLRRK2 does not cause dopaminergic neurodegeneration (Ren et al. 2011). Furthermore, deletion of the kinase or the WD40 domain had no impact on dopaminergic neuronal survival and did not result in locomotor deficits. This latter study is consistent with prior reports of loss-of-function or knockout studies in *Drosophila* and rodents that collectively suggest a limited role for LRRK2 in dopaminergic neuron development and maintenance.

2.3.2 Rodent *LRRK2* Models

Although invertebrate models are powerful tools to screen for pharmacological or genetic modifiers of LRRK2, it is important to mention that *Drosophila* and *C. elegans* do not contain true orthologs of human *LRRK2* (Marin 2008). Comparative genomic analyses reveal that *dLRRK* and *LRK-1* are most likely paralogs of mammalian *LRRK2*. Moreover, PD is characterized by slow and progressive neurodegeneration and alteration of basal ganglia circuitry with increasing age. The absence of basal ganglia circuitry in invertebrates and their short life span make them imperfect models for studying PD. In comparison, rodent models of LRRK2 circumvent these limitations and offer a more relevant approach to understand the pathological function of LRRK2 and to validate therapeutic targets for treating idiopathic and familial PD.

LRRK2 knockout models: Andres-Mateo et al. reported that *LRRK2* knockout (KO) mice with a partial deletion of exon 39 and complete deletion of exon 40 encoding the kinase domain are viable, grossly normal, and have an intact nigrostriatal dopaminergic pathway up to 2 years of age. Furthermore, *LRRK2* KO mice do not exhibit altered sensitivity to MPTP-induced neurotoxicity (Andres-Mateos et al. 2009). *LRRK2* KO mice with a deletion of exon 2 have also been generated that similarly are viable, fertile and do not display motoric deficits (Lin et al. 2009; Tong et al. 2010, 2012). In addition, no dopaminergic neurodegeneration or α-synuclein accumulation or aggregation within the brain is detected in 20-month-old KO animals (Tong et al. 2010). Lin et al. examined the pathological interaction between LRRK2 and α-synuclein using inducible transgenic mice with expression of human A53T α-synuclein in CamKIIα-positive forebrain neurons (Lin et al. 2009). Overexpression of A53T α-synuclein led to progressive degeneration of forebrain neurons associated with motor deficits, gliosis, Golgi

fragmentation, and the somatic accumulation and aggregation of α-synuclein. The loss of *LRRK2* in these mice prevents Golgi fragmentation, α-synuclein accumulation/aggregation, microglial activation, and forebrain neuronal degeneration. The consequences of *LRRK2* deletion on motor deficits induced by A53T α-synuclein were not reported (Lin et al. 2009). In a subsequent study, Daher and colleagues employed an A53T α-synuclein transgenic mouse model under the control of the hindbrain-selective mouse prion protein (PrP) promoter to explore the pathological interaction between LRRK2 and α-synuclein (Daher et al. 2012). The deletion of *LRRK2* did not influence the lethal neurodegenerative phenotype of PrP-A53T α-synuclein transgenic mice, including premature survival, behavioral deficits, α-synuclein pathology and gliosis, suggesting that α-synuclein-mediated neurodegeneration in hindbrain neurons occurs largely independent of LRRK2 expression in mice (Daher et al. 2012). Although LRRK2 may selectively contribute to cellular aspects of α-synuclein-induced neuropathology, it is not yet clear whether inhibition of LRRK2 could be employed as a therapeutic strategy to attenuate α-synuclein-mediated neuronal damage relevant to PD.

Tong and colleagues generated two independent lines of *LRRK2* KO mice through deletion of either the promoter and exon 1 or exon 29–30 encoding the GTPase domain (Tong et al. 2010). The integrity and function of the nigrostriatal dopaminergic system was not affected in the brain of *LRRK2* null mice at 2 years of age. Neuropathological features associated with neurodegeneration, including α-synuclein or ubiquitin accumulation, gliosis or altered neuronal structure, were absent from *LRRK2* KO mice. Notably, KO mice developed age-dependent kidney abnormalities, such as reduced size due to increased apoptosis, and altered kidney morphology. KO kidneys also displayed prominent α-synuclein accumulation and aggregation, ubiquitin accumulation, and impaired activity of the autophagy-lysosomal pathway. Herzig et al. confirmed the important role of LRRK2 in the kidney and identified an additional role for LRRK2 in the lung (Herzig et al. 2011). Loss of *LRRK2* led to disrupted lysosomal homeostasis in both organs whereas no abnormalities were observed in the brain. In contrast, however, they failed to observe impaired autophagy or α-synuclein accumulation in the kidney of KO mice (Herzig et al. 2011). Recent studies suggest that the loss of *LRRK2* causes age-dependent bi-phasic alterations of autophagic activity in the kidney (Tong et al. 2012), or that *LRRK2* deletion leads to a progressive enhancement of autophagic activity in the kidney but without evidence of bi-phasic changes (Hinkle et al. 2012).

Hinkle and coworkers generated *LRRK2* KO mice by deletion of exon 41 that encodes the activation hinge of the kinase domain (Hinkle et al. 2012). At 20 months of age, *LRRK2* KO mice display no alteration of the nigrostriatal dopaminergic system and no alteration of α-synuclein or tau levels. Behavioral analysis revealed that KO mice exhibit abnormal exploratory activity in the open-field test that may indicate increased anxiety. Furthermore, although G2019S LRRK2 expression was shown to impair neurite outgrowth (MacLeod et al. 2006) and suggested to induce defects in neural stem cell proliferation and differentiation (Liu et al. 2012), the loss of *LRRK2* in mice had no effect on the spine dynamics of medium-sized spiny neurons or on neural stem cell proliferation and neuroblast cell survival in the dentate

gyrus (Hinkle et al. 2012). Paus et al. have examined adult neurogenesis and the dendritic morphology of adult newborn neurons in the dentate gyrus of *LRRK2* KO mice (Paus et al. 2013). The proliferation and survival of neural precursors is not altered in KO mice. However, the loss of *LRRK2* increases the number of double-cortin-positive migrating neuroblasts and immature neurons, although the total number of mature neurons remains unaltered. Furthermore, immature neuroblasts in KO mice displayed enhanced dendritic branching and complexity while the density of mossy fibers projecting from the dentate gyrus to the hippocampal CA3 region was increased in KO mice (Paus et al. 2013). Collectively, these studies suggest a regulatory role for LRRK2 in adult neurogenesis by modulating neural stem cell fate and by shaping dendritic branching and the axonal output of adult newborn neurons in the hippocampus.

