Parkinson Disease: Translating Insights from Molecular Mechanisms to Neuroprotection

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Abstract—Parkinson disease (PD) used to be considered a nongenetic condition. However, the identification of several autosomal dominant and recessive mutations linked to monogenic PD has changed this view. Clinically manifest PD is then thought to occur through a complex interplay between genetic mutations, many of which have incomplete penetrance, and environmental factors, both neuroprotective and

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This work was supported by grants from National Institutes of Health (NIH) National Institute of Neurological Disorders and Stroke [Grant NS38377], [Grant NS097049], [Grant NS102035] and National Institute on Aging [Grant AG059686], the JPB Foundation the Michael J. Fox Foundation and the RMS Family Foundation and through support of the Adrienne Helis Malvin and Diana Helis Medical Research Foundations.

T.M.D. serves on the Board of Directors is compensated for his roles as a consultant and interim Chief Scientific Officer of Valted Seq Inc. These arrangements have been reviewed and approved by the Johns Hopkins University in accordance with its conflict-of-interest policies.

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https://doi.org/10.1124/pharmrev.120.000189
increasing susceptibility, which variably interact to reach a threshold over which PD becomes clinically manifested. Functional studies of PD gene products have identified many cellular and molecular pathways, providing crucial insights into the nature and causes of PD. PD originates from multiple causes and a range of pathogenic processes at play, ultimately culminating in nigral dopaminergic loss and motor dysfunction. An in-depth understanding of these complex and possibly convergent pathways will pave the way for therapeutic approaches to alleviate the disease symptoms and neuroprotective strategies to prevent disease manifestations. This review is aimed at providing a comprehensive understanding of advances made in PD research based on leveraging genetic insights into the pathogenesis of PD. It further discusses novel perspectives to facilitate identification of critical molecular pathways that are central to neurodegeneration that hold the potential to develop neuroprotective and/or neurorestorative therapeutic strategies for PD.

Significance Statement—A comprehensive review of PD pathophysiology is provided on the complex interplay of genetic and environmental factors and biologic processes that contribute to PD pathogenesis. This knowledge identifies new targets that could be leveraged into disease-modifying therapies to prevent or slow neurodegeneration in PD.

I. Introduction

Approximately 1% of the population over age 65 has been diagnosed with Parkinson disease (PD), making it the second most common neurodegenerative disease. The estimated global prevalence of PD was 6.1 million in 2016, and as the population of older individuals rises, this number is projected to more than double by 2030 (Dorsey et al., 2007; Marras et al., 2018; GBD 2016 Neurology Collaborators, 2019). Individuals with PD are less likely to work and have higher nursing home placement and greater mortality than the general population (Aarsland et al., 2000). Although most PD is currently thought to be idiopathic, about 5%–10% of individuals with PD have a monogenic form of the disease with Mendelian inheritance, and more than 90 susceptibility genes have been identified to date (Bandres-Ciga et al., 2020a). The pathophysiology of these genetic contributions reflects the numerous pathways that lead to dopamine (DA) cell loss and the many potential areas of disease-modifying therapy.

PD is a chronic progressive disorder manifested by at least two of the four motor signs—tremor at rest, bradykinesia, rigidity, and postural instability—and results from the selective degeneration of the nigrostriatal pathway, which provides dopaminergic innervation to the striatum. PD is currently diagnosed based on patient history and a clinical examination showing the presence of Parkinson motor signs, coupled with no or minimal “red flags” that indicate atypical parkinsonism (i.e., slow or absent vertical saccades) or other exclusion criteria (i.e., history of

ABBREVIATIONS: 4E-BP, eukaryotic initiation factor 4E–binding protein; 5-HT, 5-hydroxytryptamine; 6-OHDA, 6-hydroxydopamine; AAV, adeno-associated virus; AD, Alzheimer’s disease; AIF, apoptosis-inducing factor; AIMP2, aminocytic-I RNA synthetase—interacting multifunctional protein type 2; ALF, lysoosomal-autophagy system; APLP1, Amyloid Beta Precursor Like Protein 1; ASO, antisense oligonucleotide; aSynL, α-synuclein mRNA transcript that retains an elongated 3’UTR; BBB, blood–brain barrier; c-Abl, ABL Proto-Oncogene 1, Non-Receptor Tyrosine Kinase; C1q, complement component 1q; Casp13, caspase-13, calcium channel, voltage-dependent, L type, alpha 1D subunit; CMA, chaperone-mediated autophagy; CNS, central nervous system; CoQ10, Coenzyme Q10; CSF, cerebrospinal fluid; DA, dopamine; DAQ, dopamine-derived quinone; D2-AR, D2-autoreceptor; DAT, DA transporter; DBS, deep brain stimulation; DMT, divalent metal transporter; DOPAL, 3,4-dihydroxyphenylacetaldehyde; DPP4, dipeptidyl peptidase; Drp1, Dynamin-Related Protein 1; DpP4, dipeptidyl peptidase; DRD1, DA D1 receptor; DRd1, D1 receptor; DRd1, D1 receptor; DUB, deubiquitinating enzyme; EAAT, excitatory amino acid transporter; eIF2, eukaryotic initiation Factor 2; eIF4E, eukaryotic initiation factor 4E; ER, endoplasmic reticulum; ERR, estrogen-related receptor; ETC, electron transport chain; FDA, Food and Drug Administration; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GABA, glutocerebrosidase; GCaese, glucocerebrosidase; GDNF, glial cell line–derived neurotrophic factor; GIT, gastrointestinal tract; GLP-1, glucagon-like peptide 1 receptor; GLUT-1, glutamate transporter 1; GTPase, guanosine triphosphatase; Herp, homocysteine-induced ER protein; HLA, human leukocyte antigen; HLA-DQ, Human Leukocyte Antigen – DQ isoform; HLA-DR, Human Leukocyte Antigen – DR isoform; IL, interleukin; iNOS, inducible nitric oxide synthase; iPSIC, induced pluripotent stem cell; K0, knockout; LANP, lymphocyte activation gene 3; LB, Lewy body; LN, Lewy neurite; LPS, lipopolysaccharide; LRRK2, leucine-rich repeat kinase 2; LTD, long-term depression; MDS-UPDRS, Movement Disorder Society-Unified Parkinson’s Disease Rating Scale; MHC, major histocompatibility class; MIF, migration inhibitory factor; miRNA, microRNA; MMP, mitochondrial membrane potential; MMP7, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; mtDNA, mitochondrial DNA; Ndfip1, Nedd4 Family Interacting Protein 1; Nedd4, Neuronal precursor cell-expressed developmental downregulated protein; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NO, nitric oxide; Ndfip1, Nedd4 Family Interacting Protein 1; Nedd4, Neuronal precursor cell-expressed developmental downregulated 4; Nf-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NINDS, National Institute of Neurologic Diseases and Stroke; NLRP3, NOD-, LRR-, and pyrin domain-containing protein 3; NMDA, N-methyl-D-aspartate; NO, nitric oxide; ORF4A2, Nuclear Receptor Subfamily 4 Group A Member 2; NSC, neuronal stem cell; PAR, poly(adenosine 5’-diphosphate-ribose); PARK, Parkinson disease genes; PARIS, Parkin interacting substrate; PARK, Parkinson’s disease; PARP, ADP-ribose transferase; PD, Parkinson disease; PDBP, Parkinson Disease Biomarker Program; PFF, preformed fibril; PGC-1, peroxisome proliferator–activated receptor γ coactivator-1α; PINK1, phosphatase and tensin–induced putative kinase; PMCA, protein misfolding cyclic amplification; PPMI, Parkinson’s Progression Markers Initiative; PR, press release; RNS, reactive nitrogen species; ROS, reactive oxygen species; S6K, S6 kinase; SN, substantia nigra; SNCA, synuclein alpha; SNP, single nucleotide polymorphism; Snp, substantia nigra pars compacta; STING, stimulator of interferon genes; SV2C, synaptic vesicle glycoprotein 2C; TFEB, transcription factor EB; TNFα, tumor necrosis factor-α; TOR, target of rapamycin; Treg, regulatory T cells; tRNA, transfer RNA; TRPC1, transient receptor potential channel 1; TSP, translocator protein; UCP, uncoupling; UDR, Urdosymmetricolic acid; UDPS, Unified Parkinson’s Disease Rating Scale; UTR, unfolded protein response; VMAT2, vesicular monoamine transporter 2; VPS35, vacuolar protein sorting-associated protein 35; VTA, ventral tegmental area; WT, wild type.
repeated strokes or long-term exposure to specific medications) (Hughes et al., 1992; Postuma et al., 2018). Using this diagnostic method, autopsy series have shown that even movement disorder specialists are incorrect about the diagnosis approximately 10% of the time. Furthermore, it is unfortunately not unusual for patients to have an up to 2-year delay between symptom onset and diagnosis. Diagnosis is complicated by two primary factors: 1) the phenotypic heterogeneity of the disease and 2) the phenotypic overlap between PD and many of the atypical parkinsonisms. In other words, parkinsonism and the associated degeneration of the nigrostriatal circuitry is a final common pathway of neurodegeneration caused by different abnormal proteins, including α-synucleinopathies such as multiple system atrophy, dementia with Lewy bodies, and PD and tauopathies such as progressive supranuclear palsy, corticobasal syndrome, and even Alzheimer disease (AD). Definitive diagnosis of PD can only occur based on autopsy tissue that shows the presence of α-synuclein and the presence of eosinophilic, intracytoplasmic proteinaceous inclusions termed Lewy bodies (LBs) and dystrophic Lewy neurites (LN)s in surviving nigral neurons (McKeith et al., 2017).

Although the cardinal symptoms of PD can be improved using currently available DA replacement strategies, many of these treatments have unacceptable side effects, and many of the nonmotor features of the disease have minimal treatments. Most importantly, treatments that provide neuroprotection and/or disease-modifying effects are an urgent unmet clinical need. Identification of neuroprotective or neurorestorative drugs requires an in-depth understanding of the molecular and biochemical mechanisms of PD pathogenesis that would provide a rationale for novel strategies for prevention or therapy as well as help identify candidates for mechanism-based targets. In this regard, unraveling the genetic etiology of PD, long considered a sporadic condition, has facilitated disease modeling and provided fundamental insights into the cellular mechanisms underlying dopaminergic degeneration. The genetic and functional studies of the rare familial genes known to date have each opened an avenue into PD pathogenesis and have implicated a number of highly interconnected and convergent molecular pathways that could serve as crucial nodal points for drug targeting. In this review, we provide a comprehensive understanding of recent advances in PD research that highlight the complex web of protein interactions and juxtaposition of molecular pathways underlying both familial and sporadic forms of PD. We further discuss novel perspectives on the emerging concepts in PD pathogenesis that could pave the way to more effectively and efficiently diagnose PD and meet our ultimate goal of halting disease progression.

II. Current Treatment Is Symptomatic

Complete appreciation of the many innovative therapeutics that are coming down the pipeline necessitates a brief discussion of current therapeutics. The current treatment of PD is solely based on symptomatic management without regard for genetic risk factors or the varying underlying pathophysiology of PD and is summarized in Table 1. Patients experience varying degrees of symptoms, with some having more rapid motor impairments and others experiencing severe psychiatric symptomatology or other primary symptoms such that effective symptomatic management is individualized, within the guidelines of the evidence-based practices discussed below. This phenotypic heterogeneity is also one of the many challenges to identifying disease-modifying therapies.

