Pathology, Prevention and Therapeutics of Neurodegenerative Disease
Pathology, Prevention and Therapeutics of Neurodegenerative Disease
Neuroscience is a large field founded on the premise that all of behavior and mental abilities have their origin in the structure and function of the nervous system. This book attempts to provide an overview of major neurodegenerative diseases with a special focus on diseases related to the central nervous system (CNS). Neurodegenerative diseases add up to tremendous medical and financial burden due to their non-partisan share for individuals of all ages, with elderly population contributing the largest share. Due to the enigmatic and complex nature of neurodegenerative diseases, therapeutic intervention to address the same is of immense challenge for the researchers. To date, research has suggested the involvement of diverse factors and complex mechanisms in disease etiology, with a bolting approach still lacking to thwart neurodegeneration. Such impuissance of researchers is mainly due to delayed appearance of behavioral symptoms: the only diagnostic marker for most of the neurodegenerative diseases presently. In fact, the visible symptoms manifest at later and peak stage of disease act as barrier for timely intervention.

Brain has postmitotic neurons thereby lacking restoration of damaged neurons. Previous studies have implicated neurogenesis mainly in the hippocampal area of the brain, while the disease pathology may encompass any brain region. Further, restoration of damaged neurons by stem cell therapy failed to achieve the desired effect due to the lack of versatile utilization for treatment and its financial impact. The prime focus of this book is to introduce students to the major CNS-related neurodegenerative diseases. The chapters aim to introduce the readers about disease pathologies, related mechanisms involved, and available therapeutics. As the disease diagnosis is a huge challenge for physicians and researchers alike, specific chapters focusing on the same have been included to assist the reader in getting a comprehensive view of the disease. Further, the book focuses on neurodegenerative diseases involving mental abilities and motor responses, specifically Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), and amyotrophic lateral sclerosis (ALS). Collectively, research to date strongly supports the view that prevention might be a better approach to fight the disease. In line with disease etiology and diagnosis, we have also endeavored to expose the readers to the existing alternative preventive therapeutic approaches. Alternative therapies derived from natural products may outweigh the side effects of the conventional approaches, thereby a potential option for long-term treatment.
We express our gratitude to all the authors for their efforts in bringing out this compilation in the field of neurosciences. We are also thankful to Eti Dinesh at Springer for her constant support throughout the project. N. S. Pandian (Senior Production Manager) and Kumar Athiappan (Project Coordinator) are also acknowledged for their contribution.

Lucknow, India  Sarika Singh
Los Angeles, CA, USA  Neeraj Joshi
### Contents

1. **Alpha Synuclein and Parkinson’s Disease** .......................... 1  
   Arti Parihar, Priyanka Parihar, Isha Solanki,  
   and Mordhwaj S. Parihar

2. **Molecular Mechanisms of Neurodegeneration: Insights from the Studies of Genetic Model of Parkinson’s Disease** .......................... 15  
   Nisha R. Dhanushkodi and M. Emdadul Haque

3. **Pathology and Cell-Based Therapy of Parkinson’s Disease** .......................... 31  
   So Young Kim, Sung S. Choi, Dong-Seok Lee,  
   Seung Hoon Lee, Sang Hoon Cha, and Hong J. Lee

4. **The Role of p53 in Alzheimer’s Disease: Impact on Tau Pathology** .......................... 39  
   Maja Jazvinščak Jembrek, Katy Newberg, and Goran Šimić

5. **Pathophysiological Mechanisms of Huntington’s Disease** .......................... 49  
   Zuleide M. Ignácio, João Quevedo, and Gislaine Z. Réus

6. **Glutamate in Amyotrophic Lateral Sclerosis: An Ageless Contestant** .......................... 61  
   Alida Spalloni, Michele Nutini, and Patrizia Longone

7. **Inherited Neurodegenerative Disorders** .......................... 73  
   Dulika S. Sumathipala and Vajira H. W. Dissanayake

8. **Astrocytes and the Synucleinopathies** .......................... 81  
   Andrew O. Koob and Paola Sacchetti

9. **The Diagnosis of Parkinson’s Disease: Current Clinical Practice and Future Trends** .......................... 103  
   Roberto López Blanco and Álvaro Sánchez Ferro

10. **Clinical Symptomatology of Huntington’s Disease** .......................... 117  
    Jan Roth

11. **Diagnosis of Amyotrophic Lateral Sclerosis/Frontotemporal Dementia Spectrum** .......................... 133  
    Vanesa Pytel and Jordi A. Matías-Guiu
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Nanocarriers for Diagnosis and Imaging of Neurodegenerative Diseases</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td>Mine Silindir-Gunay and A. Yekta Ozer</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Parkinson Disease Therapies and Drugs</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Rodolphe Hajj</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Therapeutic Application of Stem Cell and Gene Therapy in Parkinson's Disease</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>Charlotte Palmer, Raquel Coronel, Adela Bernabeu-Zornoza, and Isabel Liste</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Targeting Glucocorticoid Receptors: A New Avenue for Alzheimer's Disease Therapy</td>
<td>173</td>
</tr>
<tr>
<td></td>
<td>Geoffrey Canet, Nathalie Chevallier, Véronique Perrier, Catherine Desrumaux, and Laurent Givalois</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Multifarious Therapeutic Avenues for Alzheimer's Disease</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td>Magisetty Obulays</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Treatment Paradigms in Huntington's Disease</td>
<td>191</td>
</tr>
<tr>
<td></td>
<td>Pushkar Kulkarni and Uday Saxena</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Management of HD: Insight into Molecular Mechanisms and Potential Neuroprotective Drug Strategies</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td>Puneet Kumar, Sumit Jamwal, and Anil Kumar</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Amyotrophic Lateral Sclerosis: Current Therapeutic Perspectives</td>
<td>207</td>
</tr>
<tr>
<td></td>
<td>Vijay Kumar, Tara Kashav, and Md. Imtaiyaz Hassan</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Mechanistic Insights into Neurodegenerative Diseases: The Potential for the Development of Novel Therapeutics</td>
<td>225</td>
</tr>
<tr>
<td></td>
<td>Medhane Cumbay, Michael LaFontaine, and Sage Arbor</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Neural Stem Cell-Based Therapeutic Approaches for Brain Repair</td>
<td>241</td>
</tr>
<tr>
<td></td>
<td>Cláudia Saraiva, Tiago Santos, and Liliana Bernardino</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Recent Advances in the Antioxidant Therapies for Alzheimer's Disease: Emphasis on Natural Antioxidants</td>
<td>253</td>
</tr>
<tr>
<td></td>
<td>Namrata Singh and Kallol K. Ghosh</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Dietary Directions Against Dementia Disorders</td>
<td>265</td>
</tr>
<tr>
<td></td>
<td>Helmut M. Hügel, Anthony R. Lingham, Neale Jackson, and Trevor Rook</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Use of Herbal Products/Alternative Medicines in Neurodegenerative Diseases (Alzheimer's Disease and Parkinson's Disease)</td>
<td>279</td>
</tr>
<tr>
<td></td>
<td>Omar M. E. Abdel-Salam</td>
<td></td>
</tr>
</tbody>
</table>
Sarika Singh completed her postgraduate degree in biochemistry at Lucknow University in 2001 and subsequently received her doctoral degree from the same university for a dissertation on the role of nitric oxide in the pathology of Parkinson’s disease. In 2006, she assumed her current position as a Senior Scientist at CSIR-Central Drug Research Institute, Lucknow, Uttar Pradesh, India. She is a life member of the Indian Academy of Neurosciences and a member of the Indian Academy of Sciences. She is a recipient of international Indo-US and CSIR-Raman research fellowships and has worked toward the identification of diagnostic markers for autism and Parkinson’s disease. Having published in several international peer-reviewed journals, she also serves as an editorial board member and reviewer for various international and national journals. Her chief research focus is on investigating the neurodegenerative and neuroprotective mechanisms involved in various brain diseases.