Recently, two *LRRK2* KO rat models have been developed (Baptista et al. 2013; Ness et al. 2013). *LRRK2* KO rats displayed significant weight gain compared to wild-type rats together with morphological and histopathological alterations in kidney, liver and lung, changes in the cellular composition of the spleen, modification of serum chemistry, and subtle differences in the response to immunologic challenge. However, the consequences of *LRRK2* deficiency in the brain were not reported in these studies (Baptista et al. 2013; Ness et al. 2013). In summary, mice and rats with disruption of *LRRK2* display similar alterations in kidney homeostasis suggesting that the function of LRRK2 in the kidney may be conserved across species.

Classic LRRK2 transgenic models: Inducible transgenic mice overexpressing human LRRK2 were first described by Lin and colleagues (Wang et al. 2008b; Lin et al. 2009; Parisiadou et al. 2009). Transgenic mice expressing HA-tagged human LRRK2 under the transcriptional control of a tetracycline operator (TetO)-regulated promoter were crossed with transgenic mice expressing a Tet transactivator (tTA) from the CamKIIα promoter. In vitro studies demonstrated that primary neurons derived from G2019S LRRK2 mice display reduced axonal outgrowth and identified a role for LRRK2 in neuronal morphogenesis through F-actin remodeling (Wang et al. 2008b; Parisiadou et al. 2009). WT, G2019S and kinase domain-deficient (KD) LRRK2 transgenic mice are viable and develop normally, whereas G2019S LRRK2 lines display increased ambulatory activities starting at 12 months of age (Lin et al. 2009). No signs of neurodegeneration or neuropathology were detected in WT and G2019S LRRK2 transgenic lines at 12 and 20 months of age. Expression of WT and G2019S LRRK2 altered microtubule organization and induced Golgi fragmentation apparently through a kinase-independent mechanism (Lin et al. 2009). Despite the limited pathology in these LRRK2 transgenic mice, the overexpression of WT or G2019S LRRK2 accelerated the progression of A53T α-synuclein-mediated neuropathology and neurodegeneration in the forebrain of conditional transgenic mice (Lin et al. 2009). LRRK2 overexpression also exacerbated the toxic cellular effects of A53T α-synuclein in forebrain neurons, including Golgi fragmentation, impairment of the ubiquitin-proteasome system, and mitochondrial abnormalities (Lin et al. 2009). Collectively, this study provides support for the pathological interaction of LRRK2 and α-synuclein in PD.

The contribution of LRRK2 to regulating α-synuclein-related neuropathology could be specific to certain neuronal populations or brain regions. We and others do not observe a pathological interaction between human LRRK2 and α-synuclein when employing alternative A53T α-synuclein transgenic mice driven by the hindbrain-selective PrP or broadly expressing Thy1 promoters (Daher et al. 2012; Herzig et al. 2012). Both studies demonstrate that high levels of G2019S LRRK2 expression within neurons of the cortex, striatum, brainstem, and spinal cord of mice do not exacerbate α-synuclein-mediated neuropathology. Herzig et al. established transgenic mice expressing human WT LRRK2 or G2019S LRRK2 under the control of a Thy1 promoter, which directs widespread expression in neurons of the cortex, brainstem, and spinal cord (Herzig et al. 2012). LRRK2 transgenic expression did not induce motoric abnormalities nor altered levels of endogenous tau and α-synuclein in the brain at 15 months of age (Herzig et al. 2012). Daher and colleagues showed that expression of G2019S LRRK2 has a limited impact on the lethal neurodegenerative phenotype that develops in A53T α-synuclein transgenic mice, including premature lethality, behavioral deficits, and human α-synuclein or glial neuropathology (Daher et al. 2012). Furthermore, the co-expression of A53T α-synuclein and G2019S LRRK2 did not combine to induce nigral dopaminergic neuronal loss in this model (Daher et al. 2012). At present, it is not clear whether LRRK2 consistently enhances α-synuclein-related neuropathology in mice, or whether the pathological interaction of these two proteins is restricted to specific neuronal populations.

Ramonet and coworkers developed LRRK2 transgenic mice bearing the PD-associated R1441C or G2019S mutations (Ramonet et al. 2011). In this model, human LRRK2 transgenes are expressed under the transcriptional control of a hybrid CMV-enhanced human platelet derived growth factor β-chain (CMVe-PDGFβ) promoter. G2019S LRRK2 was highly expressed in many brain areas, including the olfactory bulb, cerebral cortex, striatum, hippocampus, and cerebellum and at lower levels in substantia nigra dopaminergic neurons, whereas surprisingly R1441C LRRK2 expression was restricted to the cerebral cortex and cerebellum (Ramonet et al. 2011). Overexpression of G2019S LRRK2 leads to the progressive and selective degeneration of nigrostriatal dopaminergic neurons ($\sim$20 %) up to 2 years of age. At 14–15 months of age, no alteration in striatal dopamine levels or locomotor activity could be detected in G2019S LRRK2 mice. Unexpectedly, R1441C LRRK2 transgenic mice exhibit impaired locomotor activity accompanied by reduced catecholamine levels in the cerebral cortex consistent with the restricted pattern of transgene expression (Ramonet et al. 2011). In vitro studies revealed that cultured primary midbrain dopaminergic neurons derived from G2019S LRRK2 mice exhibit reduced neurite complexity. G2019S LRRK2 mice failed to develop α-synuclein, ubiquitin or tau neuropathology or gliosis. Notably, G2019S or R1441C LRRK2 expression resulted in the accumulation of autophagic vacuoles and damaged mitochondria in the brains of aged mice (Ramonet et al. 2011). Collectively, this model demonstrates that the common G2019S mutation exerts deleterious effects on the nigrostriatal dopaminergic pathway in mice possibly involving abnormal autophagy.