A. Treatment of Motor Symptoms of Parkinson Disease

The mainstay treatment of the motor complications of PD remains DA replacement, usually in the form of levodopa. Levodopa was first given to patients in 1961, with growing recognition of its efficacy through the 1960s and the addition of a dopa decarboxylase inhibitor in the early 1970s (Tolosa et al., 1998). Carbidopa/levodopa remains the most effective medication for treating the motoric symptoms of PD. Unfortunately, about 50% of patients develop dyskinesias within about 5 years of treatment (Rascol et al., 2000). Additionally, over time the therapeutic window of the medication narrows (Olanow et al., 2006), necessitating increased frequency and amount of dosing. Much of the development of new medications in PD has focused on improving the motor fluctuations and dyskinesias due to this narrowing of the therapeutic window as well as novel levodopa delivery mechanisms. A new oral formulation of carbidopa/levodopa was approved by the FDA in January 2015. Marketed under the brand name Rytary, it contains both controlled-release and immediate-release carbidopa/levodopa. Patients therefore take the medication about three to four times a day, as opposed to the current carbidopa/levodopa, which may eventually require as many as 7–10 doses a day. Carbidopa/levodopa intestinal gel, approved by the FDA in 2015, follows the same principles of DA replacement but has a novel delivery system with a percutaneous jejunostomy tube that enables continuous dosing of the medication. There are additional novel delivery systems currently being tested, including an inhaled levodopa (LeWitt et al., 2016) as well as a subcutaneous levodopa (Ramot et al., 2017; Ellenbogen et al., 2020). Although these have great potential...
<table>
<thead>
<tr>
<th>Symptom</th>
<th>Mechanism of Action</th>
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<td>CR carbidopa-levodopa</td>
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<td>ER carbidopa-levodopa</td>
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<td>Ropinirole</td>
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<td>Rotigotine</td>
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<td>Monoamine oxidase-B inhibitors</td>
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<td>Rasagiline</td>
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<td><strong>Motor fluctuations</strong></td>
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<td><strong>Rescue medication</strong></td>
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<td>FDA approved</td>
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<td></td>
<td>Nonergot dopamine agonist</td>
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<tr>
<td><strong>Dyskinesias</strong></td>
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<td>Inj ectable apomorphine</td>
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<td>Anticholinergics</td>
<td>ER amantadine capsule/tablet</td>
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<td><strong>Nonmotor symptoms</strong></td>
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<td></td>
<td>NMDA receptor antagonist</td>
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<td>Psychosis</td>
<td>Memantine</td>
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<td>D2 and 5-HT2 receptor antagonist</td>
<td>Quetiapine</td>
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<td>5-HT2A and 5-HT2C receptor agonist and antagonist</td>
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<td>Depression and anxiety</td>
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<td>Selective serotonin reuptake inhibitor/serotonin norepinephrine inhibitor</td>
<td>Carbidopa/levodopa formulations</td>
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<tr>
<td></td>
<td>DA precursors</td>
<td>Carbidopa/levodopa</td>
<td>FDA approved</td>
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<td>Melatonin receptor 1A agonist</td>
<td>Melatonin</td>
<td>Supplement</td>
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<td></td>
<td>Orthostatic hypotension</td>
<td>DA precursors</td>
<td>Controlled-release carbidopa/levodopa</td>
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<td></td>
<td>Fluid retention</td>
<td>Salt tabs</td>
<td>Supplement</td>
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<td></td>
<td>DA2-receptor antagonist</td>
<td>Domperidone</td>
<td>Not FDA approved</td>
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<td></td>
<td>α1-adrenergic receptor stimulator</td>
<td>MIdodrine</td>
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<td></td>
<td>Increased Na reabsorption</td>
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<td></td>
<td>Increased norepinephrine</td>
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<td>Acetylcholinesterase inhibitor</td>
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<td></td>
<td>Constipation</td>
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<td></td>
<td>Stool bulking</td>
<td>Docusate</td>
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<td></td>
<td>Mixes stool fat and water</td>
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<td>Supplement</td>
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<tr>
<td></td>
<td>DA2-receptor antagonist</td>
<td>Polyethylene glycol</td>
<td>Not FDA approved</td>
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<tr>
<td></td>
<td>VGNA and K channel blocker, GABA receptor enhancer</td>
<td>Zonisamide</td>
<td>Off label</td>
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<td></td>
<td>Drooling</td>
<td>Anticholinergic</td>
<td>Off label</td>
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<td></td>
<td>Urinary</td>
<td>Muscarinic antagonist</td>
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<td></td>
<td>Fatigue</td>
<td>β3-adrenergic agonist</td>
<td>Off label</td>
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(continued)
to improve the dyskinesias and motor fluctuations that occur with the narrowing of the therapeutic window, at their basis they are the same medication that was identified more than 55 years ago and are not disease-modifying therapy. There are a number of other symptomatic therapies in regular use. DA agonists are also used to improve PD symptoms, although they are generally not as effective as carbidopa/levodopa. Agonists currently available in the United States include pramipexole, ropinirole, and the more recently available transdermal formulation rotigotine. These medications have a place in management (Antonini et al., 2009), often among younger patients and/or to help with the motor fluctuations that occur with carbidopa/levodopa dosing. These medications also have unacceptable side effects—about 17% of patients taking DA agonists develop compulsive behaviors including compulsive gambling, sex, or shopping (Weintraub et al., 2010). Monoamine oxidase inhibitors rasagiline and selegiline and the recently FDA-approved safinamide may also be useful to augment levodopa. Catechol-O-methyltransferase inhibitors—namely, entacapone and tolcapone—also seek to extend the life of levodopa. Finally, amantadine is useful for treatment of dyskinesias and is sometimes used for symptomatic treatment (Dietrichs and Odin, 2017).

There are also surgical options currently available for PD symptomatic treatment. These surgical treatments are most effective at improving the motor fluctuations due to the narrowing therapeutic window of levodopa and the pharmacokinetics of medication administration. The most frequently performed surgical procedure for PD is deep brain stimulation (DBS), which involves placing electrodes in either the globus pallidus internus or the subthalamic nuclei. The resulting constant stimulation attenuates the motor fluctuations that occur later in the PD course. Prior to the proven effectiveness of DBS, ablative procedures were common. The pallidotomies and thalamotomies were helpful for symptomatic treatment, but concerns over side effects and the irreversibility led to DBS becoming more common (Fasano et al., 2012). However, the last few years have seen a resurgence in ablative procedures, with the research supporting symptomatic improvement (Schlesinger et al., 2015; Zaaroor et al., 2018), and now FDA approval of magnetic resonance-guided focused ultrasound ablations (Martinez-Fernández et al., 2020). This mechanism of ablative procedures negates many of the challenges with the more traditional surgical ablations, although DBS remains the more common performed procedure and has the benefit of being adjustable over time. Carbidopa/levodopa intestinal gel (marketed as Duopa in the United States) is a newer surgical approach mentioned previously that involves placement of a percutaneous jejunostomy tube and the subsequent infusion of carbidopa/levodopa intestinal gel. Both DBS and carbidopa/levodopa intestinal gel have been shown to improve off times when motor and/or nonmotor symptoms of PD return or worsen (Merola et al., 2011), and the determination of which procedure is most appropriate is based on clinical characteristics and patient preference (Volkmann et al., 2013).

Exercise has also been proven to be critical to the treatment of the motor symptoms of PD (Goodwin et al., 2008). Specific exercises that have shown benefit include tai chi (Li et al., 2012) and a special type of physical therapy called the Lee Silverman Voice Treatment-BIG program (Ebersbach et al., 2015). Rock Steady Boxing (Combs et al., 2011), a noncontact boxing program for individuals with PD, has also developed a devoted following, who report that the exercise improves their balance and results in significant comradery with other participants. Many patients have also benefited from other forms of physical therapy, speech therapy, and occupational therapy (Sturkenboom et al., 2013), which provide tailored exercises and adaptive mechanisms to improve their health and function.

B. Treatment of Nonmotor Symptoms of Parkinson Disease

The treatment of the nonmotor symptoms of PD, similar to the treatment of the motor symptoms, aims to ameliorate the functional impact of these changes. Some of the more common and debilitating nonmotor

### Table 1—Continued

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<thead>
<tr>
<th>Symptom</th>
<th>Mechanism of Action</th>
<th>Medication</th>
<th>FDA-Approved Indication for PD or Off Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>DA and NE reuptake inhibitors</td>
<td>Methylphenidate</td>
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</tr>
<tr>
<td></td>
<td>DA reuptake inhibitor</td>
<td>Modafinil</td>
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</tr>
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<td></td>
<td>Nonergot dopamine agonists</td>
<td>Rotigotine</td>
<td>Off label</td>
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<tr>
<td></td>
<td>DA precursor</td>
<td>Carbidopa/levodopa</td>
<td>Off labelb</td>
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<td>formulations</td>
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</tbody>
</table>

CR, controlled release; ER, extended release; IR, immediate release; NE, norepinephrine; VGNA, voltage-gated sodium.

bThese medications are FDA-approved for treatment of PD, but they are often used to treat these PD-related symptoms off label.
symptoms of PD are the neuropsychiatric manifestations, with depressive symptoms and anxiety affecting at least 40% of individuals with PD (Aarsland et al., 2009b). These symptoms can lead to significant disability, and patients report that depression is a bigger determinant of health-related quality of life than motor symptoms (Soh et al., 2011). Although there is reasonable evidence for the efficacy of tricyclic antidepressants for depression in PD (Liu et al., 2013), treatment of the depression and anxiety related to PD relies primarily on the selective serotonin reuptake inhibitors (Bomasang-Layno et al., 2015) as a result of their proven efficacy and superior side effect profile. Cognitive behavior therapy has also been shown to have reasonable benefits (Dobkin et al., 2011; Pachana et al., 2013). For many patients, the anxiety symptoms also correlate with the "off" time in their motor fluctuations. For these patient’s, adjustment of their DA schedule can be very beneficial. PD-related psychosis is also common, with treatment relying primarily on quetiapine or clozapine because of the prominent extrapyramidal side effects of the other antipsychotics. In addition, the FDA recently approved the novel antipsychotic pimavanserin to treat PD-related psychosis. Pimavanserin is a selective serotonin, 5-hydroxytryptamine (5-HT)2A inverse agonist with fewer side effects because of its lack of dopaminergic, adrenergic, histaminergic, or muscarinic affinity (Cummings et al., 2014). Despite these treatment options, the depression, anxiety, and psychosis associated with PD remain debilitating for many patients, and new treatments are needed to ease the burden of these symptoms.

Cognitive impairment is also common in PD, with a cumulative incidence of dementia as high as 83% by 20 years after diagnosis (Hely et al., 2008). The dementia is likely multifactorial, with cortical α-synuclein being a primary cause of the cognitive changes, as well as comorbidities including amyloid-β, and tau pathology as well as vascular pathology (Biundo et al., 2016). Individuals with PD- and AD-type pathology have a more rapid disease course and earlier mortality than those with PD pathology alone (Irwin et al., 2017), and significant research efforts are going into identification of these individuals with multiple pathologies compared with PD pathology alone (Das et al., 2019; Wilson et al., 2020). Treatment of PD dementia is of limited benefit and currently consists of behavioral measures, management of contributors such as depression, and the medications approved for Alzheimer disease, including cholinesterase inhibitors or memantine (Meng et al., 2019).

Autonomic nervous system changes are also common in later-stage PD (Cersosimo and Benarroch, 2012), in part because of the autonomic nervous system changes and in part because of the medication side effects, such as levodopa lowering blood pressure. For individuals with orthostatic hypotension, nonpharmacologic treatments including increased hydration and compression stockings and increasing salt intake are often tried first. Although these measures were shown to not have improved outcomes (Schoffer et al., 2007), they may indeed have benefits (Sánchez-Ferro et al., 2013). The usual pharmacologic treatments are midodrine, fludrocortisone, and droxidopa, the latter of which was FDA-approved more recently and is specific for treatment of orthostasis in PD. These medications act on the adrenergic receptors, inflammatory cytokines, and peripheral arterial and venous vasoconstriction (Seppi et al., 2011). They are also associated with supine hypertension, although droxidopa is supposed to have less of an instance of that particular complication. Occasionally, pyridostigmine may be beneficial in certain patients with neurogenic orthostatic hypotension, and it has a lower propensity to induce supine hypertension (Low and Singer, 2008). For urinary urgency, frequency, and nocturia, often with accompanying bladder spasms, anticholinergics have been found to be reasonably effective, although they may have cognitive side effects, and some patients may benefit from botulinum toxin injections into the bladder muscle (Giannantoni et al., 2009). Botulinum toxin has also been used in controlling motor symptoms in PD, including dystonias and levodopa-induced dyskinesias, and pilot studies have also used botulinum toxin for freezing (Camargo and Teive, 2019).

Pain is also common in PD and may be due to the motor fluctuations, dyskinesias, off medication times, and central limb pain (Chaudhuri and Schapira, 2009), as well as a neuropathy, which is common in PD (Rajabally and Martey, 2011). The treatment of many of the pain symptoms is therefore adjustment of levodopa to reduce the fluctuations and total off times, as well as some nighttime carbidopa/levodopa to assist with the pain at night (Chaudhuri and Schapira, 2009). Gabapentin and other neuropathy treatments are also useful for the neuropathic pain. Fatigue is another frequent concern among patients with PD. Treatment of fatigue involves first a comprehensive assessment of the etiology of the fatigue, including a good sleep history, as well as an assessment of additional contributors to fatigue, including depression and other medical comorbidities such as pulmonary and cardiac function changes. Treatment can then be targeted at the etiology and general work toward improvements in endurance.