Neeraj Joshi received his master’s (M.Sc.) degree in biochemistry from Lucknow University, India, in 2001, after which he was recruited to Bhabha Atomic Research Center (BARC), Mumbai, India. After completing the Orientation Course in Nuclear Science and Engineering at BARC, he worked at its Radiation Biology and Health Sciences Division as a Scientific Officer from 2002 to 2006. His research at BARC focused on investigating DNA damage repair and radiation hormesis in the context of cancer biology and neurodegeneration. To further understand the mechanisms of genomic integrity, Neeraj chose to pursue his doctorate at Cleveland State University, USA, where he explored the mechanistic aspects of meiotic chromosome segregation. This resulted in (1) unraveling the role of genome architecture in DNA damage repair (DDR), (2) the discovery of a new, ultrasensitive DNA damage responsive checkpoint system, and (3) the development of a novel molecular assay: “Homolog Pairing Capture.”

Collectively, his doctoral studies provided a new perspective on cellular DDR mechanisms and the indirect involvement of proteasome in the DDR process. From 2015 to 2017, his postdoctoral work at the University of California-San Francisco (UCSF), USA, centered on investigating both the selective and comprehensive repertoire of Cullin-RING-like (CRL) ubiquitin ligases under defined stress conditions.
Alpha Synuclein and Parkinson’s Disease

Arti Parihar, Priyanka Parihar, Isha Solanki, and Mordhwaj S. Parihar

1.1 Introduction

Parkinson’s disease (PD) is the age-related neurodegenerative disorder diagnosed by tremor at rest, rigidity, and bradykinesia symptoms. The prevalence of PD increases with the increase in age and about 2–3% population worldwide suffer from the disease ≥65 years [1]. The major neuropathology of PD patients is the deficit of dopaminergic neurons the substantia nigra pars compacta (SNpc) region of the midbrain. The lesions caused in these brain regions cause severe depletion of striatal dopamine. Non-motor symptoms like dementia, depression, anxiety, insomnia, excessive daytime sleepiness, rapid eye movement sleep disorder, constipation, difficulty in swallowing, and dyspepsia may also be involved in PD symptoms and pathology. Histological characteristic of PD includes the occurrence of Lewy bodies (LBs) in existing neurons [2]. However, little is known about the formation of LBs. The rising evidence revealed that LB biogenesis may involve neuroprotective reactions [3]. Numerous studies have been executed to elucidate the role of α-synuclein in the pathogenesis of PD.

Reports have shown the expression of α-synuclein in neurons which abundantly distributed in presynaptic neuronal terminals of synapses [4]. The distribution of α-synuclein in the synaptic terminals indicates that this protein may take an important role in synaptic plasticity, kinetics of vesicle, and in the dopamine synthesis and its release. The role of α-synuclein in the pathogenesis of PD has been extensively analyzed. The observation of fibrillar α-synuclein in LBs and the occurrence of mutations in the α-synuclein gene in familial forms of PD have led to the belief that this protein has a critical role in PD pathology. The relationship of α-synuclein and PD has been identified by a genetic finding of A53T mutation of α-synuclein gene (SNCA) in a family with autosomal-dominant familial PD [5]. Furthermore the implication of α-synuclein in PD has been corroborated by the discovery of the other mutations of SNCA, involving A30P and E46K in other families with inherited PD [6, 7]. The function of α-synuclein in PD was further strengthened by the investigation in which presence of this protein was found as the primary structural constituent of LBs [8]. Here, we present an overview of existing knowledge on the physiological functions, oligomerization, and aggregation of α-synuclein and its pathological
role in PD. Considering the nature of the various α-synuclein structures and its mechanism of toxicity may be important in developing attractive treatment options against the pathologic hallmarks of PD and α-synucleinopathies.

1.2 Localization and the Structure of α-Synuclein

The varied forms of synuclein protein, α, β, and γ are expressed at numerous locations in the nervous system [9]. Synuclein α- and β-forms are chiefly present in nerve terminals, near synaptic vesicles in the central nervous system [10], whereas γ-synuclein is present in neuronal cells of the peripheral nervous system [10]. α-Synuclein is mainly located in the cytoplasm but extracellular α-synuclein has also been studied [11]. In PD, the levels of α-synuclein are higher in cerebrospinal fluid (CSF) than age matched controls [12], indicating that α-synuclein is also present in extracellular brain fluids. Most significantly, α-synuclein oligomers have abundantly distributed in the extracellular space in PD. The presence of α-synuclein both at intracellular and extracellular spaces could explain that the extracellular α-synuclein oligomers may disperse from one neuron to another, and this movement might channelize the succession of the disease from one brain region to other regions.

α-Synuclein is a 14 kDa protein (140 amino acids; pKa of 4.7) expressed by the SNCA gene on human chromosome 4 [13]. It is the cytoplasmic and/or membrane-bound protein found in presynaptic terminals of neurons [14] categorized by an amphipathic lysine-rich amino terminus (residues 1–60), a central hydrophobic region (61–95), so-called NAC (non-Aβ component), and a carboxyl terminus which is extremely negatively charged (Fig. 1.2) and is prone to be unstructured [20]. The N-terminal domain is particularly significant for the pathological dysfunction of α-synuclein as the rare point mutations like Ala53Thr, Ala30Pro, Glu46Lys, His50Gln, Gly51Asp, and Ala53Glu are present in this region [21]. However, NAC domain is accountable for the aggregation attributes of α-synuclein via inhibition of its degradation and promotion of its fibrillation [22]. Although the normal physiological role of α-synuclein is not known, still it appears to be involved in compartmentalization, storage, and recycling of neurotransmitters [23]. α-Synuclein has been shown to interrelate directly with the membrane phospholipids, especially vesicles and have a role in the vesicle trafficking during the neurotransmission release. It also appears to be associated with directive of various enzymes and tends to augment the integer of dopamine transporter molecules [24]. In addition, recombinantly α- and β-syneclins inhibit mammalian phosphatidylcholine (PC)-specific phospholipases D2 activity in vitro [25], suggests that inhibition of PLD2 may be a function of syneclins.