Chen et al. generated similar transgenic mice expressing HA-tagged human WT or G2019S LRRK2 from the same CMV-enhanced PDGFβ promoter. The G2019S LRRK2 mice also display a progressive, late-onset degeneration of substantia nigra dopaminergic neurons (Chen et al. 2012). In addition, G2019S LRRK2 expression increased tau phosphorylation, whereas the levels of ubiquitin or α-synuclein were unaltered. G2019S LRRK2 mice exhibit decreased striatal dopaminergic fiber density accompanied by reduced dopamine reuptake in the striatum. Furthermore, G2019S LRRK2 mice displayed L-DOPA-responsive progressive motor deficits. Interestingly, a comparative phosphoproteomic analysis between WT and G2019S LRRK2 brains revealed that MAP kinase kinase 4 (MKK4) could be a potential substrate of LRRK2 kinase activity in the substantia nigra (Chen et al. 2012). G2019S LRRK2 mice displayed increased MKK4 phosphorylation at 12 months of age, which correlated with abnormal activation of the MKK4-JNK-c-Jun-mediated cell death pathway. Collectively, this transgenic model could recapitulate some of the key features of PD subjects harboring the G2019S mutation and suggests a potential role for MKK4 phosphorylation in G2019S LRRK2-mediated dopaminergic neurodegeneration (Chen et al. 2012).

Maekawa and coworkers generated transgenic mice constitutively expressing V5-tagged human I2020T LRRK2 from a CMV promoter (Maekawa et al. 2012). I2020T LRRK2 was expressed at $\sim$1.5 fold the level of endogenous LRRK2 in all brain areas examined, including the striatum and substantia nigra. I2020T LRRK2 mice are viable and exhibit normal weight and fertility. Furthermore, expression of I2020T LRRK2 had no influence on nigral dopaminergic neuronal number or striatal dopaminergic fiber density. I2020T LRRK2 mice display a transient impairment of locomotor activity, reduced striatal dopamine content, fragmented Golgi apparatus, and an elevated degree of tubulin polymerization, which was not mediated through increased tau phosphorylation. Primary dopaminergic neurons derived from the ventral midbrain of I2020T LRRK2 mice display increased vulnerability in vitro and reduced neurite length.

Zhou et al. reported the first rat transgenic model expressing G2019S LRRK2 (Zhou et al. 2011). They created constitutive and inducible lines to temporally control G2019S LRRK2 expression. Constitutive expression of LRRK2 in rats failed to induce any behavioral phenotype, whereas the conditional adult-onset expression of LRRK2 caused abnormal locomotor activity in aged animals possibly through altered striatal dopamine reuptake. Despite this behavioral alteration, LRRK2 expression had no effect on the number of dopaminergic and noradrenergic neurons or on striatal dopamine content. No inclusions positive for α-synuclein, ubiquitin, or phosphorylated tau were detected in the brains of G2019S LRRK2 rats. Hence, inducible LRRK2 transgenic rats manifest early dopaminergic neuronal dysfunction potentially akin to asymptomatic subjects carrying the G2019S mutation (Zhou et al. 2011).

Bacterial artificial chromosome (BAC) LRRK2 transgenic models: One of the criticisms of classical transgenic models that employ mini-gene cassettes relates to the non-physiological levels of transgene expression in cell populations that do not closely reflect the normal endogenous pattern of gene expression. Furthermore,

chromosome-position effects resulting from the random integration of small transgenes within the host genome can often result in transgenic founder animals with distinct transgene expression levels and patterns that may account for the diverse phenotypes observed among different mouse models. Models that employ bacterial artificial chromosome (BACs) constructs circumvent many of these issues as they utilize the endogenous promoter and regulatory elements to recapitulate the normal expression pattern of a (trans)gene of interest, and are less susceptible to chromosome-position effects at the genomic integration site owing to their large size (~150–200 Kb) and nature. Furthermore, BAC transgenic constructs typically integrate within the genome at lower copy number (~1–10 copies) than mini-gene cassettes, thereby more closely recapitulating expression levels of the endogenous gene. This, however, can also be a disadvantage as in many cases high non-physiological levels of transgene expression are required to precipitate robust neurological phenotypes within the life span of mice.

Li and colleagues described the first BAC transgenic mice expressing human WT or R1441G LRRK2 using a BAC clone containing the human *LRRK2* genomic sequence (Li et al. 2009). R1441G LRRK2 was expressed in the cortex, cerebellum, striatum, and ventral midbrain at 5–10 fold the level of endogenous LRRK2. WT and R1441G LRRK2 mice develop normally without alteration in body or brain weight. R1441G LRRK2 mice displayed age-dependent and progressive L-DOPA-responsive motor deficits typified by impaired vertical rearing activity and akinesia at 10–12 months of age, accompanied by a modest reduction of striatal dopamine release. R1441G LRRK2 expression did not affect the number of nigrostriatal dopaminergic neurons or their striatal nerve terminals in mice at 9–10 months of age, although a modest reduction in cell body size and the density of TH-positive dendrites of nigral dopaminergic neurons was observed. Furthermore, some evidence of dopaminergic axonal damage, including axonal varicosities, spheroids, and dystrophic neurites, could be detected in the striatum and piriform cortex that are enriched with dopaminergic projections. R1441G LRRK2 BAC mice did not display alterations in α-synuclein and ubiquitin, whereas neuronal processes positive for hyperphosphorylated tau were detected in the striatum and cortex. It is not clear how the subtle dopaminergic neuropathology of R1441G LRRK2 mice precipitates the profound motor deficits in this BAC model, but these observations suggest either dysfunction of nigrostriatal dopaminergic neurotransmission or an extra-nigral origin of the dopamine-dependent motor deficits such as from the prefrontal cortex. Further dissection and validation of this motor phenotype is required especially since two recent studies could not replicate the original motor deficits in this BAC model that were initially evident at 10 months of age. Dranka et al. reported that the same R1441G LRRK2 BAC mice alternatively display deficits in motor coordination in the Rotarod and pole tests at 16 months of age (Dranka et al. 2013), whereas Bichler et al. reported that R1441G LRRK2 mice developed mild hypokinesia in the open-field arena at 16 months with gastrointestinal dysfunctions beginning at 6 months (Bichler et al. 2013). R1441G LRRK2 mice do not additionally display non-motor phenotypes,

including depression and anxiety-like behaviors, altered pain sensitivity and olfaction, or impaired learning and memory (Bichler et al. 2013).