III. Challenges to the Identification of Disease-Modifying Therapies

The lack of disease-modifying therapies is due to both the significant work required to elucidate the pathophysiology of PD and the significant hurdles
translating the basic science and preclinical and clinical knowledge into effective therapeutics. First and foremost, the phenotypic and likely pathophysiologic heterogeneity of the disease means that a therapy that is potentially efficacious for one subset of the disease is drowned out by its lack of effect on other disease subsets. Importantly, if the potential disease-modifying therapy had a large effect on some or all PD pathophysiology pathways, the heterogeneity of the disease would not cause as many challenges for determining the efficacy of the therapeutic (Espay et al., 2020a). In other words, this heterogeneity is an issue because the effect size of the therapeutics tested thus far is either small or nonexistent.

There are also significant challenges related to study design. Since PD is a slowly progressive disease, study designs must follow patients for enough time for them to demonstrate clinical changes. In the absence of a large disease modification or even disease reversal of the therapy, we would anticipate patients in both the therapeutic and placebo arms to be clinically worse at the end than at the beginning of a trial. We therefore have to compare relative differences in disease progression. Different trial designs, including a delayed-start design, have been used to try to overcome this issue, but it remains a challenge in all trials (Espay et al., 2020b). Again, the known heterogeneity of PD and its rate of progression will complicate this calculation.

Finally, clinical trials rise and fall based on the quality of their outcome measurements. At this time, the primary motor outcome in every PD study is the Unified Parkinson’s Disease Rating Scale (UPDRS) or the more recent Movement Disorder Society-UPDRS (MDS-UPDRS). The MDS-UPDRS relies on patient responses and physician assessments to quantify a patient’s nonmotor and motor deficits. Although there is significant literature validating the tool and an online course that administrators of the tool need to pass, it remains subjective. This subjectivity is made even more challenging in PD by the motor fluctuations of the disease—patients’ MDS-UPDRS scores may vary widely within even an hour of testing (Evers et al., 2019). Significant research identifying more objective clinical outcomes with the use of wearables and other technologies is ongoing (Godinho et al., 2016; Mancini and Horak, 2016). Dividing patients into clinical phenotypes, with presumptively similar underlying pathophysiologies, would significantly improve progress in clinical trials. Biomarkers would make a fundamental difference in identification of a disease-modifying therapy by allowing for the stratification of patients with PD into different pathophysiologic categories and would provide objective outcome measurements and measures of medication target engagement (Chen-Plotkin et al., 2018). Despite these challenges, there are a number of agents that are in clinical trials (Table 2).

IV. Biomarkers

A biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic response to a therapeutic intervention” (Biomarkers Definitions Working Group, 2001). A classic example of a disease biomarker is fasting blood glucose levels for diabetes mellitus—blood glucose levels diagnose diabetes, track disease progression, and monitor response to therapeutics. An ideal biomarker for PD would be similar. A biomarker for PD would be a similarly objective measurement that allows us to diagnose PD, follow disease progression, and monitor response to therapeutics. In practice, it is likely that we will need a collection of different compounds for each of these types of biomarkers. In addition, given the phenotypic heterogeneity of PD, different phenotypes of PD may require different markers. A PD biomarker marker may be biofluid or biochemical, clinical, genetic, or imaging-based (Chen-Plotkin et al., 2018).

One of the many challenges to biomarker identification is the variability of the methodology used to collect and measure the marker. There are many large research programs within PD that are seeking to address this challenge through the standardization of biofluid acquisition protocols. The Harvard Biomarker Study, established in 2008 (Mohammadi, 2013), developed a longitudinal biobank of clinical data with associated blood, cerebrospinal fluid (CSF), RNA, DNA, and ultimately autopsy tissue. Its primary goal is to identify biomarkers for PD and other neurodegenerative diseases. The Michael J. Fox Foundation’s Parkinson’s Progression Markers Initiative (PPMI) began in 2010 and has enrolled more than 1000 participants in the many different subgroups of the study (Simuni et al., 2020b; Weintraub et al., 2020). PPMI seeks to identify progression markers of PD and serves as a validation cohort for biomarkers discovered through other research studies, including the Harvard Biomarker Study and the newer studies BioFIND and the Parkinson’s Disease Biomarker Program (PDBP) (Rosenthal et al., 2016). The BioFIND study, funded by the Michael J. Fox Foundation and the National Institute of Neurologic Diseases and Stroke (NINDS), enrolled individuals with moderate-stage PD in a cross-sectional, case-control study and included blood and CSF collection. The PDBP, also funded by NINDS, is a longitudinal biofluid collection from individuals with all stages of PD and controls as well as some atypical parkinsonism patients. Biofluids from each of these investigations are submitted to a central repository, and qualified researchers can apply through a central mechanism to use these biofluids to discover and validate new biomarkers.
<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Name of Therapeutic</th>
<th>Sponsor</th>
<th>Phase</th>
<th>End Date</th>
<th>Recruitment Status</th>
<th>Clinicaltrials.gov ID</th>
<th>Results</th>
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<td>Denali Therapeutics</td>
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<td>Biogen</td>
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(continued)
TABLE 2—Continued

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<tr>
<th>Mechanism</th>
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<th>Recruitment Status</th>
<th>Clinicaltrials.gov ID Number</th>
<th>Results*</th>
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</table>

*Ongoing studies are those that are recruiting or active; promising studies are those that will be or should be moving to the next phase of testing based on either a press release, abstract, or published journal article.


GIT, gastrointestinal tract; ASO, antisense oligonucleotide; NSC, neuronal stem cell; PR, press release; UDCA, Ursodeoxycholic acid.
A detailed review of all assessed biomarkers is beyond the scope of this manuscript, and readers are referred to other articles discussing biomarkers (Chahine et al., 2013; Espay et al., 2017; Gwinn et al., 2017; Chen-Plotkin et al., 2018; Tropea and Chen-Plotkin, 2018; Parnetti et al., 2019). We will discuss a few of the more promising protein and biofluid-based biomarkers, as these are potential targets for new therapeutics. The most frequently tested biofluid biomarker in PD is \( \alpha \)-synuclein because \( \alpha \)-synuclein is believed to be the pathologic protein associated with neuronal cell loss in PD. Total \( \alpha \)-synuclein levels have been found to be lower in the CSF of individuals with PD (Mollenhauer et al., 2010, 2017), although levels do not seem to change with increasing disease severity. Pathologic tissue concentration of \( \alpha \)-synuclein also seems to separate individuals with PD compared with controls. Gastric (Sánchez-Ferro et al., 2015; Yan et al., 2018), rectal, and colonic (Pouclet et al., 2012) biopsies demonstrated significant concentration of \( \alpha \)-synuclein in the tissue of individuals with PD compared with controls and may be reasonable biomarkers. \( \alpha \)-Synuclein has also been found in the skin (Zange et al., 2015) and peripheral autonomic nerve fibers that innervate the heart (Iwanaga et al., 1999), abdominopelvic organs (Minguez-Castellanos et al., 2007), and the paraspinal sympathetic ganglia and endocrine organs (Beach et al., 2010). In addition, presence of post-translationally modified \( \alpha \)-synuclein (including phosphoserine 129 (Wang et al., 2012c) and phosphotyrosine 39 in the CSF (Na et al., 2020) and blood) may separate individuals with PD from controls (Vicente Miranda et al., 2017), and skin nerve fibers differentiate between PD and multiple system atrophy (Zange et al., 2015). Another promising marker for PD is the DJ-1 protein. DJ-1 likely has many roles, including inhibiting \( \alpha \)-synuclein aggregation. Total DJ-1 levels are lower in the CSF of individuals with PD (Hong et al., 2010), and higher total DJ-1 levels were found to correlate with greater disease severity in the saliva of patients with PD (Masters et al., 2015). However, total DJ-1 levels in the plasma are not significantly different between those with PD and those with Alzheimer disease or controls (Shi et al., 2010), limiting DJ-1’s utility as a biomarker. Seven DJ-1 post-translationally modified isoforms in the whole blood may be biomarker candidates, and further research is forthcoming. Both \( \alpha \)-synuclein and DJ-1 are also part of protein panels that, when looked at together, had reasonable sensitivity (\( >92\% \)) and specificity (up to 60%) for both PD versus controls and PD versus some of the atypical Parkinsonism (sensitivity about 99% and specificity about 90%) (Shi et al., 2011). Other potential markers of PD diagnosis or motor progression include Apolipoprotein A1 (Qiang et al., 2013; Swanson et al., 2015), vitamin D (Ding et al., 2013), and urate levels (Ascherio et al., 2009), although none of these markers have held up under significant further scrutiny.

Biochemical markers for PD dementia include the most common markers for both PD and Alzheimer disease, specifically amyloid-\( \beta \), tau, and phospho-tau. Lower total \( \alpha \)-synuclein levels predicted preservation of cognitive function in PD (Stewart et al., 2014). Furthermore, up to one-half of individuals with PD dementia had the biomarker signature of Alzheimer disease with amyloid-\( \beta \) 42 levels reduced (Montine et al., 2010). Still, others have noted that a combination of CSF amyloid-\( \beta \) 42 as well as age, rapid eye movement behavior symptomatology, smell testing, and striatal uptake on DaTScan imaging predicted dementia within 2 years (Schrag et al., 2017). Although there are CSF-based markers for vascular injury, including most prominently e-selectin and vascular cell adhesion molecule-1 (Li et al., 2015a), neither of these have been tested in the context of PD-related cognitive impairment.

There are many other markers that are undergoing testing and validation in new cohorts. Importantly, none of the markers that we have discussed, nor any of these new markers, are ready to be used in standard clinical care.

V. Genetics of Parkinson Disease

Until the end of the last century, PD was considered a nongenetic condition that was presumably caused by synergistic environmental factors. In 1996, reporting of a family showing Mendelian segregation of PD and mapping of the associated genetic factor to chromosome 4 (PD genes (PARK1) locus) (Polymeropoulos et al., 1996) lend credence to the notion that a strong genetic component might underlie PD. The subsequent identification of an A53T mutation in the synuclein alpha (SNCA) gene encoding \( \alpha \)-synuclein (Polymeropoulos et al., 1997) further prompted a surge of interest in PD genomics that has continued to date (Kumaran and Cookson, 2015). Currently, mutations in seven genes are known to conclusively cause PD: \( \alpha \)-synuclein (SNCA), leucine-rich repeat kinase 2 (LRRK2), and vacuolar protein sorting-associated protein 35 (VPS35) are causal for autosomal dominant PD, whereas autosomal recessive forms result from loss of function of PARKIN, phosphatase and tensin-induced putative kinase 1 (PINK1), and DJ-1. Glucocerebrosidase (GBA) is causal for both autosomal dominant and autosomal recessive forms, with the latter being the most common genetic risk factor for PD (Sidransky and Lopez, 2012; Ribolli and Di Fonzo, 2019). A few other causal genes have been reported, and mutations in these genes have been found only in rare families and were not able to be replicated in larger consortium (Bandres-Ciga et al., 2020a). Their actual contribution to the disease...
etiology in the larger PD population warrants further investigation. Although these monogenic mutations known to date have been instrumental in dissecting the genetic etiology of PD, they account for less than 10% of familial PD, leaving a large proportion of PD cases unexplained. It is conceivable that in the vast majority of patients with PD, including those with a negative family history, a multifactorial inheritance underlies the disease wherein interactions between several genes, environmental factors, and gene-environment interplay all append varying layers of complexity and variability that ultimately reach a threshold to cause the disease (Gasser, 2005; Bandres-Ciga et al., 2020a). Candidate gene association studies and recent genome-wide association studies have collectively identified common variants across at least 90 independent loci that modify disease risk, with strong associations replicated for three loci—namely, SNCA, LRRK2, and GBA (Bandres-Ciga et al., 2020a). These 90 single nucleotide polymorphisms (SNPs) represent 16%–36% of the heritability of PD. The true significance of many of these loci remains to be examined, as many of the SNPs at these loci are associated with PD by virtue of being in linkage disequilibrium with a polymorphism known to alter the disease risk. As such, whether such SNPs are the actual functional variant and their relative contribution to PD pathogenesis warrants further investigation. Additionally, some of these loci contain a second risk allele exerting an effect independent of the primary allele, indicating that multiple genetic variants, even with low penetrance, can have a cumulative effect toward the risk of developing PD (Kumaran and Cookson, 2015). Consequently, the identity of the true causative genes at these loci remains ambiguous. Some loci, like Bone Marrow Stromal Cell Antigen 1 and Microtubule-Associated Protein Tau, appear to exhibit population-specific variability, indicating that population-specific differences also contribute to the genetic heterogeneity in PD (Satake et al., 2009; Simón-Sánchez et al., 2011).