In aqueous solution, α-synuclein normally has natively unfolded protein structure but may assume oligomeric and/or fibrillar conformations in definite pathological conditions like mutations in the SNCA gene, overexpression, oxidative stress, and posttranslational amendment (Fig. 1.3a–d). Studies indicate that the pathogenic species of α-synuclein involve the posttranslationally modified, mutant, oligomeric, or aggregated forms that could induce adverse effects by disturbing the physiological function of α-synuclein in release of neurotransmitters [26, 27]. Pathological form of α-synuclein may impair mitochondrial functions and mitophagy [28, 29]. It may also result in endoplasmic reticulum (ER) stress by disrupting ER-Golgi vesicular transport [30, 31] and vitiating the effectiveness of some protein degradation pathways [32]. Thus α-synuclein adversely affects the cellular physiology which consequently causes cellular injury and death.
1.3 The Transmission and Release of α-Synuclein in Brain Cells

α-Synuclein has self-propagating property, therefore it extends gradually among interconnected brain regions. Different brain regions have the presence of pathological α-synuclein aggregates involving both the peripheral nervous system (PNS) and central nervous system (CNS) [33]. Several observations in human samples revealed the transmission and secretion of α-synuclein in the brain cells. Together monomeric and oligomeric forms of α-synuclein species have been observed in samples of human plasma and cerebrospinal fluid [11, 34], which suggests that α-synuclein can be secreted in brain cells. The exact machinery of α-synuclein release is not entirely understood; however, it is well identified that α-synuclein can be secreted into the culture medium by varied types of neuronal cells [35, 36]. Internalization of α-synuclein has also been demonstrated [37–39], possibly through passive diffusion by enacting with membranes and lipids [40]. Majority of experiments verified that α-synuclein may be spread from one cell to
another by a cell-to-cell transmission machinery [41]. The study confirmed that diverse forms of human α-synuclein, involving monomers, oligomers, and fibrils, might be absorbed by neurons in vivo by endocytosis [42]. In addition, host-to-graft transmission of human α-synuclein has also been demonstrated in rats [43].

1.4 α-Synuclein Physiological Functions

The physiological functions of α-synuclein are the subject most debated in the neuroscience field. However, several researches in the field suggest that α-synuclein enacts at the presynaptic terminal and controls the synaptic transmission. The subcellular localization of α-synuclein at the synapse supports this idea [44, 45]. Evidences suggest that α-synuclein perform many functions at the synapse, i.e., in the rhythm of synaptic vesicles, regulating the vesicle pool size, militarization, and endocytosis [4, 46]. C-terminus region of α-synuclein has been observed to interact with the synaptobrevin-2 (VAMP2) [47], a central player in synaptic exocytosis [48]. Burre et al. [47] reported that the N-terminus of the protein might bind to phospholipids and endorse soluble N-ethylmaleimide-sensitive factor attachment protein receptor
Fig. 1.3  Aggregation of α-synuclein in human neuroblastoma cells. (a) Human neuroblastoma cells were overexpressed with wild-type α-synuclein and immunostained for α-synuclein using monoclonal antibody. (b) Mitochondria were labeled with mitotracker red. (c) Merge shows mitochondria and α-synuclein images overlaid. Aggregates are shown by arrows. (d) Silver-stained SDS-PAGE of cell homogenates [lane (a) unaggregated (control) α-synuclein, lane (b) aggregated α-synuclein (mutant A53T), lane (c) aggregated α-synuclein (A30P), and lane (d) aggregated α-synuclein (wild type). Unaggregated α-synuclein migrated at about 19 kDa, consistent with monomeric size. Aggregated α-synuclein showed both low and high molecular mass]
(SNARE) complexes assembly. SNARE proteins encounter important roles in synaptic vesicle exocytosis [49]. Study by Diao et al. [50] revealed that α-synuclein was involved in synaptic transmission by increasing vesicle clustering. These studies suggest that the α-synuclein may delay vesicle trafficking by enhancing vesicle clustering. These studies support the complex multimerization-dependent function of α-synuclein, which is vastly reliant on its lipid-binding domains. α-Synuclein can continuously transport between cytosolic monomeric and membrane-bound multimers. α-Synuclein also has an important role in the nucleus. The N- and C-termini of α-synuclein have a signal-like role for its nuclear translocation. Familial mutations and oxidative stress has been found to increase its nuclear localization [51–53]. However, the mechanism of nuclear import of α-synuclein is still not understood. Once α-synuclein enters the nucleus, it may participate in the regulation of transcription. It has been observed that α-synuclein binds to the GC1α promoter, a vital mitochondrial transcription factor, eventually having a negative effect on mitochondria homeostasis [54, 55]. Although several questions are still unclear, currently there is strong evidence for the role of α-synuclein in intracellular trafficking, with particular focus on synaptic vesicle trafficking.

α-Synuclein has been shown to defend dopaminergic cells against apoptosis by signaling pathways involving protein kinase C (PKC). PKC is a serine-threonine kinase involved in phosphorylation of different target proteins and therefore controls many cellular mechanisms, such as apoptosis. PKC is very sensitive to oxidative stress and triggers an apoptotic cascade in dopaminergic cells. α-Synuclein has been shown to be a PKC downregulator that can protect dopaminergic cells against apoptosis. α-Synuclein has been shown to switch off the proteolytic cascade by downregulation of PKCα expression. Thus in dopaminergic cells, α-synuclein may be considered to be a neuroprotective protein [56]. α-Synuclein regulates different cellular functions via activation of Ras. The activated Ras can activate other signaling molecules including the ERK/MAPK pathway which is involved in sending a signal of growth factor from the cell receptor to transcription factors in the nucleus [57].

α-Synuclein expression has also been recorded in many other cell types, involving cells pertaining to secretory processes. α-Synuclein interacts with insulin-containing secretory granules KATP channels that leads to the inhibition of insulin secretion triggered by glucose stimulation. These observations suggest a function of α-synuclein in diabetes. Moreover, it has been shown that in type 2 diabetes, there is a deposition of amyloidogenic protein in pancreatic β-cells and these patients are most likely to develop PD. However, when α-synuclein combines to amyloid fibrils, an amyloidogenic protein deposits in pancreatic β-cells and forms irreversible damaging complexes in dopaminergic cells [58]. Another important function of α-synuclein has been suggested for modulation of calmodulin (CaM) activity. Calmodulin (CaM) is a messenger protein that can be activated through binding to Ca2+ ions and triggers various mechanisms such as those involved in short- and long-term memory. Studies have revealed that both wild-type and mutant α-synuclein can interrelate with CaM both in vitro and in vivo. This interaction of CAM with wild-type and mutant α-synuclein causes α-synuclein fibrillation. α-Synuclein interacts with many cellular proteins and acts as a molecular chaperone, because it comprises regions that are homologous with 14-3-3 proteins which interact with many cellular proteins. Chaperone activity of α-Synuclein is dependent on both its N- and C-terminal regions. The N-terminus is accountable for interfacing of α-synuclein with substrate proteins, leading to the arrangement of a large complex while the C-terminus is responsible for the solubilization of that complex [59].

α-Synuclein may act as an antioxidant in precluding oxidation of unsaturated lipids in synaptic vesicles. Dopaminergic neurons are very sensitive to oxidative damage including the oxidants produced by the metabolism of dopamine. The α-synuclein in its monomeric form can protect lipids from oxidation by interaction with lipid membranes. Fibrillar form of α-synuclein does not have this capability of protecting lipids from oxidation. Thus monomeric form of
α-synuclein could act as an antioxidant which has a significant role in dopaminergic neurons to protect them against oxidative damage [60]. Monomeric α-synuclein can prevent apoptosis by binding to cytochrome c oxidase in mitochondrial membrane and inhibits liberation of cytochrome c from mitochondria to cytosol [61].

