Preface

The *PARK8* locus on chromosome 12, identified from linkage analyses of a large Japanese family with individuals from multiple generations diagnosed with the progressive neurodegenerative disorder Parkinson's disease (PD), was first reported in 2002 by Funayama and colleagues. It was not until two years later that independent groups led by A. Singleton and T. Gasser cloned the responsible gene within this locus known as leucine-rich repeat kinase 2, or *LRRK2*. Typically, PD develops sporadically with the greatest risk factor being age. However, for approximately 10% of cases, a classical Mendelian inheritance with both autosomal dominant and recessive transmissions is present. The six clearly pathogenic single amino acid substitutions that are causative for PD collectively comprise the most frequent genetic causes of PD, with the most common mutation being Gly2019Ser.

The protein encoded by the LRRK2 gene is a large 2527 amino acid multi-domain protein comprised of several well-defined protein interaction domains as well as a Ser/Thr kinase domain and a small GTPase-like domain (ROC; Ras of complex proteins). The kinase and GTPase domains are interrupted by a COR (C-terminal of ROC) domain characteristic of the ROCO protein family. Likewise, the domain structure and kinase domain in particular of LRRK2, and the related LRRK1, bear significant similarity to the receptor-interacting protein kinase (RIPK) family. Clearly, its complex domain structure and widespread expression, including, in addition to the brain, high levels in the kidney and lung as well as circulating immune cells, predicts a wide range of cellular functions and activities. In the short period of time since its identification, our understanding of LRRK2 biology, as well as our tools to investigate its function, is rapidly evolving. Historically, only the G2019S mutant form of LRRK2, when overexpressed in cell lines, would exhibit a significant alteration in kinase activity, displaying approximately two- to fivefold increases in autophosphorylation or, later on, phosphorylation of model peptide substrates. Multiple studies of the remaining pathogenic LRRK2 mutations reported mixed effects on kinase function. Recently, however, multiple members of the large Rab GTPase family of proteins were identified as true physiological phospho-substrates of LRRK2; and strikingly, virtually all of the pathogenic LRRK2 mutants, as well as several risk factor variants, show elevated phosphorylation of these

substrates. While this represents an important step in our understanding of LRRK2 function, what is still lacking is a better understanding of how mutations in LRRK2 alter its function in such a way, in what cell types, and in response to what external stimuli that results in the progressive loss of dopamine neurons of the substantia nigra pars compacta—the neurodegenerative hallmark underlying the motor symptoms of PD. With a prominent focus on its role in PD, but always in the larger context of a broader range of activities of LRRK2, this book was envisioned to provide a window into the current state of understanding of this complex protein by some of the leaders in the field of LRRK2.

In Part I, we begin with discussions of the genetic and clinical considerations of LRRK2-associated PD, with contributions by Di Fonzo and colleagues and Alcalay and colleagues, respectively. In a gene of this size, there are, expectedly, dozens of sequence variants that show varying degrees of association with developing PD. However, relatively few such mutations, located primarily within the central ROC-COR-kinase signaling core of the protein, demonstrate clear pathogenicity. Even among carriers of those specific mutations, there is considerable variability in the penetrance across different ethnic backgrounds. Despite this, the clinical features of LRRK2-associated PD are remarkably similar to the much more common idiopathic manifestation of the disease; however, some important clinical and pathological differences have been reported among the distinct mutations, including altered progression of motor symptoms as well as the absence of classical Lewy body-like inclusions in some cases.

LRRK2 possesses an extraordinarily broad range of cellular functions, dictated not only by its expression in specific cell types but also by a complicated and coordinated regulation of its activity. This aspect of LRRK2 biology is covered in detail in Part II of the book. One regulatory mechanism occurs via the phosphorylation of LRRK2, by itself and other kinases, at multiple domains throughout the protein (discussed in the chapter by Nichols). This is in turn kept in check via the action of specific cellular phosphatases (Taymans). While the kinase activity of LRRK2 has received considerable attention in terms of substrate profiling, its requirement for neurodegeneration, and the obvious opportunity for targeted therapeutic strategies, our understanding of the GTPase function of LRRK2, in terms of its reciprocal regulation of kinase activity as well as its activity underlying the pathological effects of mutant LRRK2, is rapidly increasing. This is discussed in detail in the chapter by Moore and colleagues. One of the earliest systems shown to be affected by LRRK2 function, or dysfunction, is autophagic/lysosomal protein degradation. Interestingly, as is the case for many proteins, including another dominantly inherited gene linked to PD, α-synuclein, in addition to being degraded in part through by the autophagic machinery, LRRK2 (particularly mutant forms of the protein) can also modulate the activity of multiple forms of autophagy (Lewis and colleagues).