Additional LRRK2 BAC transgenic mouse models have also been developed (Li et al. 2007, 2010). Li and coworkers generated transgenic mice using a BAC clone encompassing the entire mouse *LRRK2* (WT and G2019S) genomic sequence. FLAG-tagged WT and G2019S LRRK2 transgenes were expressed at similar levels in the cerebral cortex, ventral tegmental area, amygdala, and hippocampus, and could be detected in substantia nigra dopaminergic neurons. WT or G2019S LRRK2 expression did not affect nigrostriatal dopaminergic neuron survival or nerve terminal morphology in mice at 20 months of age. WT LRRK2 BAC mice displayed a reduced number of phospho-tau-positive cells in the striatum compared to control or G2019S LRRK2 mice, suggesting that LRRK2 might prevent the accumulation of phosphorylated tau in the brain (Li et al. 2010). However, the significance of this tau phenotype is unclear since wild-type mice do not normally contain detectable hyperphosphorylated tau in the brain. WT and G2019S LRRK2 transgenic expression altered striatal dopamine transmission in an opposite manner. WT LRRK2 mice had enhanced striatal dopamine release with unaltered dopamine uptake or tissue content, and accordingly WT LRRK2 mice were hyperactive and showed enhanced motor performance. Conversely, G2019S LRRK2 mice showed normal motor function, but displayed an age-dependent decrease in striatal dopamine content and decreased striatal dopamine release (Li et al. 2010). Collectively, these BAC mice reveal that LRRK2 regulates dopaminergic neurotransmission in the striatum and contributes to the control of motor activity.

Melrose et al. also developed BAC mice expressing human WT and G2019S LRRK2 (Melrose et al. 2010). LRRK2 transgene and endogenous LRRK2 expression were similar throughout the brain, except in the hippocampus, where transgene expression was higher. Expression of LRRK2 had no influence on nigral dopaminergic neuron number but correlated with reduced extracellular dopamine levels in the striatum. In contrast to R1441C knockin mice (see below; Tong et al. 2009), decreased dopamine levels in these BAC mice were not caused by alteration of D2 autoreceptor-mediated inhibition of dopamine synthesis and release (Melrose et al. 2010). G2019S LRRK2 mice display normal sensorimotor function but exhibit reduced exploratory behaviors, which may reflect increased anxiety. Pathologically, G2019S LRRK2 mice display an age-dependent increase in tau levels and phosphorylation suggesting altered tau metabolism (Melrose et al. 2010). G2019S LRRK2 BAC mice also exhibit reduced adult neurogenesis, which could be partially rescued by physical exercise (Winner et al. 2011). G2019S LRRK2 expression altered the proliferation and migration of neuroblasts in neurogenic niches of the adult brain, and exerted a negative impact on neurite outgrowth and spine density of hippocampal newborn neurons.

In summary, BAC transgenic models expressing LRRK2 mutations exhibit a consistent yet mild neuropathological phenotype characterized by dysfunction of nigrostriatal dopaminergic neurotransmission, altered locomotor activity, and increased tau expression and/or phosphorylation. These phenotypes could potentially represent the earliest derangements of the nigrostriatal dopaminergic

pathway in PD. BAC LRRK2 models fail, however, to recapitulate other cardinal features of PD, including dopaminergic neuronal degeneration (or indeed any evidence of neuronal loss) and α-synuclein accumulation and aggregation. BAC LRRK2 mice could therefore be considered an early pre-symptomatic model of PD prior to manifestation of neuronal degeneration and associated motor deficits. These mild phenotypes may result from insufficient levels of LRRK2 transgene overexpression in nigral dopaminergic neurons compared to some classical transgenic models that exhibit neurodegeneration (Ramonet et al. 2011; Chen et al. 2012). Collectively, studies in BAC mice support a role for LRRK2 in the regulation of striatal dopaminergic neurotransmission and motor control.

LRRK2 knockin mice: Tong and colleagues generated LRRK2 R1441C knockin (KI) mice by introducing the R1441C mutation into exon 31, thereby allowing its expression under the control of endogenous regulatory elements (Tong et al. 2009). R1441C LRRK2 KI mice are viable, fertile, and appear grossly normal. The R1441C mutation had no impact on dopaminergic neuron number or morphology in the substantia nigra, or upon noradrenergic neurons in the locus coeruleus. Striatal dopamine levels and dopamine turnover are normal in R1441C KI mice. Gliosis and the accumulation or abnormal phosphorylation of α-synuclein, ubiquitin, and tau are not altered in 22-month-old KI mice. R1441C KI mice displayed normal spontaneous locomotor activity, but exhibited impaired amphetamine-stimulated locomotor activity, altered dopamine D2 receptor-mediated function in the striatum and reduced catecholamine release in cultured chromaffin cells (Tong et al. 2009). Together, the R1441C mutation in mice impairs stimulated nigrostriatal dopaminergic transmission and D2 receptor function.

Liu and colleagues developed R1441G LRRK2 KI mice and investigated the effects of the R1441G mutation on the nigrostriatal dopaminergic pathway (Liu et al. 2014). R1441G KI mice are viable, fertile, and have normal body weight, brain size, and locomotor activity. R1441G KI mice do not display any alteration in the number and morphology of substantia nigra dopaminergic neurons or the density of striatal dopaminergic nerve terminals. Alterations in autophagy or abnormal α-synuclein, tau, or ubiquitin aggregation or accumulation could not be detected in the brains of R1441G KI mice. R1441G KI mice do exhibit an increased vulnerability to, and slower recovery from, reserpine-induced synaptic dopamine depletion and locomotor impairment (Liu et al. 2014). Collectively, observations in R1441C and R1441G LRRK2 KI mice indicate that pathogenic mutations in the ROC GTPase domain cause striatal dopaminergic synaptic vulnerability and perturbed nigrostriatal dopaminergic neurotransmission.