One of the key purposes of α-synuclein has been suggested in the determination of dopamine biosynthesis. α-Synuclein acts as the downregulator of tyrosine hydroxylase (TH) activity that may regulate dopamine production and manage its cellular levels. Reduced expression of α-synuclein or its aggregated form may lead to enhanced dopamine synthesis that may lead to oxidative stress caused by dopamine metabolism. Both overexpression of α-synuclein and mutations were demonstrated to upregulate the inhibitory effect of α-synuclein on TH and dopamine levels, leading to downregulation of dopamine synthesis and release [62].

### 1.5 α-Synuclein Misfolding and Aggregation

Inherently perturbed proteins typically contain primary sequences that preclude aggregation. They are commonly high in charged residues and prolines, and divested of long hydrophobic stretches [63]. The NAC domain of α-synuclein is the main aggregation sensitive region. This region is partially sheltered by the positive and negatives charges of the both N- and C-terminus of the protein. In fact, α-synuclein exhibits vibrant conformations stabilized by retentive interactions which offer considerable degree of compactness [64]. The retentive interactions that happen between the C-terminus and the NAC region, and among the N- and C-termi, may prevent protein aggregation [64]. However, the native compactness of α-synuclein might be disturbed due to the mutations, alterations in environmental conditions, and/or posttranslational modifications, that may lead to misfolding and aggregation of the protein. In an experimental study involving wild type and mutants (A53T, A30P), we showed that α-synuclein aggregates when overexpressed in human neuroblastoma cells (Fig. 1.3) [65]. In another detailed study, we showed that aggregated α-synuclein binds specifically to the membranes including mitochondrial membrane [65]. We showed that overexpressions of wild-type and/or mutants (A53T, A30P) α-synuclein increase the aggregation in cells (Fig. 1.3) and affinity of membrane binding which is exaggerated during oxidative stress [66]. It has been shown that the aggregation tendency of α-synuclein is augmented by the E46K, H50Q, and A53T mutations, whereas the opposite occurs in the G51D and A53E variants. A30P seems to be more susceptible to oligomerization, at the disbursement of fibrillization [67–73]. The oligomeric species detected in patients pretend by synucleinopathies [74–76] has been shown to be the most toxic forms of α-synuclein [77–79]. In addition to the toxicity by oligomeric species, observations sustaining toxicity for fibrillar and mature α-synuclein species are also being described [80–82].

### 1.6 α-Synuclein and Parkinson’s Disease

The role of α-synuclein in PD pathogenesis is controversial. Several data described that the mutations in gene encoding α-synuclein results in familial PD, whereas the SNCA polymorphism results in sporadic PD [83]. Transgenic mice overexpressed with α-syn showed reduction in dopamine reuptake, impairments in exocytosis in synaptic vesicles, reduced mass of synaptic vesicle reusing pool, and a reduction in neurotransmitter release [84]. SNCA knockout mice causes disablement in hippocampal synaptic responses [26] that shows that synuclein participate to the extended regulation and preservation of the nerve terminal function [85]. The pathogenic effect of both synthetic and murine disease-associated forms of α-synuclein has been demonstrated to cause PD-like α-synuclein pathology in vivo [80]. Brain homogenates obtained from old α-synuclein transgenic mice when injected intracerebrally into the neocortex and striatum of young asymptomatic transgenic mice, there occur the accrument of the pathological α-synuclein in diverse parts of the brain includ-
ing the spinal cord. The accumulation was connected with the cellular loss in the substantia nigra and caused debilitated motor coordination [86]. In similar experiment synthetic recombinant α-synuclein preformed fibrils when injected to young asymptomatic transgenic mice, the animal produced the α-synuclein pathology in vivo. In an experiment a normal mice were shown to exhibit the α-synuclein pathology after administration of the homogenates from patients with other synucleinopathies, like dementia with LB [81]. Reports have also referenced the probable transmission of α-synuclein pathology from the periphery to the brain. Monomeric and oligomeric α-synuclein are readily taken up by brain cells [87] although to a lesser extent the fibrillar α-synuclein was also taken up by brain cells. Human α-synuclein was also seen in little microglial cells in the olfactory bulb, anterior olfactory nucleus, and frontal cortex. Accumulation of α-synuclein inside microglia signifies that microglia could offer clearing process of the human α-synuclein present into the extracellular space by the neuronal cells.

In cases of autosomal-dominant forms of PD, six different missense mutations have been recognized in the gene encrypting for α-synuclein. These are p.A53T, p.A30P, p.E64K, p.H50Q, p.G51D, and p.A53E [88]. Mutations (A53T, A30P, and E46K) or duplication or triplication of WT α-synuclein have been connected with unusual forms of familial PD [5]. Many α-synuclein transgenic mouse models of the familial forms of PD due to mutations in α-synuclein have been produced [89] which replicate many of the features of α-synucleinopathy-induced neurodegeneration, observed in human PD and diffuse LB disease [90]. Posttranslational alterations of α-synuclein such as nitrosylation, oxidation, and phosphorylation have a role in amending α-synuclein aggregation and toxicity [91].

1.7 Cellular Toxicity of Wild-Type and Mutated α-Synuclein

Numerous ex vivo and in vivo findings showed that in vitro generated α-synuclein species have significant toxic effects on cells [92]. Oligomers were revealed to have different destructive effects on cells in culture conditions. The mechanism of toxicity in inducing cell death was proposed through disturbance of cellular ion homeostasis by a pore-forming mechanism. The increased permeability and influx of ions, as a result of disturbance in pore-forming machinery, from the extracellular space may cause cell death [92]. Oligomers formed by recombinant α-synuclein were exposed to form pores in a synthetic bilayer assay. These protofibril-shaped species when exposed to primary cortical neurons induced a depolarization of the cellular membrane. Another mechanism proposed that α-synuclein could directly penetrate in cells and cause amplified protein aggregation [92]. A significant neurotoxic effect was noted when C. elegans and D. melanogaster were exposed to in vitro produced α-synuclein oligomers [77]. The transgenic mice exhibiting the artificial α-synuclein variants E57K and E35K caused oligomer formation and demonstrated an extreme loss of dopaminergic neurons as compared to standard α-synuclein transgenic mice, overexpressing wild-type α-synuclein [78].

Oligomeric α-synuclein may cause a direct synaptotoxic effect [93]. Exogenously added α-synuclein oligomers on hippocampal brain slices from rats cause an impairment on neuronal signaling [94]. Preincubation of tissue with α-synuclein oligomers caused an enhancement in synaptic transmission offering to a suppression of long-term potentiation. In a recent study by Kaufmann et al., [95] two dissimilar types of oligomers were made either by polymerization of monomers or by sonication of fibrils. Despite of variations in the structure of these species, both exhibited similar pessimistic impact on the neuronal excitability. In vivo experiments were also confirmed the outcome of α-synuclein oligomers on the synaptic dysfunctions. In one such experiment, mice expressing the α-synuclein mutants E57K showed widespread synaptic and dendritic pathology in conjunction with the loss of synapsin 1 and reduction in synaptic vesicles [96]. These observations indicate the α-synuclein induced the disruption of presynaptic neurotransmitter release machinery by
the reduction of neuronal synaptic vesicles. The buildup of oligomers chiefly occurs at the synaptic sites and is critical for the neuronal network activity. These oligomeric or fibrillary α-synuclein forms can propagate from one type of neurons to other types and can produce toxic effects in the recipient neurons [97].