Specific mutations also influence the PD phenotypes that develop. LRRK2 carriers, for example, have slower progression in their motor scores and less cognitive impairment than the general PD population (Srivatsal et al., 2015). GBA mutation carriers, including those with the E326K polymorphism, demonstrate a faster motor and cognition progression (Davis et al., 2016) and significantly more anxiety (Chahine et al., 2013) than patients with PD without those mutations. α-Synuclein triplication leads to early-onset PD, with accompanying depression, behavior disturbances, and cognitive decline (Olgiati et al., 2015). Apolipoprotein E mutation carriers have decreased cognitive performance (Mata et al., 2014).

Most PD-associated SNPs fall into noncoding regions, raising ambiguities with regard to how these variants impact nearby genes. In some cases, a single variant is associated with the expression and/or methylation of more than one gene, as observed in the case of the PD risk–associated SNP rs199347 on chromosome 7, which leads to both decreased methylation of the nearest gene, Glycoprotein non-metastatic b, and increased expression of another nearby gene, Nucleoporin like 2 (Nalls et al., 2014). This suggests that a single polymorphism could differentially regulate one or more proximally located genes, thereby heightening the disease risk. Some common variants within candidate PD genes also appear to be high-risk susceptibility factors in sporadic PD. For instance, within the SNCA gene, over 800 SNPs are reported, and nearly half of these show strong association with sporadic PD, indicating that genetic variability across the SNCA locus modulates susceptibility to PD (Edwards et al., 2010). There are at least two genetic variations that lead to increased α-synuclein and, taken together, may indicate that chronic increased α-synuclein levels could confer an increased risk of developing late-onset sporadic PD (Singleton et al., 2003). Expansion of a complex polymorphic microsatellite repeat (Rep1) located in the promoter region ~10 Kb upstream of the SNCA start site (Maraganore et al., 2006), also increases α-synuclein expression in the human brain and transgenic mouse models (Fuchs et al., 2008; Linnertz et al., 2009). In addition, duplication and triplication of the SNCA locus result in elevated α-synuclein levels that increase the penetrance and lower the age of disease onset (Hernandez et al., 2016). Mutations in LRRK2, a multimodule kinase protein, are by far the most common cause of autosomal dominant PD and also persist in patients with sporadic PD. LRRK2 is expressed in various tissues and cell types, with physiologic roles that range from maintenance of nuclear membrane integrity (Shani et al., 2019) and endolysosomal trafficking (Erb and Moore, 2020) to regulation of ciliogenesis (Steger et al., 2017), cytoskeletal and vesicle dynamics (Marku et al., 2020), and immune pathways (Wallings and Tansey, 2019). Of note, an association between increased LRRK2 kinase activity and neuronal toxicity is well documented (Smith et al., 2006; Taymans and Greggio, 2016). Unlike SNCA multiplications, gene dosage effects have not been observed with LRRK2. However, at least five PD-associated SNPs have been reported to lie upstream of the LRRK2 locus (Satake et al., 2009). It is likely that these proximally located SNPs play a role in transcriptional upregulation of LRRK2 to levels that are toxic to dopaminergic neurons. A recent screening of the 3′ untranslated region (UTR) of LRRK2 identified a PD-associated polymorphism (rs66737902) that leads to increased LRRK2...
mRNA levels, presumably through disruption of binding site for the microRNA miR-138.2 (Cardo et al., 2014), indicating that common variants located in the LRRK2 UTR could also regulate LRRK2 expression through microRNA binding.

However, besides the loci containing the Mendelian genes, many of the other loci lack candidates that can be strongly prioritized, and the functional effects of PD-associated risk variants are unclear for the most part (Kumaran and Cookson, 2015). Nevertheless, in light of the studies discussed above, it is conceivable that each of the presumed PD loci encompassing highly penetrant mutations as well as low-risk variants working through specific molecular pathways collectively have a small to moderate impact toward a lifetime risk of developing PD. Overall, this summation that several different susceptibility SNPs increase or decrease an individuals’ risk of developing PD and that these mutations change the functioning of specific PD pathways (Bandres-Ciga et al., 2020b) ties together the genetic findings and the extensive research indicating alterations in specific molecular pathways as the etiology of PD. These molecular pathways include protein misfolding and aggregation, endosomal-lysosomal dysfunction, post-translational protein modification, synaptic transmission, lipid and vitamin metabolism, immune response, and membrane and intracellular trafficking (Bandres-Ciga et al., 2020b).

The genes and proteins linked to PD underlie a complex network of molecular pathways, suggesting a common pathogenic mechanism may underlie both familial and sporadic forms. In the following sections, we present a synthetic overview of cellular mechanisms that likely participate in the demise of DA neurons and neurodegeneration in other neuronal systems in both sporadic and familial PD and the therapeutic targets that have emerged from the underlying pathways. In particular, we describe how damages to vulnerable neurons via cell-autonomous and non–cell-autonomous mechanisms could arise from cellular disturbances caused by accumulation of misfolded and aggregated proteins, mitochondrial dysfunctions, uncontrolled nitrosative and oxidative stress, loss of calcium homeostasis, alterations in protein translation, and synaptic dysfunctions, as well as the detrimental consequences of neuroinflammatory responses and the insidious spread of toxic α-synuclein species have on neuron survival. When relevant, we also discuss how the concerted actions of some of these mechanisms could promote neuronal death.

VI. Environmental Contributors to Disease Progression

There is emerging evidence that PD is the fastest growing neurologic disorder in the world as a result of the byproducts of the industrial revolution, such as heavy metals, solvent, and pesticides (Dorsey et al., 2018). Consistent with this notion is the observation that the incidence and prevalence of PD cannot simply be explained by individuals living longer as a result of advances in health care. (Dorsey et al., 2018). Dietary factors and toxin exposures are also associated with modulating disease progression. There has been significant evidence showing that higher caffeine intake is associated with decreased risk of PD development (Hamza et al., 2011; Popat et al., 2011) and that caffeine intake helps individuals with their PD symptoms, likely because of the caffeine targeting the adenosine receptor. Elevated vitamin D levels have also been found to be associated with PD development, and a number of lines of research support these findings. Researchers have found that 1) there is an increased expression of the vitamin D receptor gene among individuals with PD (Scherzer et al., 2007), 2) 25-hydroxy vitamin D3 reduces 6-hydroxydopamine–induced neurotoxicity (Wang et al., 2001), and 3) there are lower levels of total vitamin D and vitamin D metabolites among individuals with PD (Ding et al., 2013). Total vitamin D is a combination of 25-hydroxy-vitamin D2 and 25-hydroxy-vitamin D3. Although vitamin D2 is obtained from exogenous sources, most frequently in the United States from fortified milk and cereal, vitamin D3 is endogenous and obtained from conversion of cholesterol in the skin after sun exposure. Vitamin D3 levels are thought to be the primary driver of the negative association between vitamin D levels and PD development and severity, further underscoring the importance of sun exposure among individuals with PD and/or vitamin D supplementation (Ding et al., 2013).

Nicotine may also change PD risk, with some research suggesting that nicotine use is protective (Ma et al., 2017), but a subsequent open-label, blinded-endpoint study of high-dose transdermal nicotine failed to show improvement in UPDRS motor scores (Villafane et al., 2018). Others postulate that individuals who are in the prodromal phase of PD and are going to go on to develop PD are more risk averse or maybe even less sensitive to tobacco’s effects, therefore making them less likely to smoke (or to be able to quit easier) (Ritz et al., 2014). Overall, given the well-proven risks of smoking tobacco, cigarette smoking is not a good method of PD disease prevention.

There are other exposures that have been shown to be toxic and accelerate disease development. One of the more clinically important toxins is Agent Orange (2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid along with contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin). Agent Orange was used extensively by United States forces during the Vietnam war as part of wider crop destruction efforts believed to benefit United States war goals. Agent Orange
exposure has subsequently been linked to the development of numerous diseases, including likely increased risk of PD. One large multicenter case-control study found a more than 2-fold increased odds of PD among those exposed to Agent Orange (Tanner et al., 2009), but other studies found no association (Kamel et al., 2007). The exact mechanism of neurotoxicity of the chemicals in Agent Orange has not been worked out in great detail, but it is believed that 2,4-dichlorophenoxyacetic acid inhibits microtubule assembly and 2,3,7,8-tetrachlorodibenzodioxin may increase oxidative stress. Importantly, the Veterans Administration lists PD on the list of diseases due to Agent Orange exposure and therefore patients are eligible for increased Veterans Administration benefits.

There are numerous other toxins that have been shown to be associated with PD, as previously discussed, including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), paraquat, and rotenone. Polychlorinated biphenyls used as lubricants and coolants have also been associated with PD, as have specific solvents. For each of these and many other toxins, there is likely a complex interplay between the toxin and genetic influences, resulting in some individuals being more susceptible than others. For example, paraquat has been shown to be more toxic among individuals with homozygous deletions encoding glutathione S-transferase T1 (Goldman et al., 2012), and organochlorines interact with the ATP Binding Cassette Subfamily B Member 1 gene (Dutheil et al., 2010). This complex interplay of genes and environment will add to our understanding of PD pathophysiology and what preventative measures need to be taken to decrease PD risk (Goldman, 2014).

VII. Targeting Pathways that Promote Clearance of α-Synuclein

The presence of intraneuronal and cellular proteinaceous inclusions, sometimes enriched with ubiquitin, in afflicted regions of the PD brain perhaps provides the most glaring evidence of abnormal protein processing and accumulation of mutant, misfolded, or damaged intracellular proteins. Notably, α-synuclein that is prone to form amyloid-like aggregates is a major constituent of LBs and Lewy neurites in sporadic and inherited PD. α-Synuclein is widely expressed in neuronal and non-neuronal cell types in the brain. The normal functions of α-synuclein remain poorly understood. Its presynaptic localization and association with synaptic vesicles, however, indicate a role in synaptic plasticity and neurotransmission (Burre, 2015). The structural features and interacting partners of α-synuclein also suggest putative roles in fatty acid binding (Lucke et al., 2006), transcriptional regulation (Bernal-Conde et al., 2012), maintenance of mitochondrial calcium homeostasis (Cali et al., 2012), and physiologic regulation of enzymes like tyrosine hydroxylase (Perez et al., 2002) and mitochondrial ATP synthase (Ludtmann et al., 2016). The aggregation potential of α-synuclein has nevertheless gained much attention as a possible molecular cause underlying most forms of PD. Supporting this notion are observations that familial PD–associated SNCA point mutations—namely, A53T, A30P, E46K, and H50Q—all increase the propensity of α-synuclein to aggregate in vitro (Narhi et al., 1999; Li et al., 2001a; Khalaf et al., 2014). SNCA triplications also facilitate the aggregation process, likely as a result of increased protein load and consequent macromolecular crowding (Miller et al., 2004). Truncated, aggregation-prone forms and oligomer forming variants of α-synuclein promote dopaminergic neurotoxicity in vivo, implicating the aggregated species in pathogenesis (Periquet et al., 2007; Winner et al., 2011). Furthermore, a number of factors presumed to play a contributing role in sporadic PD, including oxidative and nitrosative stress, accelerate α-synuclein misfolding. Nevertheless, the presence of aggregated α-synuclein by and of itself does not prove whether protein misfolding and aggregation exhibit a causal relationship with neuronal death or represent a secondary step in the course of the disease. In fact, although overexpression of wild-type or mutant α-synuclein produces neurologic defects in mice and rats, none of these models recapitulate the entire spectrum of PD phenotypes. Of note, with the exception of invertebrate models, neither LB-like inclusions nor nigral degeneration is observable in many other transgenic α-synuclein models, and in most cases, massive overexpression of synuclein or additional stresses are required to see any effects (Dawson and Dawson, 2010). Furthermore, LBs are not common to all genetic forms of PD. Although mutations in GBA are associated with LBs and most cases of LRRK2 have α-synuclein inclusions, some LRRK2 mutations are devoid of Lewy pathology. For as many cases of PARKIN with α-synuclein inclusions, an equal number without Lewy pathology have been reported. Many transgenic α-synuclein mouse models exhibit various functional abnormalities in the nigrostriatal system in the absence of overt dopaminergic degeneration. Some of abnormalities also exhibit DA responsiveness. Accumulating evidence indicates that α-synuclein oligomers can themselves have detrimental effects on various physiologic functions that culminate in neurotoxicity. Although the underlying mechanisms of aggregate toxicity are poorly understood, one possibility is that α-synuclein aggregates composed of more heterogeneous oligomers may promote aberrant interactions by exposing flexible hydrophobic surfaces that facilitate sequestration of other cellular proteins, resulting in functional impairment (Bolognesi et al., 2010; Campioni et al., 2010). In fact, a quantitative proteomic analysis revealed that amyloid-like
aggregates tend to sequester numerous metastable proteins that occupy hub positions in cellular protein networks ranging from chromatin regulation, transcription, translation, and protein quality control and thus could impact multiple essential cellular functions (Olzscha et al., 2011). Impaired endoplasmic reticulum (ER) folding and chronic stress have been found to result from interaction and sequestration of ER chaperones by α-synuclein oligomers in the ER fractions (Colla et al., 2012). Aggregated α-synuclein also efficiently binds to subunits of the 20S proteasome complex, resulting in the selective inhibition of proteasomal activity (Linderosson et al., 2004). Such inhibition of central protein quality control and clearance mechanisms can lead to further propagation of folding defects and setting forth feed-forward mechanisms of further neuronal injury. Oligomeric intermediates of α-synuclein can also compromise the integrity of various membrane structures in the cell and cause neurotoxicity through pore-like membrane permeabilization (Kayed et al., 2004) or by destabilization of the membrane, allowing nonspecific ion transport (Danzer et al., 2007). In particular, mitochondrial membranes appear to be more vulnerable to such permeabilization effects (Stefanovic et al., 2014) and could account for the mitochondrial dysfunctions observed due to abnormal associations of α-synuclein with the mitochondria (Martin et al., 2006; Devi et al., 2008). Thus, disruptions to basic cellular functions that interface with the unique biology of dopaminergic neurons could result in multifactorial toxicity, with prolonged periods of such alterations eventually causing neuronal death (Fig. 1).