Herzig and coworkers generated G2019S LRRK2 KI mice (Herzig et al. 2011). In contrast to *LRRK2* KO mice, G2019S KI mice do not display any morphological alterations in kidney and lung tissues, suggesting that the G2019S mutation does not manifest a loss-of-function, but KI mice exhibit increased diastolic pressure (Herzig et al. 2011). Analysis of G2019S KI mouse brain revealed that the G2019S mutation does not cause any remarkable neuropathology and had no influence on the nigrostriatal dopaminergic pathway. Furthermore, G2019S KI mice display

normal drug-induced locomotor activity (Herzig et al. 2011). Collectively, the G2019S mutation in mice has minimal impact on the nigrostriatal dopaminergic system, suggesting that LRRK2 KI mice do not represent robust models of PD.

Viral-mediated gene transfer of LRRK2 in rodents: Viral-mediated gene delivery direct to the rodent brain offers several advantages over the conventional transgenic rodents outlined above, including their simple and rapid generation compared to transgenic animals, the possibility to deliver the transgene during adulthood to avoid potential developmental compensatory effects, and the capacity to directly compare multiple variants of the same transgene with equivalent expression levels and patterns (Low and Aebischer 2012). Gene delivery of viral vectors also permits direct targeting of specific neuronal populations that may not be readily accessible using transgene cassettes with defined promoter elements in mice. In addition, whereas transgenic models are typically limited to mice and rats, viral models can be developed in rodents and non-human primates, which serve to hasten translation to human diseases.

In the context of PD, viral models offer additional advantages. First, investigating the specific effects of transgene expression in substantia nigra dopaminergic neurons can be achieved through direct stereotactic injection. Despite a limited diffusion of the virus to adjacent regions, the transgene expression remains localized to the targeted area, and the viral serotype or promoter element can be altered to improve and optimize neuronal-specific transgene expression. Conversely, a strict control of transgene expression is difficult to achieve in transgenic rodents whereby widespread transgene expression can often lead to confounding extra-nigral phenotypes, whereas alternatively it has also proved difficult to achieve sufficient levels of transgene expression in nigral dopaminergic neurons using available promoter elements. Furthermore, the unilateral injection of viral vectors into one hemisphere of the brain allows one to determine the impact of transgene expression on dopaminergic neuron survival and physiology by direct comparison to the non-injected, unaltered hemisphere of the same animal. High-level transgene expression can also be achieved, which will help to accelerate the onset and progression of dopaminergic neurodegeneration. This aspect may be critical since neurodegeneration can typically be observed a few weeks after viral delivery to the brain, whereas transgenic models require substantial time before neuronal loss (if any) becomes apparent. Additionally, the integration and copy number of transgene per cell can be modulated by adjusting the viral titer injected, which allows one to experimentally correlate phenotype severity with transgene dosage. Finally, viral-mediated gene transfer models can be easily applied to existing environmental and/or genetic animal models of PD to study genetic or pathological interactions, or to validate therapeutic targets.

Adeno-associated viral (AAV)-based models of α-synuclein-induced toxicity in nigral dopaminergic neurons have been successfully established as important models of PD (Kirik et al. 2002; Klein et al. 2002; Lo Bianco et al. 2002; Low and Aebischer 2012). Due to the limited packaging capacity of AAV vectors, similar approaches could not be used to deliver the human *LRRK2* gene into midbrain dopaminergic neurons. Two rodent models of viral-mediated LRRK2 expression have so far been reported. Lee et al. developed a herpes simplex virus (HSV)

amplicon-based mouse model of G2019S LRRK2-induced dopaminergic neurotoxicity, whereas Dusonchet et al. generated a rat model of progressive dopaminergic neurodegeneration using a second-generation human adenovirus serotype 5 expressing human G2019S LRRK2 (Lee et al. 2010a; Dusonchet et al. 2011).

The model described by Lee and coworkers is based upon a single unilateral striatal injection of HSV expressing a CMV-driven GFP reporter and untagged human LRRK2 from the immediate-early 4/5 gene promoter (Lee et al. 2010a). Injection of HSV was performed in the striatum to avoid non-specific inflammatory damage to the substantia nigra and resulted in the transduction of ~75 % of the dopaminergic neurons in the ipsilateral nigra. The nigrostriatal expression of WT LRRK2 induced modest nigral dopaminergic neurodegeneration (~10–20%), whereas expression of the kinase-hyperactive G2019S LRRK2 resulted in ~50% neuronal loss in the ipsilateral nigra associated with reduced striatal dopaminergic fiber density at 3 weeks post-injection. Furthermore, expression of a kinase-inactive variant, G2019S-D1994A, which abolished the elevated kinase activity of the familial G2019S mutation, did not induce dopaminergic neuronal loss (Lee et al. 2010a). Collectively, this study confirms prior findings in G2019S LRRK2 transgenic mice (Ramonet et al. 2011; Chen et al. 2012) and strongly supports a critical role for elevated kinase activity in mediating the G2019S LRRK2-dependent degeneration of dopaminergic neurons in mice (Lee et al. 2010a). To further validate the kinase activity of LRRK2 as a potential therapeutic target for LRRK2-related PD, the protective effects of pharmacological inhibition of LRRK2 kinase activity were evaluated in this HSV model. Using a library of kinase inhibitors, two potent inhibitors of LRRK2 kinase activity (GW5074 and indirubin-3′-monooxime) were identified in vitro (Lee et al. 2010a). The twice daily intraperitoneal injection of either inhibitor in mice injected with HSV-LRRK2 G2019S attenuated dopaminergic neuronal degeneration. Although these findings are promising, it is not clear from this study whether these kinase inhibitors act directly on LRRK2 since both compounds are selective for and more potently inhibit a number of additional kinases. It will be important to further evaluate this HSV LRRK2 rodent model using highly selective, potent, and brain-penetrant LRRK2 kinase inhibitors that have recently been developed (Choi et al. 2012; Reith et al. 2012; Sheng et al. 2012). Altogether, this study suggests that pharmacological inhibition of LRRK2 kinase activity is a promising therapeutic approach for the treatment of neurodegeneration in *LRRK2*-associated PD. Despite the promising neurodegenerative phenotype, the authors do not describe the behavioral or cytopathological consequences of G2019S LRRK2 expression in the nigrostriatal pathway (Lee et al. 2010a).