An important regulatory protein interacting with LRRK2 is 14-3-3. It binds to a cluster of phosphorylated residues located in the N-terminal region of the protein. While the phosphorylation of these residues is dependent upon LRRK2 kinase activity, pharmacological inhibition leads to dephosphorylation at these sites; they are not true autophosphorylation sites within LRRK2. Multiple other kinases,

downstream of LRRK2 activity, have been identified that can phosphorylate these residues. The phospho-dependent binding of 14-3-3 at these sites appears to play a critical role in the subcellular localization of LRRK2. Upon dissociation, LRRK2 redistributes into discrete filamentous structures of unknown function, similar to those seen upon overexpression of certain mutant forms of LRRK2. Although it remains unclear what the composition of these structures is, there is strong evidence supporting the existence of LRRK2 in a dimeric state. Further, it is believed that, at least in terms of kinase activation, the LRRK2 dimer is the active conformation. A comprehensive review of the evidence, and implications, of LRRK2 dimer formation is provided in the chapter by Greggio and colleagues. Beyond its role in neurodegeneration, LRRK2 plays an important role in many other pathways as well as a result of its prominent activity in the immune system (Dzamko).

The final section of the book focuses on modeling LRRK2 neurodegeneration, the potential links to other PD-related proteins, the mechanisms of neurotoxicity and cellular implications of LRRK2 dysregulation, and efforts to develop therapeutically viable inhibitors of LRRK2 activity. Despite the generation of many in vivo models of mutant LRRK2 overexpression, including traditional transgenic models, BAC transgenic lines, and knockin lines expressing mutant LRRK2, as well as viral models, the plurality of in vivo models fails to show evidence of a progressive loss of dopaminergic neurons (Dawson and colleagues). That is not to say that gains have not been made from these efforts; far from it. Using specific promoters, or viral vector approaches, the progressive degeneration of dopaminergic neurons can be elicited by overexpression of mutant forms of LRRK2; and other neuronal pathologies have been reported in lines even in the absence of neuronal loss. Conversely, clues to the function of LRRK2 have been discerned from knockout models in non-neuronal tissues; and its important interaction in the neurodegenerative phenotype triggered by α-synuclein overexpression or inflammatory insults (Daher) has been discovered in LRRK2-deficient rats. This is highlighted by the critical discussion of non-cell-autonomous effects of LRRK2, as well as its role in vesicular trafficking (Cookson). While the nature of cell death observed in isolated neuronal cell models of LRRK2 neurodegeneration appears to be apoptotic, whether the same is true at the systems level remains to be seen. The similarity of LRRK2 to the RIP kinase protein family and its interaction with, and activation of, extrinsic death pathway components raise the possibility that other modes of cell death may contribute to the loss of neurons in PD (Rideout). Finally, the book closes with a discussion of the efforts to develop small molecule inhibitors of LRRK2 kinase activity that could potentially be utilized in the clinic (Gray and colleagues). Multiple cellular and in vivo models indicate that mutant LRRK2-induced neuronal death is dependent upon its kinase activity, although the substrates of this activity linked to cell death remain unknown. These efforts, both academic- and industry-wide, have resulted in the discovery of ever more potent and selective inhibitors of LRRK2 kinase activity that are currently being evaluated in safety studies.

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especially would like to thank Simina Calin and Jeffery Taub for their support and guidance. It is my hope and belief that this book will serve as an in-depth introduction and snapshot of the current state of the art in LRRK2 biology and, as highlighted in each of chapters' discussion of future directions, will stand as a foundation for the next steps taken in this exciting field.

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