How α-synuclein aggregation–associated proteotoxicity dominates clearance mechanisms and impinges on neuronal survival is an important question to explore, particularly when not one but three protein quality control pathways—namely, the ubiquitin proteasome system, chaperone-mediated autophagy (CMA), and macroautophagy—are involved in regulating α-synuclein levels. Monomeric α-synuclein is actively degraded by all of these pathways, which also compensate each other to maintain steady-state levels of α-synuclein (Massey et al., 2006; Kaushik et al., 2008; Koga et al., 2011). However, oligomers and aggregates are primarily degraded via macroautophagy. The A30P and A53T familial PD mutations in α-synuclein inhibit CMA through inhibitory interactions with lysosome-associated membrane protein type 2a, the lysosomal receptor that is critical for substrate degradation (Cuervo et al., 2004). This could essentially impair clearance of not only α-synuclein but also other substrates targeted by CMA. Blockage of CMA also leads to compensatory activation of macroautophagy (Massey et al., 2006), which could result in inadvertent neuronal toxicity. High levels of wild-type (WT) LRRK2 or the most common pathogenic G2019S LRRK2 mutants have a similar self-perpetuating inhibitory effect on lysosome-associated membrane protein type 2a in neuronal and non-neuronal cells that impairs clearance of CMA substrates, most notable of which is α-synuclein itself. Intriguingly, mutations in LRRK2 or α-synuclein are sufficient to elicit CMA toxic effect of the other, and in the case of α-synuclein, the resultant effect favors its oligomerization into toxic species that could underlie the LB pathology observed in patients with PD with LRRK2 mutations (Orenstein et al., 2013). LRRK2 overexpression has also been observed to inhibit the proteasome (Lichtenberg et al., 2011), which could indirectly promote accumulation of α-synuclein, among other proteasomal substrates. In fact, coexpression of WT or G2019S LRRK2 in A53T α-synuclein mice causes synergistic toxicity to forebrain neurons through impairment of the proteasomal pathway and consequently accelerates the progression of α-synuclein–mediated neuropathology (Lin et al., 2009). Overexpression of WT α-synuclein also impairs macroautophagy by inhibiting autophagosome biogenesis in a Rab1a-dependent manner (Winslow et al., 2010). Such reduction in autophagy, a major route for clearance of aggregation-prone intracytoplasmic proteins, could subsequently increase the cellular concentration of such proteins, thereby augmenting their probability of aggregation. In fact, α-synuclein induces fibrillation of tau, which then promotes synergistic fibrillation of both proteins (Giasson et al., 2003), indicating that increased α-synuclein load could serve as a template and initiate feed-forward cycles of indiscriminate aggregate formation. Given the prominent role macroautophagy plays in the clearance of dysfunctional mitochondria (a process referred to as mitophagy) [reviewed in Ryan et al. (2015)], α-synuclein–mediated inhibition of macroautophagy could have far-reaching effects on mitochondrial quality control and increase neuronal susceptibility to pro–cell death insults, all of which are implicated as pathogenic processes in PD.

Further genetic evidence linking impairment of lysosomal–autophagy pathway to PD emanates from GBA, mutations in which confer increased risk for PD (Migdalska-Richards and Schapira, 2016), and the neuronal lysosomal adenosine triphosphatase ATP13A2, loss-of-function mutations in which underlie autosomal recessive forms of PD (Ramirez et al., 2006). Loss of ATP13A2 results in lysosomal deficiency and deterioration, leading to decreased clearance of autophagosomes (Dehay et al., 2012). ATP13A2 overexpression protects against α-synuclein misfolding and toxicity in yeast, worm, and neuronal models, suggesting a link between ATP13A2 and α-synuclein pathways (Gitler et al., 2009). Additionally, elevated levels of ATP13A2 are observed in surviving dopaminergic neurons in human PD brains (Ramonet et al., 2012). GBA mutations are associated with increased intracellular α-synuclein in sporadic PD cases (Murphy et al., 2014). Similar accumulations of
α-synuclein occur in mouse models harboring GBA mutations (Sardi et al., 2011; Fishbein et al., 2014) and in iPSCs derived from patients with the pathogenic GBA N370S mutation (Fernandes et al., 2016). Interestingly, α-synuclein inhibits vesicular glucocerebrosidase (GCase) trafficking from the ER-Golgi complex to the lysosomes (Mazzulli et al., 2011). It is likely that elevated levels of α-synuclein resulting from genetic variations initiate a positive-feedback loop that impairs GCase trafficking and lysosomal function, leading to further...
elevation of intracellular \( \alpha \)-synuclein levels and aggregate formation (Kumaran and Cookson, 2015). These diverse observations indicate that the lysosomal-autophagy system (ALP), when dysregulated, can contribute to PD pathogenesis. In support of this notion, pharmacological modulation of autophagy or restoration of GCCase shuttling to lysosomes in PC12 cells using rapamycin or isofagomine, respectively, has been shown to abate \( \alpha \)-synuclein accumulation, even when mutant GBA is overexpressed (Cullen et al., 2011), highlighting the potential of therapeutic strategies targeting the ALP.

Accumulating evidence also indicates that defective protein sorting within vesicular compartments plays pathogenic roles in PD. Notably, a pathogenic link between retromer function and PD is emerging and is exemplified by linkage of rare mutations in the retromer complex protein VPS35 to rare familial forms of PD (Vilarino-Güell et al., 2011; Zimprich et al., 2011). Retromer complexes mediate the retrograde transport of cargo from the endosome to the trans-Golgi network—most prominently, lysosomal endopeptidases like cathepsin-D, the dominant endosomal-lysosomal protease for \( \alpha \)-synuclein processing (Miura et al., 2014). VPS35 dysfunction could thus decrease the active form of such lytic enzymes in the lysosomes, leading to neurotoxic accumulations of \( \alpha \)-synuclein. Indeed, augmenting retromer dysfunction through VPS35 overexpression alleviates \( \alpha \)-synuclein toxicity in mouse models (Zavodszy et al., 2014) and also rescues lysosomal deficits in LRRK2 mutant Drosophila and rat neuronal culture models (MacLeod et al., 2013; Linhart et al., 2014). LRRK2’s role within the endolysosomal compartments is mediated through interactions with the guanosine triphosphatase (GTPase) Rab7L1, one of the five genes within the PARK16 nonfamilial PD risk–associated locus. LRRK2 mutations or polymorphisms in the PARK16 locus lead to the formation of truncated Rab7L1 protein and defective Rab7L1-LRRK2 pathway, resulting in abnormal lysosomal structures (MacLeod et al., 2013). Auxilin-2 (also known as Cyclin G-associated kinase), encoded by DNAJ Heat Shock Protein Family (Hsp40) Member C6, yet another gene implicated in familial PD, may also contribute to this pathway by facilitating clathrin-mediated trafficking between the Golgi and lysosomes (Edvardson et al., 2012). Thus, multiple PD genes and risk factors appear to interface upon pathways that regulate vesicular transport and initiation of autophagy. Of relevance to the disease pathology, defective retromer function appears to be the proximal site of action for these proteins, indicating that inducing retromer function could offer therapeutic benefit.

Post-translational modifications of PD-linked proteins also appear to impact its autophagic clearance. In the case of \( \alpha \)-synuclein, Polo-like kinase 2–mediated phosphorylation of \( \alpha \)-synuclein at (S129) facilitates its autophagic clearance (Inglis et al., 2009; Oueslati et al., 2013), whereas phosphorylation of \( \alpha \)-synuclein at Y39 by the ABL Proto-Oncogene 1, Non-Receptor Tyrosine Kinase (c-Abl) increases its propensity to aggregate (Mahul-Mellier et al., 2014; Brahmachari et al., 2016). Of note, phospho-Y39 \( \alpha \)-synuclein accumulation is observed in substantia nigra (SN) and striatum in post-mortem PD brains as well as in Lewy bodies of patients with PD. Moreover, c-Abl overexpression in wild-type mice leads to DA neuron degeneration with concomitant elevation of phospho-Y39 \( \alpha \)-synuclein and pathogenic \( \alpha \)-synuclein accumulation. Although c-Abl overexpression accelerates behavioral abnormalities and pathology of human A53T transgenic mice, these defects are ameliorated by c-Abl knockout, indicative of the pathogenic contribution of c-Abl to \( \alpha \)-synuclein neurodegeneration (Brahmachari et al., 2016). Various oxidative, nitrosative, and dopaminergic stressors implicated in PD are known to activate c-Abl (Sun et al., 2000; Ko et al., 2010; Imam et al., 2011) and could in part explain the nigrostriatal neuronal injury and pathogenic process underlying sporadic forms of PD. These studies indicate that selective inhibition of c-Abl could be neuroprotective. Indeed, the c-Abl inhibitor, nilotinib, ameliorates striatal motor deficits in an MPTP mouse model of PD (Tanabe et al., 2014) and DA neuron loss in a viral mouse model of \( \alpha \)-synuclein toxicity (Hebron et al., 2013). Early neuroinflammatory responses to \( \alpha \)-synuclein also appear to be modulated by c-Abl inhibitors (Hebron et al., 2014). However, a major caveat to be considered with these studies is that the inhibitors used are nonspecific kinase inhibitors with broad activity profiles toward a wide range of kinases and are thus likely to have unforeseen side effects with substantial toxicity. The presence of markers of c-Abl activation like phospho-Y39 \( \alpha \)-synuclein in the PD brain nevertheless provide a rationale for developing specific c-Abl inhibitors with better safety records. By preventing \( \alpha \)-synuclein Y39 phosphorylation and its propensity to aggregate, therapeutics based on c-Abl inhibition could promote \( \alpha \)-synuclein clearance and thereby counteract toxicity associated with \( \alpha \)-synucleinopathies.