The toxicity of α-synuclein depends on its properties of binding to cytoplasmic organelles possibly via N-terminal region. Our previous studies [66] clearly showed the binding of α-synuclein with the mitochondrial membrane when aggregated. The overexpression of either wild-type or mutants (A53T, A30P) forms of α-synuclein in human neuroblastoma cells increases the accumulation of proteins. The accumulated forms of α-synuclein upon binding to the mitochondria cause decline in the mitochondrial membrane potential and hamper the respiration [66]. α-Synuclein oligomers have been shown to block the proteins import into the mitochondria by communicating with the translocase of the outer membrane 20 (TOM 20) [98]. In addition, the accumulations of α-synuclein oligomers in the endoplasmic reticulum (ER) cause ER stress and perturb its functions including the ER–mitochondria associations [99]. ER possesses interconnected chaperone proteins that guide the correct folding of secreted proteins. These ER chaperones including the grp94, grp78, and PDI have been found to be compromised in the brain stem and spinal cord of an α-synuclein A53T transgenic mouse model [100], thus suggesting that α-synuclein may interfere with the process of folding, translocation, or degradation of protein in neurons.

1.8 Mechanisms of α-Synuclein Toxicity

Insufficient is known about the machinery of toxicity innate to cell-to-cell transmitted α-synuclein. The approach of α-synuclein transmission and the variations of the SNCA gene manipulate α-synuclein transmission remain to be explored. The cell-to-cell imparted and endogenously expressed α-synuclein both contribute to cytotoxic mechanisms that straightly influence neuronal survival. α-Synuclein aggregation and its cell-to-cell transmission particularly affect neuronal physiological mechanisms like vesicle trafficking including neurotransmitter release and recycling [26, 27]. Its membrane binding affinity with cytoplasmic organelles especially mitochondria and thus consequent dysfunction of mitochondria perturbs not only the metabolic procedures but also degradative mechanisms [101, 102]. The interruption of vesicular transport machinery, specifically those that activate endoplasmic reticulum stress [30] is another important negative effect of α-synuclein. It was reported that extracellular α-synuclein causes activation of astrocytes and microglia in vitro and in vivo executing neuroinflammatory response alike observed in PD pathology [80]. Apparently neurons are extremely susceptible to glial cells-derived proinflammatory factors, consequently representing a substitutional neurotoxic process generated by cells that have attained α-synuclein from the extracellular milieu. α-Synuclein produces both protective and damaging effects. α-Synuclein secreted by neurons could provoke toxicity inside the cytoplasm of neighboring cells and also in the extracellular space. This may cause activation of glial cells in the brain that may induce chronic inflammation, thus participating to the succession of the pathology throughout the brain. Glial cells including both astrocytes and microglia are able to absorb and degrade synthetic recombinant α-synuclein [103]. In fact, α-synuclein can be exchanged among neurons and glial cells in vitro [104]. Neuron-derived α-synuclein exposed to rat primary astrocytes [104] and microglia [105] resulted in induction of an inflammatory reaction. α-Synuclein in aggregated form activates the microglia and thus originates inflammation and damage of exaggerated neurons [105, 106]. α-Synuclein was found to activate the microglia in a primary mesencephalic neuron-glial culture system, which was followed by enhancement of dopaminergic neurodegeneration [105]. In another study, when cultured microglial cells were incubated with protofibrils of α-synuclein, proinflammatory signaling mechanisms involving p38, ERK1/2 MAP
kinases and NF-κB turned out to be activated. Administration of α-synuclein protofibrils into the substantia nigra of adult rats induced the activation of microglia in addition to neuronal cell loss, which could be inhibited by the MAP kinase inhibitor semapimod [106]. These findings suggest that oligomeric/protofibrillar α-synuclein could exert few of its adverse effects by enhancing inflammatory responses in the pretended tissue.

Abnormally high level of α-synuclein may also disturb mitophagy. Postmortem brain tissues obtained from PD patients showed the aggregation of α-synuclein. This aggregation increases oxidative stress and agitates mitochondrial function [47]. Our own work showed that mitochondria are very sensitive for oxidative stress induced by wild-type and mutated α-synuclein [65, 66]. Furthermore, both in vivo and in vitro, expression of α isoforms of α-synuclein in neuronal cells induces the dysfunction of mitochondria, which will ultimately lead to the declined respiration and neuronal cell death [107]. Overexpression of α-synuclein may be restricted in mitochondria and interrupt the mitochondrial membrane potential by opening the mitochondrial permeability transition pore (mPTP) [66], thus developing mitophagy [108].

1.9 Concluding Remarks

α-Synuclein plays an imperative role in various physiological purposes involving regulation of dopamine neurotransmitter, synaptic transmission, inhibiting oxidation of unsaturated lipids in synaptic vesicles. α-Synuclein accumulates in PD brains, in neuronal cells of the substantia nigra, pons, medulla, and gut leading to inflammation and cellular death and subsequently difficulties in movement, digestion, circulation, and sleep. Experimental studies also support the hypothesis that mutations in SNCA gene and α-synuclein oligomers have a vital role in the pathology of PD and other age-related disorders [109]. The mechanisms of toxic and damaging effects of prefibrillar species of α-synuclein have been recognized using molecular and biochemical methods. The main mechanisms of oligomeric α-synuclein cellular toxicity include: mitochondrial impairments, ER stress, synaptic impairment, and affected cell membrane functionality. Furthermore, oligomers of α-synuclein may act as seeds for the arrangement of aggregates and also appear to be prone to transfer among cells. Stopping the α-synuclein from aggregation is the most potential target for treatment of PD. The strong evidence in favor of α-synuclein oligomers indicates that they are predominantly accountable for the dissemination of pathology. Therefore such oligomeric species of α-synuclein should be appropriate targets for early therapeutic intervention in Parkinson’s disease and other age-related disorders. Immunotherapy which efficiently interferes with uptake of extracellular α-synuclein has also been recently tried [110].

References


Molecular Mechanisms of Neurodegeneration: Insights from the Studies of Genetic Model of Parkinson’s Disease

Nisha R. Dhanushkodi and M. Emdadul Haque

2.1 Introduction

Parkinson’s disease (PD) is the second most common neurodegenerative disease with clinical motor syndrome characterized by bradykinesia, resting tremor, muscle rigidity, and postural instability caused by reduced level of dopamine [1]. Although the cause of the disease remains elusive, recent studies suggest that mitochondrial dysfunction, oxidative stress, neuroinflammation, misfolded protein stress, and lysosomal defects lead to the death of dopamine (DA) producing neurons in the SNc (substantia nigra pars compacta) area. Genetic studies over the past 20 years identified several genes mutation which lead to familial forms of the disease. Parkinson’s disease may be caused by single gene mutation in autosomal dominant or recessive fashion and these genetic mutations account for about 10–15% of the cases of PD. In the current PD genetics nomenclature, 18 specific chromosomal regions, are termed PARK (to denote their putative link to PD), and numbered in chronological order of their identification (PARK1, PARK2, PARK3, etc., where PARK1 and PARK4 are the same gene, SNCA) [2]. Mutations in autosomal recessively inherited genes, namely parkin, PINK1, and DJ-1, typically lead to early onset of PD. The genes PINK1 and parkin appear to work in the same pathway that controls mitochondrial quality and integrity during cellular oxidative stress. Dominantly inherited mutations in leucine-rich repeat kinase 2 (LRRK2) and α-Synuclein (α-SYN) cause late-onset PD and have characteristic Lewy body pathology. Recent GWAS (genome-wide association studies) study suggests that genetic variants of α-SYN and LRRK2 confer an increased risk for late-onset sporadic PD [3].