In a second study, Dusonchet and colleagues generated a LRRK2 model based on the unilateral injection of recombinant, second-generation human serotype 5 adenoviral (rAd) vectors expressing FLAG-tagged human WT or G2019S LRRK2 driven by a neuronal-specific human synapsin-1 promoter (Dusonchet et al. 2011). Injections of the rAd vectors were performed at 6 distinct sites in the striatum of adult rats as adenoviral particles undergo efficient retrograde axonal transport to dopaminergic neurons of the substantia nigra, whereas direct injections into the

nigra result in poor transduction efficiency. At 10 days post-injection, ~30 % of nigral dopaminergic neurons exhibited strong transgene expression that persisted up to 42 days albeit with a progressive reduction in expression over time. Despite the moderate proportion of dopaminergic neurons transduced, the expression of human LRRK2 was 2-fold greater than endogenous LRRK2 in the substantia nigra suggesting that infected cells express high levels of the transgene. WT LRRK2 or GFP did not cause dopaminergic neurodegeneration, whereas G2019S LRRK2 expression induced the progressive loss of ~20% of dopaminergic neurons in the ipsilateral substantia nigra over 42 days, but without a corresponding reduction of striatal dopaminergic fiber density (Dusonchet et al. 2011). The expression of WT and G2019S LRRK2 was transiently associated with the abnormal hyperphosphorylation of tau in dystrophic dopaminergic neuritic processes, thereby uncoupling tau pathology from neurodegeneration. However, the accumulation or aggregation of ubiquitin or α-synuclein could not be detected.

Collectively, this study demonstrates the feasibility of developing viral models of LRRK2-mediated neurodegeneration in rodents and provides strong in vivo support for the contribution of elevated kinase activity to LRRK2-dependent neurotoxicity. Interestingly, the correlation between LRRK2 expression levels and tau pathology recapitulates observations in some G2019S *LRRK2* PD subjects and suggests that this viral model may be useful for exploring the interaction between LRRK2 and tau in PD. However, the limited retrograde transport of adenovirus to the substantia nigra together with a progressive reduction of transgene expression over time makes this model unsuitable for long-term assessments of the pathological effects of G2019S LRRK2 expression in vivo.

Recently, Beilina and coworkers demonstrated that LRRK2 forms a protein complex with Rab7L1, Cyclin-G-associated kinase (GAK), and Bcl2-associated athanogene 5 (BAG5) to promote the clearance of *trans*-Golgi network (TGN)-derived vesicles (Beilina et al. 2014). Pathogenic mutations in LRRK2 that increase kinase activity or disrupt GTPase activity showed an enhanced clearance of the Golgi in cultured cells. To corroborate these findings in vivo, the authors injected lentiviral vectors expressing GFP-tagged G2019S LRRK2 in the striatum of adult mice. At 2 weeks post-injection, the viral-mediated expression of G2019S LRRK2 caused a marked reduction in GLG1 immunoreactivity, a TGN marker, suggesting a potential role for LRRK2 in TGN turnover (Beilina et al. 2014). Prior studies have also described perturbations to Golgi morphology induced by G2019S LRRK2 expression (Lin et al. 2009; Stafa et al. 2012). It is not yet clear whether increased TGN turnover is specific for G2019S LRRK2 compared to WT or other variants of LRRK2, involves kinase activity, selectively occurs in particular neuronal populations, or is required for LRRK2-dependent neuronal degeneration. Nevertheless, the impact of mutant LRRK2 on Golgi-mediated vesicular trafficking could provide a promising avenue for future investigations.

2.3.3 *LRRK2*-Induced Pluripotent Stem Cells (iPSc)

In 2007, the first human-induced pluripotent stem cells (iPSc) were described providing a new approach to studying human disease (Takahashi et al. 2007; Yu et al. 2007). Prior translational research efforts were based on expression of human genes in immortalized cell lines, primary cultures, or animal models. Now, it is possible to investigate the consequences of genetic mutations in several patient-derived cell subtypes whereby the genome and its transcriptional control are mostly intact. Compared to animal models, iPSc models allow one to study the consequences of genetic mutations directly on human cellular physiology and therefore provide an important yet complementary model in which to understand disease mechanisms. However, like any cultured cell, iPS cells provide limited information and cannot recapitulate the complexity of brain circuits and the physiological diversity of neuronal populations of the intact mammalian brain. Nevertheless, iPSc provide useful disease-relevant cellular models that incorporate human genetic diversity. In PD, iPSc can provide highly relevant models because of the clear involvement of nigrostriatal dopaminergic neurons in disease and the well-developed capacity to generate iPSc-derived dopaminergic neurons.

The first models of PD developed from iPSc were derived from idiopathic PD subjects due to limited accessibility to fibroblasts from PD subjects with familial mutations (Park et al. 2008; Soldner et al. 2009). Nguyen et al. described the first monogenic PD model using iPSc derived from a skin biopsy of a 60-year-old female patient with early-onset and typical L-DOPA-responsive PD harboring a homozygous G2019S mutation in *LRRK2* (Nguyen et al. 2011). iPS cells were expanded for 8 months and a small proportion of cells could be differentiated into TH-positive dopaminergic neurons ($\sim$3.6–5%). G2019S *LRRK2* iPSc-derived TH-positive neurons selectively exhibited accumulation of α-synuclein, up-regulation of key oxidative stress response genes and increased vulnerability to neurotoxins, including hydrogen peroxide, MG132, and 6-OHDA. Sánchez-Danés and coworkers generated dopaminergic neurons from four G2019S *LRRK2* PD subjects (Sanchez-Danes et al. 2012). Following long-term culture, G2019S *LRRK2* iPSc-derived dopaminergic neurons displayed α-synuclein accumulation, altered morphology with fewer and shorter neurites, and compromised autophagosome maturation suggesting deficits in the autophagy pathway. Therefore, G2019S *LRRK2* iPS cells could recapitulate some pathological features of LRRK2 transgenic animal models and idiopathic and *LRRK2*-associated PD, thereby validating them as potential models of PD.