In a parallel pathway, stress-induced activation of c-Abl also has an inhibitory effect on the multifunctional E3 ligase PARKIN (Ko et al., 2010; Imam et al., 2011), mutations in which are the most common cause of autosomal recessive PD (Khan et al., 2003). Different ubiquitin lysine linkages are employed by Parkin to regulate protein homeostasis. Although Parkin-mediated polyubiquitination of substrates via lysine 63 promotes inclusion body formation and autophagy (Olzmann and Chin, 2008; Chin et al., 2010), lysine 48 linkages promote proteasomal clearance of Parkin substrates (Dawson et al., 2010). Tyrosine phosphorylation of Parkin by c-Abl inactivates this catalytic function of Parkin, resulting in toxic
accumulation of Parkin substrates such as aminoacyltransfer RNA (tRNA) synthetase–interacting multifunctional protein type 2 (AIMP2) (Corti et al., 2003; Ko et al., 2005; Lee et al., 2013b), fuse-binding protein 1 (Ko et al., 2006; McCoy et al., 2006) and Parkin interacting substrate (PARIS) (Shin et al., 2011) that cause dopaminergic neurodegeneration. Mutations in PARIS cause early onset PD (Li et al., 2021). Of note, PARIS and AIMP2 accumulate in sporadic PD, familial PD causing early onset PD (Ko et al., 2006; McCoy et al., 2006) and Parkin interactome models of neurodegeneration, underscoring their pathogenic relevance (Shin et al., 2011; Lee et al., 2013b; Brahmachari et al., 2019). Thus, modifying the phosphorylation status of Parkin by interfering with c-Abl activation provides a unique opportunity to maintain Parkin in a catalytically active state and prevent accumulation of its pathogenic substrates. In this regard, c-Abl inhibitor imatinib, INNO-406, reduces tyrosine phosphorylation of Parkin and suppresses upregulation of Parkin substrates such as fuse-binding protein 1 and AIMP2, thereby slowing PD progression (Ko et al., 2010; Imam et al., 2011, 2013). Other c-Abl inhibitors, such as nilotinib, reduce c-Abl activation and levels of PARIS in an MPTP-induced model of PD (Karuppagounder et al., 2014). Radotinib prevents pathologic a-synuclein–induced loss of DA neurons (Lee et al., 2018b). Nilotinib, imatinib, and other c-Abl inhibitors are already approved by the FDA for the treatment of chronic myelogenous leukemia and other disorders with Philadelphia chromosome translocations. Given their potentially promising mechanism of action, c-Abl inhibitors have already begun to be tested as potentially disease-modifying therapy in individuals with PD. Nilotinib, with its comparatively reasonable side effect profile, was tested the most extensively in humans. A small, open-label study of nilotinib in PD was initially promising, showing both a reasonable clinical response and a reduction in a-synuclein levels in the CSF (Pagan et al., 2016). The pilot data also indicated that nilotinib had the potential to be safe in this population. There were subsequently two randomized controlled trials of nilotinib versus placebo in patients with PD to determine its safety and potential efficacy as disease-modifying therapy in PD. One study was multisite and concluded that although nilotinib was safe in the PD population, there was not enough evidence of efficacy to warrant a phase III study (Simuni et al., 2020a). The other investigation from a single site, however, concluded that there was sufficient evidence to warrant a phase III study (Pagan et al., 2020). Therefore, although research is ongoing regarding whether nilotinib is indeed disease-modifying, the c-Abl mechanism of action warrants further investigation in humans. The development of more targeted c-Abl inhibitors would facilitate further explorations of the therapeutic utility of this pathway in PD (Dawson and Dawson, 2019). Furthermore, if markers of c-Abl activation, for instance, phosphorylation of Y245 of c-Abl, Y39 of a-synuclein, or Y143 of Parkin, are detectable in biologic fluids such as the CSF or serum of patients with PD, they could be used as biomarkers of disease progression as well as to monitor the efficacy of c-Abl inhibition–based treatments (Brahmachari et al., 2016). In fact, a recently developed enrichment method detected pY39 a-synuclein peptides in human CSF and demonstrated a significant increase in the ratio of pY39 a-synuclein to Y39 a-syn in the CSF of patients with PD (Na et al., 2020).

Although Parkin overexpression ameliorates a-synuclein toxicity in rat, Drosophila, and cellular models, so far there is no biochemical evidence indicative of a-synuclein to be a Parkin substrate, and native unmodified a-synuclein and Parkin do not appear to interact (Chung et al., 2001). Furthermore, a-synuclein toxicity and phenotype are unaffected by lack of Parkin in genetic models overexpressing WT a-synuclein in Parkin null background. This may suggest that endogenous Parkin does not modulate a-synuclein toxicity (von Coelln et al., 2006) or Parkin is inactivated in a-synuclein models through c-Abl phosphorylation of Y143 (Brahmachari et al., 2016; Brahmachari et al., 2019). Thus, the protective effects Parkin has in the various synuclein models could be related to its well documented role as a multipurpose cytoprotective agent against a variety of stress paradigms and could be mediated through as yet unclear complex mechanisms that are distinct from its endogenous physiologic functions (Dawson et al., 2010). Nevertheless, preventing tyrosine phosphorylation of Parkin could in essence maintain its cytoprotective functions that appear to counter a-synuclein toxicity. In fact, tyrosine kinase inhibition has been recently reported to increase ubiquitination of Parkin, consequently leading to increased protein solubility and stability (Lonskaya et al., 2013), which could augment the cellular availability of Parkin to mediate its neuroprotective effects. These studies together provide a rationale for testing the pharmacodynamic properties and efficacy of other brain-permeable tyrosine kinase inhibitors as potential disease-modifying therapeutic agents.

VIII. Mitochondrial Pathways to Neuroprotection

A mitochondrial axis in PD pathogenesis is exemplified by the fact that many PD genes encode proteins that localize to the mitochondria with direct or indirect link to mitochondrial homeostasis. Among these genes, PINK1 and PARKIN display the most
compelling link to mitochondrial functions, operating in common pathways to regulate multiple domains of the mitochondrial quality control process including fission/fusion dynamics, mitophagy, and trafficking of mitochondria [reviewed in Scarffe et al. (2014)] and are thus essential to the maintenance of a functional pool of healthy mitochondria. Additionally, DJ-1 localizes to the mitochondria and functions as a redox sensor and chaperone (Shendelman et al., 2004), and pathogenic mutations in α-synuclein and LRRK2 also modulate mitochondrial functions (Wallings et al., 2015; Zaltieri et al., 2015).

Several lines of evidence have implicated mitochondrial dysfunctions in PD pathogenesis (Dawson and Dawson, 2003), most notably a modest decrease (in the range of 30%) in mitochondrial electron transport chain (ETC) complex I activity in the substantia nigra pars compacta (SNpc) and frontal cortex of postmortem brains of patients with PD (Schapira et al., 1990; Parker et al., 2008). Similar complex I deficits are observed in platelets, lymphocytes (Yoshino et al., 1992), and to a lesser extent in muscle tissues from patients with PD (Taylor et al., 1994; Penn et al., 1995), indicating a systemic, low-grade inhibition of complex I activity in PD. Consistent with this, complex I inhibitors such as rotenone (Alam and Schmidt, 2002; Fleming et al., 2004) and MPTP (Langston et al., 1999; Dauer and Przedborski, 2003) cause neuropathological and behavioral changes in rodent and mouse models that are similar to human PD. Alterations to complex I activity can in turn have deleterious consequences, including reduced ATP production, increased oxidant generation, and cytochrome C release. Alterations to complex I activity can in turn reduce ATP production and increase oxidant generation and cytochrome C release, with deleterious consequences. In mitochondrial preparations from PD frontal cortex samples, mitochondrial and nuclear encoded complex I subunits exhibit oxidative damage (Keeney et al., 2006), indicating that the ensuing oxidative stress could cause disassembly or reduced stability of complex I subunits. Complex I deficiencies are also known to impact DA homeostasis. For instance, loss of the complex I subunit NADH:Ubiquinone Oxidoreductase Subunit S4 in DA neurons leads to increased turnover rates of DA in the striatum and decreased release of DA from striatal axon terminals with no observable effect on DA neuron survival (Sterky et al., 2012). Such striatal DA alterations could result from decreased vesicular uptake of DA (Caudle et al., 2007), a process that is particularly susceptible to ATP deficiency (Wimalasena, 2011). This could in turn lead to enhanced cytosolic DA metabolism, adding to neuronal oxidative stress and further compromising mitochondrial capacity.

Mitochondrial oxidative phosphorylation depends on proteins encoded by both the nuclear and mitochondrial genomes. Notably, seven of the complex I subunits are encoded by the mitochondrial DNA (mtDNA) (Schon et al., 2012). Several lines of evidence indicate that mtDNA damage and/or mutations accrued with aging or over the course of the disease impair mitochondrial respiration and potentiate nigrostriatal dysfunction. In fact, mtDNA damage and mutations resulting from oxidative stress lead to progressive respiratory deficiency (Shokolenko et al., 2009). Co-segregation of parkinsonism with polymorphisms in the human mtDNA polymerase γ that reduce mtDNA copy number is observable in several families (Gui et al., 2015). In mice, dopaminergic deletion of the mitochondrial transcription factor A, which is essential for mitochondrial transcription and mtDNA maintenance, recapitulates key parkinsonian phenotypes—namely, adult onset, progressive motor deficits, and loss of midbrain DA neurons (Ekstrand et al., 2007). The mitochondrially targeted endonuclease PstI, which induces double-stranded breaks in mtDNA, also causes progressive neuronal degeneration and striatal DA depletion when expressed in DA neurons (Pickrell et al., 2011).

Alterations in mitochondrial dynamics mediated by fission and fusion events have also been documented in several genetic PD models. Acute loss of PINK1 or Parkin in cultured mammalian cells and primary neurons causes Dynamin-Related Protein 1 (Drp1)-dependent mitochondrial fragmentation and reductions in mitochondrial membrane potential and ATP production (Exner et al., 2007; Dagda et al., 2009; Lutz et al., 2009; Sandebring et al., 2009). Mitochondrial fragmentation and depolarization are also induced by DJ-1 deficiency in cultured cells, primary neurons, patient fibroblasts, or lymphoblasts (Wang et al., 2012a). Increased kinase activity of pathogenic LRRK2 mutations is also associated with increased mitochondrial fission, an effect mediated through direct interaction of LRRK2 mutants with Drp1 (Wang et al., 2012b). Notably, blocking Drp1 function or promoting mitochondrial fusion in these systems is sufficient to attenuate mitochondrial dysfunction and neurotoxicity. Furthermore, neurotoxic molecules such as rotenone (Barsoum et al., 2006), MPP⁺ (Wang et al., 2011a), methamphetamine (Tian et al., 2009), and 6-hydroxydopamine (Gomez-Lazaro et al., 2008) damage the nigrostriatal pathway and cause mitochondrial fragmentation and neurotoxicity that can also be attenuated by inhibiting fission or increasing fusion. Blockage of fission is also neuroprotective in PINK1 knockout and MPTP mouse models and restores striatal DA release deficits observable in these animal models (Rappold et al., 2014). Genetic studies in Drosophila models, however, indicate that PINK1 and Parkin function in a linear pathway to promote mitochondrial fission rather than fusion (Deng et al., 2008; Poole et al., 2008). This discrepancy could be attributed in part to temporal differences in
morphologic analyses between the different model systems and/or the activation of different compensatory mechanisms in these models (Rappold et al., 2014). In fact, in Drosophila S2 cells, mitochondrial fragmentation occurs immediately in response to transient silencing of PINK1/Parkin function and is rapidly followed by hyperfusion (Lutz et al., 2009). It is likely that such early fission events are part of compensatory strategies induced in the absence of PINK1 or parkin to prevent irreversible cellular damage in Parkin- or PINK1-deficient cells. Nevertheless, mitochondrial fusion could subsequently be activated in an attempt to allow for complementation with functional mitochondria and thereby ameliorate the effects of defective mitochondria. Such functional complementation events could also underlie the rescue effects mediated by genetic or pharmacologic induction of mitochondrial fusion (Youle and van der Bliek, 2012). These studies collectively indicate that tipping the balance in favor of mitochondrial fusion along the nigrostriatal pathway could have therapeutic benefits.