2.1.1 Neuropathology of PD

The neuropathology of PD is characterized by a specific pattern of DA-producing neuronal loss in substantia nigra pars compacta (SNc) with Lewy bodies (LB) rich in α-SYN in the surviving neurons [4]. It has been suggested that PD may begin in the lower brainstem and olfactory bulb where the substantia nigra only becoming affected during the middle stages of the disease [5]. However, not all the clinical features of PD are attributable to the degeneration of DA neurons [6]. Non-dopaminergic neuron degeneration accounts for other features of the disease like depression, dementia, sleep, olfactory and balance problems [7] that typically occur in advanced stages of PD. The non-dopaminergic features of PD are often the most disabling, and current treatment

N. R. Dhanushkodi · M. E. Haque (*)
Department of Biochemistry, College of Medicine and Health Sciences, UAE University, Al Ain, UAE
e-mail: ehaque@uaeu.ac.ae
with L-DOPA obviously does not cure these symptoms [8]. Most recently, it has been suggested that connection between spreading of Lewy pathology and development of clinical PD is very weak [9].

2.2 Genetic Models of PD

It is noteworthy that patient-based genetic studies identified the role of genetics in PD which further justify to generate model organisms to elucidate the function of those genes. Animal models are advantageous since it allows manipulation of the condition and yields result in short time. Currently, there are many genetic models of PD, including vertebrate organisms like rat, mice, and zebrafish; invertebrate organisms like Drosophila melanogaster and Caenorhabditis elegans. These genetic PD models have been informative in understanding molecular pathways and pathological changes in PD [10].

2.2.1 Vertebrate Models of PD

Mouse is the most preferred model to study neurodegenerative diseases such as PD disorders. This is because mouse possesses human alike neuronal networks and genetic homologs [11]. Since PD is a chronic disorder, promoter should be strong as well as constitutively active throughout the lifetime of mice. Conditional temporal expression of a transgene can also be used to control the expression [12]. A more advanced technique is the tetracycline (Tet)-regulated transgenic switch in which expression of the transgene follows the activity pattern of the promoter in the driver construct. The ability of Tet-transactivator protein (tTA) to change its conformation and affinity for Tet-resistant protein (tetP) by doxycycline allows temporal on/off control of transgene induction [10, 13, 14]. Stereotaxic viral injection of different viruses (e.g., lentivirus, recombinant adeno-associated virus [rAAV], and herpes simplex virus [HSV]) can be used for the expression of desired transgene. Gene knockout (KO) and knock-in (KI) can also be achieved by homologous recombination. In the conventional transgenic system, a gene is overexpressed under the control of a promoter that drives the expression in preferred organ like brain. Usually dominant mutants (i.e., A53T, A30P, and E46K for α-SYN; G2019S and R1441C/G mutants for LRRK2) are preferred to be overexpressed because the mode of inheritance supports a gain of toxicity and hence an exaggeration of its endogenous function. Deletion of important exons or introduction of premature termination should be able to simulate early-onset PD caused by autosomal-recessive gene. Deletion of parkin, PINK1, and DJ-1 has however not yielded mouse with desired phenotype [10]. Even knocking out all three genes together has proved ineffective [15] possibly due to potential compensatory mechanisms elicited in mouse model. The Cre–loxP-mediated conditional KO approach is widely used when embryonic lethality prevents studying deletion of a gene in adult animals [16]. Rat models of PINK1, DJ-1, and Parkin genes have been generated using zinc finger technology. The phenotype of these rats showed progressive neurodegeneration and early behavioral deficits, suggesting that these recessive genes may be essential for the survival of dopaminergic neurons in the SNc area [17]. The neuroanatomy of zebrafish is typical that of vertebrates with forebrain, hindbrain, and spinal cord, and their genes are highly homologous to that of humans and hence it has been used as a PD model organism. For example, transient knockdown of DJ-1 using morpholino antisense oligonucleotides has shown loss of function of DJ-1 in zebrafish.

2.2.2 Invertebrate Models of PD

C. elegans and D. melanogaster are small, inexpensive to culture models with short life spans and hence time effective. Although they lack α-SYN homolog and have limited repertoire of cell death effectors, these models offer the advantage of identifying evolutionarily conserved pathways. However, the validity of these studies is a variable on their reproducibility in human system. A large number of mutant strains are available as stocks for the researchers to use them effectively.

N. R. Dhanushkodi and M. E. Haque
to provide insights on the pathways involved. In reverse genetic approach, RNA interference (RNAi) to knockdown target genes is achieved by simply injecting, soaking, or feeding the C. elegans with dsRNA which is complementary to the targeted gene. Similar to human CNS, dopamine plays important functions like locomotion, feeding, sleep/circadian rhythms, and learning in drosophila. The nervous system dysfunction in these models can be studied using the changes in resting and synaptic potentials and linking these changes to behavioral deficits and loss of DA neurons [18, 19]. The Drosophila genome encodes homologs of DJ-1, PINK1, PARKIN, LRRK2, and VPS35. Expression of human SYN in Drosophila results in dopaminergic neuronal loss [20, 21]. Table 2.1 summarizes various genetic models of PD and their observable phenotype.

### 2.3 Autosomal-Dominant PD

#### 2.3.1 SNCA (PARK1/4)

Missense mutation in the SNCA gene identified by Polymeropoulos et al. [22] is the first PD-associated gene identified. Soon, Spillantini and colleagues established that α-SYN protein is the major component of the LB. The three dominant α-SYN mutations identified in separate family studies are A30P, A53T, and E46K [22–24]. Duplication and triplication of the wild-type α-SYN locus are also shown to cause familial PD [25]. Polymorphisms around the SNCA locus are also significantly associated in two recent genome-wide association studies of sporadic PD [3, 26]. Thus, α-SYN plays critical role in both familial and sporadic PD [27].
The α-SYN, a presynaptic phosphoprotein, although found to be unstructured in free state, also exists in a variety of structures, including oligomers, protofibrils, fibrils, and filaments. The soluble protofibrils and fibrils seem to be the most toxic forms [28] than the insoluble aggregate present in Lewy bodies [29]. In mouse with pan-neuronal or DA-specific promoters driven expression of α-SYN wild type (WT) or mutants causes the severity and age of onset of disease depended heavily on the promoter and levels of transgene expression. These mouse models however lack the key pathological feature of PD which is DA neuronal loss. However, they show neurodegeneration in other anatomical sites and functional abnormalities in the nigrostriatal system [16, 30]. The mouse prion promoter (mPrP) driven A53T transgenic mice exhibit more pathological phenotype which includes increased phosphorylation, ubiquitination, and aggregation of α-SYN, leading to progressive neurodegeneration [31]. Although the anatomical site is not the one traditionally associated with PD, these systems may however serve to study mechanisms of α-SYN-induced neurodegeneration. Since there is no obvious DA neuronal loss in most α-SYN transgenic mice, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) intoxication is used additionally [32]. Through mitochondrial dysfunction it is sufficient to induce α-SYN aggregation and DA neuronal loss [16]. Interestingly, expression of human WT, A53T, and A30P α-SYN in drosophila produced many pathological hallmarks of PD like the age-dependent DA neuron loss, Lewy body like inclusions containing α-SYN, and other locomotor deficits [20]. Although C. elegans overexpressing α-SYN has been shown to cause loss of DA neurons, they lack significant Lewy body like pathology [33, 34].