Cooper et al. generated iPSc-derived neural cells from subjects carrying homozygous G2019S mutations or heterozygous R1441C substitutions in *LRRK2* (Cooper et al. 2012). This study observed that iPSc-derived *LRRK2* neural cells display alterations in cellular basal oxygen consumption, mitochondrial dynamics and morphology, and increased vulnerability to a number of cellular stressors known to induce mitochondrial dysfunction. Furthermore, co-treatment with rapamycin or LRRK2 kinase inhibitors increased the resistance of *LRRK2* neural cells to cellular stressors. Interestingly, the sensitivity to chemical stressors increased as

neural cells became more functionally similar to vulnerable cell types in PD, that is, iPSc-derived neural cells and neurons were more sensitive to mitochondrial stress than fibroblasts from the same patient. Recently, Sanders and colleagues report that iPSc-derived neural cells from subjects carrying G2019S or R1441C mutations in *LRRK2* exhibit elevated mitochondrial DNA (mtDNA) damage. These effects were specific to neural cell types and could be abolished by zinc finger nuclease-mediated correction of *LRRK2* genomic sequence (Sanders et al. 2014). Collectively, these observations connect *LRRK2* mutations with impaired mitochondrial function and indicate that *LRRK2* iPSc-derived neural cells are useful models for exploring neuronal vulnerability to stress and the identification of neuroprotective molecules. Studies of neural stem cells (NSCs) derived from G2019S PD subjects support a role for the nucleus as a cellular compartment implicated in PD (Liu et al. 2012). Liu et al. showed that G2019S *LRRK2* iPSc-derived NSCs display increased susceptibility to proteasomal stress, as well as passage-dependent deficiencies in nuclear envelope organization, clonal expansion, and neuronal differentiation. The passage-dependent alterations in nuclear morphology of G2019S NSCs correlated with epigenetic changes such as those observed during cellular aging and could potentially be mediated through phosphorylation of B-type lamins. Additionally, late-passage G2019S NSCs exhibited increased phosphorylation of LRRK2 and its putative substrate 4E-BP1. Pharmacological inhibition of LRRK2 kinase activity rescued the aberrant nuclear morphology and clonogenic capacity in G2019S iPSc-derived NSCs and restored a gene expression signature similar to WT *LRRK2* NSCs. In contrast to prior studies (Cooper et al. 2012), no evidence of altered mitochondria morphology was observed, which could result from differences in cellular differentiation protocols and/or the neural cell-type studied. Analysis of neuronal nuclear architecture in brains of idiopathic and G2019S *LRRK2* PD revealed that dentate gyrus neurons display altered nuclear morphology, whereas neurons of non-neurogenic areas do not exhibit alterations in nuclear envelope organization (Liu et al. 2012). These findings suggest a role for LRRK2 in neural stem cells and neural progenitors in adult neurogenic niches.

A recent report describes multiple phenotypes for iPSc-derived dopaminergic neurons derived from G2019S *LRRK2* PD subjects, including reduced neurite outgrowth and increased sensitivity to rotenone and 6-OHDA toxicity, which could be ameliorated by inhibition of LRRK2 kinase activity or by direct gene correction of the G2019S mutation (Reinhardt et al. 2013). G2019S iPSc-derived dopaminergic neurons displayed increased tau and α-synuclein protein levels, but without evidence of increased α-synuclein transcription. This finding suggests that the G2019S mutation may impair the degradation of α-synuclein. A second study in iPS cells further suggests an inhibitory role for LRRK2 in the clearance of α-synuclein by chaperone-mediated autophagy (Orenstein et al. 2013). Gene correction of the G2019S mutation in iPS cells enabled the identification of differentially regulated genes in G2019S *LRRK2* iPSc-derived dopaminergic neurons (Reinhardt et al. 2013). Alterations in the ERK signaling pathway and the *CPNE8*, *ANXA1*, *MAP7*, *CADPS2*, *MAPT,* and *UHRF2* genes were suggested to play a role in G2019S LRRK2-induced dopaminergic neuronal toxicity.

Mitochondrial and lysosomal dysfunction have also been reported in dopaminergic neurons derived from G2019S *LRRK2* iPS cells, such as loss of mitochondrial membrane potential, increased mitochondrial reactive oxygen species (mitoROS), and lysosomal hyperactivity (Su and Qi 2013). These effects are mediated by dynamin-related protein 1 (Drp1), a key regulator of mitochondrial fission. Drp1 inhibition corrected mitochondrial and lysosomal dysfunction and increased neurite length in G2019S iPSc-derived dopaminergic neurons. Inhibiting fission or increasing fusion of mitochondria may offer one potential approach to rescue mitochondrial dysfunction and enhance dopaminergic neuronal survival in G2019S *LRRK2* PD patients.

Collectively, iPSc-derived neurons provide important cellular models for understanding *LRRK2*-associated PD. iPSc-derived dopaminergic neurons display reduced neurite length, accumulation of α-synuclein and tau, increased vulnerability to cellular stress, and impaired autophagy and mitochondrial function. Inhibition of LRRK2 kinase activity rescued many of the pathological properties of the G2019S mutation, thereby confirming prior studies in LRRK2 animal models (Lee et al. 2010a). Interestingly, pathological features of *LRRK2* iPSc first appeared in aged cultures, thus suggesting age as a critical factor in their development as in PD. Furthermore, iPSc-based models can be successfully employed to discover and validate novel molecular pathways, organelles, and cellular populations potentially involved in *LRRK2*-associated PD. LRRK2 is abundantly expressed throughout the human brain, but is poorly expressed in dopamine-containing neurons (Galter et al. 2006). Therefore, the effects of familial mutations in endogenous LRRK2 might be subtle and difficult to identify in iPSc-derived dopaminergic neurons. It would be of interest to investigate the pathological consequences of LRRK2 mutations in other neuronal populations with higher endogenous expression of *LRRK2*, such as the cerebral cortex, striatum, hippocampus, or cerebellum. A major limitation of iPS cells is the poor efficiency of differentiating stem cells into specific neuronal subtypes such as functional dopaminergic neurons. Drug screening and target validation would be difficult to implement due to the scarcity of differentiated dopaminergic neurons in iPSc-derived cultures. Research efforts continue to focus on identifying key factors involved in human neural differentiation, and on the improvement of methods to produce specific neuronal subtypes that are relevant to PD.