Studies in Drosophila models show that although PINK1 or Parkin loss affects mitochondrial integrity, such defects are evident in only a subset of dopaminergic neurons, persist in diverse other cell types, and do not always correlate with DA neuron loss (Greene et al., 2003; Pesah et al., 2004; Clark et al., 2006). PINK1 or Parkin deficiency in mammalian cells is also well compensated under basal conditions, in line with PINK1- or Parkin-deficient mice, which lack the major alterations in mitochondrial morphology (Palacino et al., 2004; Kitada et al., 2007; Gispert et al., 2006). PINK1 or Parkin deficiency in mammalian cells is also well compensated under basal conditions, in line with PINK1- or Parkin-deficient mice, which lack the major alterations in mitochondrial morphology (Palacino et al., 2004; Kitada et al., 2007; Gispert et al., 2006). Despite these inconsistent observations pertaining to mitochondrial morphology, loss of PINK1 or Parkin function still cause mitochondrial dysfunction across various models, suggesting that effects on mitochondrial morphology could be secondary to other mitochondrial insults that ultimately impinge on mitochondrial functions. In support of this notion, PINK1-deficient phenotypes such as defective neurotransmitter release, ATP depletion, and loss of mitochondrial membrane potential (MMP) in Drosophila neurons are not corrected by Drp1 expression (Liu et al., 2011; Vilain et al., 2012) but by genes that restore proton motive force (Vos et al., 2012) or by the yeast internal NADH dehydrogenase 1P that can bypass electron transport in complex I in mammalian cells (Vilain et al., 2012). Comprehensive analysis of mitochondrial respiratory activity and protein abundance in PINK1 knockout fibroblasts also reveals deficiencies in complex I, III, and IV activities (Amo et al., 2014). Furthermore, loss of complex I reductase activity is also observable under resting conditions in PINK1 knockout cells and iPSCs derived from patients with PINK1 mutations in the absence of discernible effects on mitochondrial morphology leading to a decrease in MMP (Morais et al., 2014). DJ-1 knockout (KO) mice do not display nigrostriatal degeneration either (Andres-Mateos et al., 2007). However, DJ-1 KO mouse–derived brain or skeletal muscle mitochondria display increased formation of reactive oxygen species (ROS) (Andres-Mateos et al., 2007; Irrcher et al., 2010). Mitochondrial alterations observed in the absence of DJ-1 in cultured neurons and mammalian cells can also be prevented by antioxidants (Irrcher et al., 2010; Thomas et al., 2011), indicating that these mitochondrial phenotypes result from increased oxidative stress and could be attributed to DJ-1’s function as an antioxidant and an atypical peroxidoxin-like peroxidase. Together, these observations suggest that perturbations to mitochondrial dynamics are an inevitable downstream consequence of alterations in mitochondrial bioenergetics and/or membrane potential.

PINK1-dependent activation and recruitment of Parkin to mitochondria with depolarized membrane potential (Narendra et al., 2008; Narendra et al., 2010; Vives-Bauza et al., 2010) is now a well recognized route for macroautophagy of mitochondria. This process, referred to as mitophagy, is essential for removal of damaged mitochondria as part of the mitochondrial quality control process. Several advances in defining the precise mechanism of PINK1 and Parkin activation have been made in recent years and are extensively reviewed elsewhere (Exner et al., 2007; Scarffe et al., 2014; Ryan et al., 2015; Panicker et al., 2017; Ge et al., 2020). Favoring a pertinent role for mitophagy in PD pathogenesis are observations that pathogenic mutations in PINK1 or Parkin impair distinct steps in the mitophagy pathway (Geisler et al., 2010; Kawajiri et al., 2010; Lee et al., 2010c; Matsuda et al., 2010; Chan et al., 2011). The contention is that failure of this process could lead to retention of damaged mitochondria, which further contributes to cellular oxidative stress by producing ROS. However, much of the inferences on mitophagy are based on studies that employed high concentrations of chemical uncouplers to trigger mitochondrial depolarization, a paradigm that has been rather difficult to adapt to neurons. Moreover, inferences on PINK1/Parkin-mediated mitophagy have primarily emerged from studies employing immortalized tumor cell lines or mouse embryonic fibroblasts overexpressing PINK1 or Parkin. Such paradigms are unlikely to reliably model the gradual accretion of pathophysiological insults in vivo that likely lead to progressive impairment of mitochondrial functions in PD. If, in fact, a primary function of PINK1 and Parkin is to promote mitophagy, loss of either gene can be expected to result in accumulation of mitochondria. However, recent studies in wild-type mice and Drosophila using mitochondrially targeted pH-sensitive fluorescent reporters show that
mitophagy in vivo is rather constitutive and occurs in multiple other energy-demanding tissues besides the DA neurons, with loss of PINK1 or parkin minimally affecting the process (Lee et al., 2018a; McWilliams et al., 2016, 2018; Kim et al., 2019b). These observations indicate that PINK1/Parkin-mediated mitophagy, even if operational, could be a rather rare event in vivo (Sterky et al., 2011). It is likely that other compensatory mechanisms that can substitute for PINK/Parkin-dependent mitophagy are at play in neurons of higher organisms. For instance, PINK1 and Parkin may exert a subtler control over mitochondrial turnover by facilitating the generation of mitochondria-derived vesicles in response to mild oxidative stress that are then targeted for lysosomal degradation, allowing for mitochondrial repair instead of replacement (McLelland et al., 2014). PINK1 and Parkin null flies exhibit protein turnover defects, in particular, electron transport chain proteins indicative of PINK1 and Parkin regulating mitochondrial homeostasis through
mechanisms other than mitophagy (Vincow et al., 2013) (Fig. 2).

Equally important to the removal of damaged mitochondria is the generation of functional mitochondria. The process of mitochondrial biogenesis is largely regulated by peroxisome proliferator–activated receptor-γ coactivator-1α (PGC-1α), which serves as a transcriptional coactivator for numerous mitochondrial proteins and fine-tunes mitochondrial functions to meet cellular bioenergetic needs and mediate protection from oxidative injury [reviewed in Zhu et al. (2013)]. PINK1 and Parkin control activation of PGC-1α through proteasomal regulation of the pathogenic substrate PARIS, a Kruppel-associated box and zinc finger protein that transcriptionally represses PGC-1α. Acting in a linear pathway, PINK1 phosphorylates PARIS and primes it for proteasomal degradation by Parkin. Loss or inactivation of PINK1 or Parkin leads to PARIS accumulation, which negatively impacts mitochondrial biogenesis by transcriptionally repressing PGC-1α (Shin et al., 2011; Lee et al., 2017). Moreover, PARIS accumulation in Drosophila (Pirozynia et al., 2020) and mouse models of adult conditional PINK1 or Parkin knockdown recapitulates key features of PD pathogenesis, most notably, age-dependent degeneration of dopaminergic neurons (Shin et al., 2011; Stevens et al., 2015; Lee et al., 2017). Ventral midbrain neurons derived from the conditional Parkin knockout mice also exhibit marked decreases in mitochondrial number, size, and mitochondrial protein markers, indicative of defects in mitochondrial biogenesis (Stevens et al., 2015). PARIS also accumulates in sporadic PD and in patients with Parkin mutations, indicating that impairment of PGC-1α–dependent mitochondrial respiration could be a molecular mechanism for neurodegeneration in PD (Shin et al., 2011). Similar findings have been observed in human DA neurons lacking PARKIN (Kumar et al., 2020). The inactivation of Parkin, upregulation of PARIS, and downregulation of PGC-1α–dependent pathways also play a prominent role in pathologic α-synuclein models of neurodegeneration (Brahmachari et al., 2019). Maintaining Parkin’s E3 ligase activity through c-AbI inactivation is dramatically protective in these pathologic α-synuclein models of neurodegeneration via the reduction of PARIS levels and maintenance of PGC-1α levels (Brahmachari et al., 2019). Consistent with this notion is the observation that knockout of PARIS prevents the neurodegeneration induced in these pathologic α-synuclein models by maintenance of PGC-1α levels (Brahmachari et al., 2019).

The PARKIN-PARIS-PGC-1α–dependent pathways also play important roles in other models of PD (Sididi et al., 2015, 2016). Several lines of evidence support a role for PGC-1α defects in PD pathogenesis. For instance, PGC-1α levels are reduced in the SNpc of patients with PD, and PGC-1α polymorphisms impact the age of onset and risk for PD (Sardi et al., 2011). Adult conditional knockout of PGC-1α isoforms also leads to dopaminergic neurodegeneration, and brain enriched isoforms of PGC-1α are markedly reduced in human postmortem PD SN (Jiang et al., 2016). PGC-1α overexpression mitigates neurotoxicity associated with PARIS accumulation (Shin et al., 2011) and MPTP, rotenone, oxidative stress, and α-synuclein–induced neurodegeneration (Zhu et al., 2013). Further substantiating a causative role for reduced PGC-1α levels in PD pathogenesis are observations that PGC-1α responsive genes are under-represented in microdissected DA neurons from patients with PD (Zheng et al., 2010). PGC-1α regulates a wide range of nuclear genes encoding components of all five respiratory chain complexes (Scarpulla, 2008). Impaired synthesis and/or assembly of respiratory chain subunits could thus result from biogenesis defects and contribute to the reduced levels of activities of complexes I, III, and IV observed in patients with PD and the PD models discussed above. PARIS-mediated inhibition of PGC-1α has also been observed to decrease expression of transcription factor EB (TFEB), a master regulator of the autophagy-lysosomal pathway, and negatively impact mitophagy (Sididi et al., 2015). Nuclear translocation of TFEB is significantly reduced in postmortem PD brains, and TFEB targets are also reduced in SNpc of animal models (Decressac et al., 2013), indicating that PARIS/PGC-1α signaling events could be the point of convergence for mitochondrial and lysosomal biogenesis pathways, with implications for both mitochondrial homeostasis and protein turnover. Collectively, these observations indicate that alleviating biogenesis defects by augmenting PGC-1α signaling could have therapeutic benefit for PD caused by loss of PINK1 or Parkin activity (Fig. 2). It will be of interest to determine whether mitochondrial biogenesis pathways could be a point of convergence for other genetic and chemical mediators of PD pathogenesis. Nevertheless, the above studies provide a strong rationale for designing and testing small molecules to modulate endogenous pathways controlling mitochondrial content and activity so as to potentiate the mitochondrial biogenesis response. For instance, farnesol is beneficial in animal models of PD by restoring PGC-1α activity and enhancing mitochondrial biogenesis. Mechanistically this occurs through the farnesylation of PARIS, which prevents PARIS from repressing PGC-1α transcription (Jo et al., 2021). In addition to maintaining Parkin E3 ligase activity through c-AbI inhibition (Ko et al., 2010), efforts are underway to identify other Parkin activators, as well as PINK1 activators to prevent the deleterious consequences of inhibiting mitochondrial quality control (Miller and Muqit, 2019). Although markedly different mechanisms of mitochondrial dysfunctions are evident in different genetic models of PD,
the functional outcome of these processes—increased bioenergetics stress and accumulative oxidative burden—are common to all forms of PD and likely increase the preferential vulnerability of DA neurons to the degenerative process (Ryan et al., 2015).

IX. Mitochondrial-Based Therapeutic Approaches

The abundance of mechanistic information currently available on the mitochondrial functions of PD-linked proteins has identified multiple nodal points for therapeutic interventions that range from direct modulation of the mutated proteins to targeting downstream mediators of mitochondrial stress that subsequently impinge on mitochondrial functions. However, there have been many negative phase III randomized controlled trials of therapeutics that stabilize or improve mitochondrial function. One of the earliest negative studies was the Pramipexole in Patients with Early Parkinson’s Disease study, followed by the Coenzyme Q10 in Early Parkinson Disease study of high doses of coenzyme Q10, a potential mitochondrial antioxidant, and the NINDS Exploratory Trials in PD of creatinine. Pramipexole is a D2/D3 DA receptor agonist and is a commonly used medication for symptomatic PD treatment. It has potential neuroprotective effects in part from a mitochondrial-mediated antiapoptotic mechanism. (Schapira et al., 2013) The Pramipexole in Patients with Early Parkinson’s Disease study sought to determine whether pramipexole was neuroprotective. The 15-month study subsequently found that individuals who started pramipexole immediately did no better than those who started the medication 6–9 months later (Schapira et al., 2013). The Coenzyme Q10 Phase III randomized controlled trial began with significant excitement over the preclinical and phase I and II data. Specifically, one study of 80 patients with PD found that those taking higher doses of Coenzyme Q10 (CoQ10) had less of a decline in their UPDRS total scores over 16 months compared with those taking placebo (Shults et al., 2002). However, a subsequent study then found that CoQ10 treatment did not meet the predetermined outcome measure of change in total UPDRS scores after 1 year (NINDS NET-PD Investigators, 2007). Because of these discrepancies, and the need to identify disease-modifying therapy, a large double blind, placebo-controlled trial of CoQ10 in 600 participants was undertaken. This study was meant to be definitive regarding the value of CoQ10, and it was ultimately stopped early after it became clear that further treatment was futile (Beal et al., 2014). The attempts to determine whether creatine is disease-modifying followed a similar course as pramipexole and CoQ10, with very promising preliminary data (Parker et al., 1989; Schapira et al., 1990; Krige et al., 1992; Matthews et al., 1999; Klivenyi et al., 2003) and then a subsequently disappointing large, randomized controlled trial (Kieburtz et al., 2015).