2.3.1.1 Mitochondrial Dysfunction Causes α-SYN Accumulation

Malfunction in mitochondrial complex I is constantly observed in sporadic PD and this established the link between mitochondrial dysfunction and synucleinopathy [35]. Various studies have established that inhibition of mitochondrial complex I causes selective DA neurodegeneration (Banerjee et al.2009). Impairment of proteasomal and lysosomal systems can lead to α-synucleinopathy, since they play a significant role in catabolizing α-SYN. However, it leads to general neurodegeneration without selectivity for DA neuronal system. But mitochondrial complex I inhibition selectively impairs DA neurons and is thus a causative event while impairment of the proteasomal and lysosomal systems are most probably only the downstream events in the pathogenesis of PD. Further, α-SYN knockout mouse is resistant to the DA neurotoxicity caused by MPTP and other mitochondrial toxins [36]. Transgenic mice overexpressing human A53T α-SYN mutant exhibit mitochondrial abnormalities including mitochondrial DNA damage and degeneration [37]. It has been reported that silencing of SYN prevents dopamine neurons loss when exposed with mitochondrial complex I inhibitor, MPTP [38]. Overexpression of α-SYN, on the other hand, makes mouse more susceptible to mitochondrial toxins like paraquat [39]. In this context, new treatment modalities preventing mitochondrial damage may be more promising for controlling PD.

DA-specific mitochondrial transcription factor A (TFAM) knockout in mouse leads to DA neurodegeneration and Lewy body formation. Moreover, it has been shown that PD patients accumulate α-SYN in the SNc and the striatum has a decreased mitochondrial complex I activity [40]. α-SYN transgenic mice not only show mitochondrial impairment but also enhance DNA damage in which poly (ADP-ribose) polymerase (PARP-1) is overactivated leading to cell death [41]. PARP deletion also protects DA neurons from MPTP-induced toxicity [42]. Thus it may be postulated that α-SYN aggregation may probably impair the regulation of PARP1.

2.3.1.2 SYN Mutation Induces Lysosomal and Autosomal Dysfunction

The autophagy-lysosome pathway plays a critical role in degrading proteins with longer half-lives [43]. Lysosomal pathway inhibition leads to accumulation of α-SYN, suggesting that α-SYN
catabolism is not solely mediated by proteasomal pathway. The chaperone-mediated lysosomal uptake pathway also mediates α-SYN binding to lysosomal membrane receptors. Mutant α-SYN shows deficient translocation blocking uptake of other substrates as well, thus causing misfolded protein stress [44]. Although not directly involved in proteasomal degradation, α-SYN overexpression causes inhibition of proteasome function [44]. A recent study in drosophila showed that expression of Rab11, a regulator of exocytosis could reverse the severe phenotype caused by overexpression of wild-type SYN [18].

2.3.1.3 SYN in Neuronal Synapse

α-SYN was initially identified as a synaptic and nuclear protein. Although their role remains elusive, evidence suggests that the protein plays a role in maintenance of synaptic vesicle pools and activity-dependent dopamine release [45]. The presynaptic cysteine-string protein knock-out in mice causes severe phenotype that is rescued by α-SYN overexpression [46], providing evidence that α-SYN might modulate synaptic vesicle function.

2.3.2 LRRK2 (PARK8)

In 2004, the genetic cause of chromosome 12 linked to PD was attributed to mutations in the LRRK2 (Leucine-rich repeat kinase-2) gene [47]. Mutations in LRRK2 cause autosomal-dominant PD. LRRK2, a large protein with multidomain is ubiquitously expressed in neurons and localizes with membranes and lipid rafts [48]. A deletion mutant for C.elegans lrk-1 (lrk-1 is similar to human LRRK1, homolog of LRRK2), indicates its significant role in localizing synaptic vesicle proteins to terminals [49]. The most common mutation in LRRK2 is G2019S with a frequency of 1% in sporadic PD and 4% in hereditary PD and the risk increases with age. Autopsy of patients with LRRK2-associated PD shows α-SYN inclusions, and hence LRRK2 and α-SYN might share common pathogenic mechanisms. Unlike α-SYN mutation, dosage effect is not seen in LRRK2 mutations and the disease in homozygotes is clinically identical to heterozygotes carrying the mutation. The toxicity of LRRK2 mutation in vitro is kinase and GTP-binding dependent and this piece of information is invaluable for probable therapeutic interventions [50]. In C. elegans, overexpression of either wild-type or G2019S LRRK2 caused DA neuronal loss [51]. LRRK2 G2019S mutation also shows vulnerability to rotenone toxicity compared to wild type. In drosophila, overexpression of human LRRK2 impairs DA-dependent locomotor activity and also causes loss of DA neurons [52]. The Lrrk loss-of-function mutant homolog in drosophila also shows deficits in synaptic transmission. In another independent study, using a different clone of Lrrk loss-of-function mutant, no difference in EJP (excitatory junction potential) amplitude was found between the mutants and wild type.

The current transgenic mouse models of PD are not very robust in producing PD phenotype compared to cellular and drosophila model systems. Despite several transgenic techniques like conventional, bacterial artificial chromosome (BAC) transgenic, mutant LRRK2 Knock-In, and tet-inducible transgenic, only one of the LRRK2 models reproduced age-dependent DA neuron death in the nigrostriatal system [53]. BAC transgenic mice and conditional expression of LRRK2 WT and LRRK2 G2019S showed no characteristic phenotype and no neuronal loss [54, 55]. R1441C mutation in LRRK2 has been shown to impair stimulated dopamine neurotransmission and D2 receptor function [56]. The R1441G BAC mouse model shows a strong phenotype with akinesia, reversible with L-Dopa and dopamine agonist apomorphine treatment [57]. It is noteworthy that mice that express mutant LRRK2 using varying promoters have different levels of expression. Thus, most LRRK2 transgenic animals although lacking neuronal loss manifest earliest deficits like DA transmission and DA-responsive behavior defects. The aberrant kinase activity of LRRK2 might cause phosphorylation of substrates that misregulates binding partners and other regulators. In HSV-LRRK2 G2019S viral induced DA neurodegeneration models, the aberrant kinase activity of LRRK2 G2019SS was
prevented by inhibitor of LRRK2 kinase activity that abolished its toxicity [58]. Thus identification of phosphosubstrates involved would help in deciphering the pathogenic mechanisms induced by LRRK2 mutations [2, 16].