3 Conclusion

3.1 What Have we Learned from LRRK2 Animal Models?

Despite the broad range of species employed for creating LRRK2 models, we are still some way from understanding the biological and pathophysiological function of LRRK2. The inability to consistently reproduce key phenotypes across different LRRK2 models has impeded the identification of common cellular processes regulated by LRRK2. LRRK2 animal models have provided support for a key role of kinase and GTPase activities in mediating LRRK2-dependent neuronal damage, and

have identified an important regulatory role for LRRK2 in striatal dopaminergic neurotransmission. Pharmacological inhibition of LRRK2 kinase activity in fly and rodent models could successfully attenuate dopaminergic neuronal degeneration induced by G2019S LRRK2, thereby supporting LRRK2 kinase activity as a key therapeutic target for treating PD. Studies are now warranted in animal models using newly developed kinase inhibitors with improved selectivity and potency for LRRK2 (Choi et al. 2012; Reith et al. 2012; Sheng et al. 2012), and for the development and validation of compounds that target the GTPase domain (Tsika and Moore 2013). Existing LRRK2 animal models provide a suitable resource in which to identify and validate therapeutic agents that modify LRRK2 activity and function as potential treatments for *LRRK2*-associated and potentially idiopathic PD.

LRRK2 animal models collectively recapitulate many of the key clinical and neuropathological features of *LRRK2*-associated PD, including the degeneration of nigrostriatal dopaminergic neurons, cytopathology, tau accumulation and hyperphosphorylation, abnormal striatal dopaminergic neurotransmission, and motoric deficits. However, the caveat is that many of these phenotypes do not often occur together in the same animal model, are not entirely robust, or do not act upon the nigrostriatal dopaminergic pathway. Although there is evidence for a pathological interaction between α-synuclein and LRRK2, studies in animal models are conflicting and difficult to reconcile, and it remains unclear whether LRRK2 is critical for the development of α-synuclein pathology. Whether α-synuclein is oppositely required for mediating LRRK2-induced neuropathology has not yet been evaluated. Mechanistically, LRRK2 plays an important role in neurite outgrowth and remodeling, and in cellular pathways previously implicated in the pathophysiology of PD including the oxidative stress response, autophagy, mitochondrial function, cytoskeleton organization and vesicular protein sorting. Animal models have also identified an unexpected biological role for LRRK2 in kidney and lung homeostasis, and in adult neurogenesis, which could potentially contribute to non-motor symptoms in PD (i.e., anxiety and depression).

3.2 Is There an Optimal LRRK2 Animal Model?

It is worth noting that despite the multitude of LRRK2 models developed so far, a single model is not able to faithfully recapitulate all clinical and neuropathological features of *LRRK2*-associated PD. The incomplete penetrance of *LRRK2* mutations and the diversity of clinical symptoms and neuropathology in *LRRK2* PD subjects may indicate a potential contribution of genetic and/or environmental factors to precipitating LRRK2-dependent neurotoxicity. What criteria should therefore be used to define an ideal animal model of LRRK2 pathobiology? The answer is that no animal model is perfect, but that all current LRRK2 models offer something different and all have their often unique differences and utilities, as we have attempted to emphasize throughout. In attempting to identify therapeutic agents for treating PD, the focus naturally levitates towards preventing or attenuating

neurodegeneration, one of the defining hallmarks of PD. Animal models that display LRRK2-mediated neurodegeneration should be considered as the most adequate for this task, although alternative robust LRRK2-related phenotypes may also prove sufficient (i.e., motoric deficits, cytopathology, or abnormal dopaminergic neurotransmission). Model organisms such as worms and flies, certain transgenic rodent models, and rodent viral models show sustained dopaminergic neurodegeneration upon overexpression of human G2019S LRRK2. Fly and worm models provide important and rapid tools for identifying and dissecting the novel molecular mechanisms underlying LRRK2-mediated neurodegeneration, whereas it is extremely time-consuming and laborious to perform such studies in transgenic rodent models. It is possible to readily evaluate the contribution of genetic and environmental factors to LRRK2-induced neurotoxicity in worm and fly models. However, the absence of a true LRRK2 homolog in *Drosophila* and *C. elegans* and the lack of conservation in brain complexity, organization, and neuronal circuitry, represent major deficiencies for the pre-clinical evaluation of therapeutic agents. Rodent models of LRRK2 are therefore necessary to corroborate findings from simpler organisms. Alternatively, iPSc-derived neuronal models permit one to study the consequences of *LRRK2* mutations directly on the cellular physiology of disease-relevant human neurons. Despite the promise of patient-derived iPS cells, the technology is not yet sufficiently advanced for high-throughput drug screening efforts, but already enables important insight into the cellular pathobiology of *LRRK2* mutations. Finally, viral-mediated gene transfer rodent models for human LRRK2 provide a promising approach to modeling PD. Adenovirus-mediated *LRRK2* gene delivery causes progressive dopaminergic neuronal degeneration within an acceptably short time frame ($\sim$6 weeks) and can be used to rapidly validate neuroprotective therapeutic targets and chemical agents. Importantly, this viral model can be applied to other environmental and/or genetic models of PD, which will broaden our understanding of the pathological pathways associated with LRRK2 in PD. Collectively, *LRRK2* animal models provide an important resource for the elucidation of novel disease mechanisms, the identification and validation of therapeutic targets, and for the evaluation of disease-modifying therapeutic agents.

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