### 2.3.2.1 LRRK2 Affects Mitochondrial Function

In *C. elegans*, RNAi-mediated silencing of *lrk-1* increased the toxicity to rotenone treatment and overexpression of wild-type LRRK2 significantly increased resistance against mitochondrial toxins such as rotenone and paraquat [51]. Similar observation was made in drosophila overexpressing human mutant LRRK2. However, LRRK2 knockout mice are not more sensitive to mitochondrial toxin, MPTP [59].

### 2.3.2.2 LRRK2 in Neuronal Morphogenesis

LRRK2 knockout mice exhibit normal numbers of dopaminergic neurons in the SNc area without any behavioral deficits. However, in vitro studies strongly suggest a role of LRRK2 in the neurogenesis of dopamine producing neurons by controlling cell cycle. Taken together, it is evident that LRRK2 is required for dopamine neuron genesis or survival in adult animals [49, 59]; however, its substrates, regulators, and binding partners remain elusive. Similar studies in mice suggest no role of LRRK2 in neurogenesis, and drosophila studies show disparate results [49, 55, 60]. LRRK2 plays multiple roles as in neuronal morphogenesis and in other peripheral processes like kidney functions in rats and mice. LRRK2 knockdown in zebrafish causes neuronal loss, developmental abnormalities like axis curvature defects, and ocular abnormalities [61]. Also by its localization to presynaptic vesicles and endosomes, LRRK2 is shown to regulate synaptic vesicle endocytosis by directly interacting with the early endosome marker protein Rab5 [62].

### 2.4 Autosomal-Recessive PD

The first identified genetic cause of autosomal-recessive juvenile Parkinsonism is the *Parkin* mutation reported in a Japanese family study [63]. With nearly 100 reported mutation (seen in 50% of familial PD cases and in 20% of young-onset sporadic PD), it is the most frequent autosomal-recessive mutation [64]. Mutations in PINK1 gene is the second most common autosomal-recessive mutation (1–7% of early-onset PD) and mutations in DJ-1 are a rare cause of PD [65, 66]. For all the three genes, whole exon deletions cause loss of protein while point mutations destabilize or yield functionally inactive proteins. Various studies in a number of animal and cellular models for parkin, PINK1, and DJ-1 have led to tremendous insight into the role of these proteins in PD. In the recent GWAS, none of the autosomal recessively inherited PD genes (Parkin, PINK1 or DJ-1) have been reported as a risk factor, but such studies might identify only strongly associated genes [3, 26].

### 2.4.1 PARKIN (PARK2)

Kitada and colleagues [63] first identified mutations in *parkin*, (maps to 6q25–q27), as one of the causes of juvenile Parkinsonism. To date, 100 different *parkin* mutations have been reported both in familial and sporadic PD. This gene extends to about 1.3 Mb of DNA with 12 exons encoding a 465 amino acid protein, with high degree of mutations. Penetrance appears to be complete in individuals with two disease-causing mutations in Parkin [64]. Parkin which is considered as an E3 ubiquitin ligase participates in the ubiquitin-proteasome system [67]. It has an ubiquitin-like (Ubl) domain at the N-terminus followed by two RING finger domains separated by an inbetween RING (IBR) domain, each of which bind two Zn$^{2+}$ atoms. Being vulnerable to oxidative and nitrosative stress, Parkin plays a key role in sporadic PD [68, 69].

In drosophila *Parkin* knock out generates defective flies with reduced climbing ability, life span, and male sterility [70, 71]. Abnormalities in muscle and sperm mitochondria ultimately result in cell death due to activation of autophagy. DA neurodegeneration with reduced TH (tyrosine hydroxylase) level was also observed along with DA-responsive locomotor deficit. To study the role of Parkin in PD, several groups have
generated parkin KO mice [72, 73]. Although these knockouts lack substantial dopaminergic or behavioral abnormalities, they show subtle changes in either the DA nigrostriatal circuit or the locus coeruleus (pons nucleus) noradrenergic system [72–75]. Parkin knockout mice had reduced mitochondrial respiratory chain proteins and stress response proteins. Parkin substrates like AIMP2, FBP1, and PARIS were shown to accumulate in the ventral midbrain of parkin knockout mice [41, 76], [77]. These cellular changes may contribute to deficits in DA metabolism and hence behavior. Parkin mutants might have a dominant negative effect since overexpression of mutant human parkin causes age-dependent progressive DA neurodegeneration in fly and mouse system [78–80].

2.4.1.1 Parkin Mediates Mitochondrial Quality Control
In addition to its role as an E3-ubiquitin ligase, Parkin seems to actively play a role to clear damaged mitochondria. A germ line deletion of Parkin leads to defective mitochondria, suggesting its role in mitochondrial quality control. Overexpression of parkin provides neuroprotection against MPTP toxicity while parkin knockout does not enhance the neuronal susceptibility to MPTP [74]. The mitochondrial defects and upturn wing phenotype due to Pink1 knockout in drosophila are rescued by overexpression of Parkin. This result suggests that Parkin maybe thus essential for clearance or might rescue defective mitochondria to reduce toxicity. However, in vitro mammalian cell culture study shows that Pink1 is essential for recruitment of Parkin to eliminate defective mitochondria [81]. Thus, the protective effect of Parkin during Pink1 knockout suggests that mitochondrial quality control pathways in drosophila can also function independent of Pink1.

2.4.1.2 Parkin Acts as an E3 Ubiquitin Ligase
The ubiquitin-proteasome pathway has been strongly linked to PD pathogenesis, highlighting the significance of the E3 ubiquitin ligase Parkin [67]. Parkin catalyzes lysine-48-mediated polyubiquitination, which targets the substrates for proteosomal degradation. Mutations in the parkin gene lead to failure of the ubiquitin-proteasome system due to impaired ligase activity that cause intracellular accumulation of parkin substrates [82]. However, in PARK2 patients, or in parkin knockout mice, the accumulation of substrate is significantly low, thus making the role of Parkin as an E3 ubiquitin ligase insignificant [84], [10]). Parkin is also involved in other forms of ubiquitination, modulating cellular processes like signal transduction, transcriptional regulation, and protein and membrane trafficking [83]. Parkin is capable of modifying proteins with different ubiquitin linkages, including monoubiquitination and polyubiquitination using both lysine-48 (involving receptor turnover, protein degradation) and lysine-63 linkages (involving protein inclusions). However, the exact role of Parkin in the context of these cellular activities is not yet clear [21].

2.4.1.3 Role in Neuronal Synapse
Although a cytoplasmic ubiquitin ligase protein involved in the cellular ubiquitination/protein degradation pathway, Parkin can also localize to the synapse and associate with membranes [85]. Interestingly, it is involved in the modulation and metabolism of several presynaptic proteins such as α-SYN and the α-SYN-binding synaptic proteins like synphilin. Parkin has been associated with the function of GPR37, aG-protein coupled receptor that interacts with the dopamine transporter DAT [44]. Reduced synaptic transmission is seen in Parkin mutant larvae in drosophila and there is reduction of both evoked and spontaneous excitatory junction potential, as well as depolarization of the resting membrane potential in flight muscles. Perturbed synaptic transmission is likely due to reduced glutamate release, because of the changes in synaptic morphology and/or ATP depletion due to mitochondrial deficits [18].

2.4.2 PINK1 (PARK6)

Mutations in the PINK1 (phosphate and tensin homolog (PTEN)-induced putative kinase 1)