Despite these negative results, many researchers remain committed to the mitochondrial hypothesis and the potential treatment of antioxidants for some patients with PD. The reduced form of CoQ10 (ubiquinol-10) was shown to improve wearing off of PD medications in a small pilot study (Yoritaka et al., 2015). In addition, a small study of 75 patients with PD with mild cognitive impairment found that combination therapy of both creatine and coQ10 significantly delayed conversion from mild cognitive impairment to dementia (Li et al., 2015b). Glutathione is another antioxidant that is being tested for disease-modifying therapy in PD. A randomized, double blind pilot study found that among 21 participants randomly assigned to intravenous glutathione or placebo, there was no significant difference in outcomes between the two groups, although the glutathione group had more improvement in the UPDRS ADL + motor scores than the placebo group ($P = 0.54$) (Hauser et al., 2009). A subsequent small investigation of intranasal glutathione showed that it was safe and well tolerated in a PD population (Mischley et al., 2015), and an additional phase IIb study of intranasal glutathione presented in abstract form demonstrated an improvement in MDS-UPDRS part III scores among individuals taking intranasal glutathione more than placebo (Mischley et al., 2017). Taken together, there is currently not enough evidence to support use of glutathione as a disease-modifying agent in PD. Further research is needed to determine its potential as a PD therapeutic molecule. Uric acid is yet another antioxidant that may be therapeutic for individuals with PD. Numerous researchers have shown that uric acid is lower in a PD population (de Lau et al., 2005; Weisskopf et al., 2007; Chen et al., 2009) and may be lower among individuals with more advanced disease (Weisskopf et al., 2007; Meammar et al., 2015). Taken together with subsequent findings that oral inosine can sufficiently elevate urate levels safely among individuals with PD (Parkinson Study Group et al., 2014), the Parkinson Study Group undertook a phase III investigation into the effectiveness of inosine as a disease-modifying therapy. The study was stopped early as a result of the failure to show disease modification, but there was some evidence that elevated inosine in women is indeed associated with slower PD disease progression (Schwarzchild et al., 2019). Put another way, there continues to be some clinical evidence supporting the mitochondrial hypothesis as a method of changing the disease course, but oral inosine is not the most effective mechanism to accomplish this goal.

The above initiatives indicate that the successful translation of mitochondria-based therapeutic approaches requires further clarification of a number of
challenging issues pertaining to mitochondrial dysfunctions. Foremost, in vivo validation of in vitro studies is needed to infer the true physiologic or pathophysiologic relevance of mitochondrial pathways implicated in the disease process. It is also vital to gain mechanistic insight into the interaction of the disease process or of the susceptibility genes with environmental factors that trigger and/or promote the neurodegenerative process. These studies would ultimately provide a rationale to target mitochondrial pathways for the development of reliable biomarkers and disease-modifying therapeutic strategies.

X. Interventions to Counter α-Synuclein Toxicity

Accumulating evidence indicates that many neurodegenerative diseases involve cell-to-cell spreading of disease-related proteins that form the hallmark lesions in their respective neurodegenerative disorders (Guo and Lee, 2014). Although such lesions were initially thought to arise in a cell-autonomous manner and be confined to selectively vulnerable brain regions, the notion that a prion-like mechanism could underlie the intercellular transmissibility of such proteins is gaining momentum. This hypothesis also fits nicely with the Braak model of PD progression (Braak et al., 2003). In the Braak model, abnormal α-synuclein forms Lewy bodies in specific, susceptible neuronal types, beginning in the peripheral and enteric nervous system and moving caudally through the brainstem, midbrain, and then eventually the cortex. Indeed, stereotypic spreading of pathologic α-synuclein from the gastrointestinal tract to the SNpc via the vagus nerve is observable in rodent models after injection of in vitro–generated α-synuclein preformed fibrils (PFFs) into the duodenum and pylorus (Kim et al., 2019a; Challis et al., 2020). Gut-to-brain propagation of pathologic α-synuclein in mice also leads to degeneration of DA neurons, reduced DA levels in the striatum, and a range of motor and nonmotor symptoms (Kim et al., 2019a; Challis et al., 2020). Neuropathological examination of α-synuclein aggregates in postmortem PD brain tissues also indicate the presence of LBs in several other regions of the brain apart from the nigra (Surmeier and Sulzer, 2013), implying that the pathogenic changes spread within the brain as the disease progresses, affecting multiple functional networks. These studies indicate that the cell-to-cell spread of α-synuclein via the extracellular milieu is a potential mechanism underlying pathologic progression in PD. This spread of the pathologic protein may go between vulnerable cell populations or between loci that are connected as part of the neuronal circuitry (Henderson et al., 2019; Henrich et al., 2020). Consistent with the impairment of multiple neural circuitry during the disease process, patients with PD describe constipation and rapid eye movement behavioral disorder that predate their motor symptoms and localize to the brainstem. After onset of motor symptoms due to SN involvement, patients may subsequently develop cognitive impairment in association with cortical involvement (Jankovic, 2008).

Even though α-synuclein is abundant in neuronal cytoplasm, a small amount of α-synuclein is, for unknown reasons, constitutively released from neuronal cells. In humans, nanomolar concentration of α-synuclein is detected in the blood plasma, brain interstitial fluid, and CSF (El-Agnaf et al., 2003, 2006). A fraction of cellular α-synuclein is partitioned into cytosolic vesicles and secreted through an unconventional exocytic pathway even from healthy mammalian cells and primary neurons in culture (Lee et al., 2005). Intriguingly, various stress conditions of proteasomal, mitochondrial, and lysosomal origin and oxidative stress increase α-synuclein secretion (Jang et al., 2010; Lee et al., 2011, 2013a) and vesicular translocation of α-synuclein. Notably, vesicular α-synuclein appears to be more prone to aggregation than cytosolic α-synuclein (Lee et al., 2005; Jang et al., 2010). Consequently, much of the α-synuclein secreted from cells under stress conditions is in oligomeric forms. Cytosolic calcium can also increase the secretion of α-synuclein, implying that neural activity can impact α-synuclein release (Emmanouilidou et al., 2010). Furthermore, neurons in culture are capable of taking up monomeric, oligomeric, or fibrillar forms of α-synuclein via endocytosis or through direct diffusion across the plasma membrane, and the transferred α-synuclein can induce death in the recipient cells (Ahn et al., 2006; Lee et al., 2008; Desplats et al., 2009; Hansen et al., 2011). In addition to interneuronal transfer, α-synuclein can also induce aggregation of endogenous α-synuclein in recipient cells, giving further credence to the notion that α-synuclein may have prion-like properties (Desplats et al., 2009). Transgenic mice overexpressing human α-synuclein exhibit transfer of the transgenic protein from host neurons to hippocampal neural stem cells grafts (Desplats et al., 2009) or to striatal grafts of dopaminergic neurons (Hansen et al., 2011). Aspects of α-synuclein transmission and aggregation can also be recapitulated in murine model systems by exogenous introduction of pathologic α-synuclein derived from diseased tissues or PFFs of α-synuclein (Volpicelli-Daley et al., 2011; Luk et al., 2012; Jones et al., 2015; Osterberg et al., 2015). The presence of miniscule quantities of aggregated or fibrillar α-synuclein in these scenarios has been demonstrated to serve as nucleation sites that seed the aggregation of endogenous α-synuclein, and this aggregation spreads along synaptically connected pathways, likely through sequential events of exocytosis and endocytosis, reminiscent of a prion-like mechanism. Moreover, α-synuclein–positive LBs and
LN5s develop in embryonic mesencephalic neurons and are associated with functional decline of the grafted dopaminergic neurons (Kordower et al., 2008a,b; Li et al., 2008), indicating that cell-cell propagation and misfolding of a-synuclein could underlie the central nervous system (CNS) spread of LBs and LN5s.

To achieve neuron-to-neuron transmission, a-synuclein must navigate within the neuron to sites conducive for interneuronal transfer, exit the originating neuron, and survive the extracellular milieu before entering the recipient neuron (Guo and Lee, 2014). Studies using microfluidic chambers show that a-synuclein can move anterogradely as well as retrogradely within a neuron, possibly via axonal transport (Freundt et al., 2012). Given the synaptic enrichment of a-synuclein and selective aggregation of a-synuclein at presynaptic terminals (Spinelli et al., 2014), trans-synaptic transmission could play crucial roles in initiating interneuronal propagation of a-synuclein. Evidence from cellular models indicates that a-synuclein is secreted to the extracellular space in association with exosomes. Exosomal a-synuclein has also been detected in human CSF (Kunadt et al., 2015), indicating that exosome-mediated release of a-synuclein is feasible in vivo. CSF exosomes from patients with PD have also been observed to induce a-synuclein oligomerization in a reporter cell line in a dose-dependent manner (Stuendl et al., 2016). Intriguingly, exosomes bearing prions facilitate prion propagation to uninfected recipient cells (Vella et al., 2007) and, when inoculated in mice, cause prion disease (Fevrier et al., 2004). Exosomes also participate in the dissemination of amyloid-β peptides of intracellular origin in Alzheimer disease (Rajendran et al., 2006), indicating that exosome-mediated exocytosis could have broader roles in the propagation of disease-related proteins within the CNS. Moreover, pharmacological and genetic inhibition of autophagy or inhibition of lysosomal function lead to a dramatic increase in exosomal release as well as increased transcellular transmission of a-synuclein (Alvarez-Erviti et al., 2011; Danzer et al., 2012) (Fig. 3). PD-linked pathogenic mutations in LRRK2 and GBA depletion that negatively impact the autophagy-lysosomal pathway also enhance a-synuclein aggregation and transmission (Kondo et al., 2011; Bae et al., 2014). Recent studies have raised the possibility that exosome secretion could serve as a novel route for rapid sequestration and removal of unwanted/toxic cellular proteins that accumulate under stress conditions (Putz et al., 2008; Yuyama et al., 2012). The misfolding-associated protein secretion that preferentially targets aberrant cytosolic proteins for secretion by using the ER-associated deubiquitylase Ubiquitin Specific Protease 19 is also observed to promote a-synuclein secretion (Lee et al., 2016). Thus, it is likely that defects in intracellular protein handling induce the preferential sorting of a-synuclein aggregates into exosomes or are targeted by misfolding-associated protein secretion in an attempt to rid the originating cell of these neurotoxic aggregates.

In the extracellular space, a-synuclein could exert cytotoxic effects while in transit. Although there are not many well structured models for underlying mechanisms of toxicity, one hypothesis is that the pore-like annular and tubular structures of oligomeric a-synuclein described in in vitro studies can disrupt membrane integrity in the neighboring cells and cause neurotoxicity (Ding et al., 2002; Lashuel et al., 2002). Another potential mode of neurotoxicity could involve neuroinflammatory responses. In fact, addition of aggregated a-synuclein to the culture medium of primary mesencephalic neuron-glial cultures causes selective dopaminergic neurotoxicity through microglial activation that initiates neuroinflammation (Zhang et al., 2005b). Preferential colocalization of microglia with aggregated a-synuclein in affected regions of the PD brain has also been noted (Croisier et al., 2005; Hirsch and Hunot, 2009). If unregulated, microglial activation could trigger the release of inflammatory mediators such as tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1, and ROS at the sites of inflammation. These chemical mediators could in turn promote aberrant protein modifications and protein misfolding in neighboring healthy neurons, thereby setting forth feed-forward cycles of neuronal damage and neuroinflammation [reviewed in Glass et al. (2010)]. Recent studies also propose that neuron-derived a-synuclein aggregates may function as chemotactants that promote directional migration of microglia toward the injured neurons via direct binding to β1-integrins (Kim et al., 2014) or the microglia receptor CD11b that leads to activation of several specific downstream proteins (Wang et al., 2015). Although such directional recruitment of microglia might temporarily benefit the CNS by removing unhealthy neurons or tissue debris, prolonged activation of microglia could nevertheless elicit chronic inflammation and inadvertently cause neuronal damage. Neuron-derived a-synuclein aggregates also serve as endogenous agonist for Toll-like receptor-2, which activates inflammatory responses in microglia (Kim et al., 2013). These observations implicate neuronal a-synuclein as a paracrine factor that mediates pathogenic interactions between neurons and glia, thereby fostering an inflammatory milieu within the brain parenchyma. As such, clearance of extracellular a-synuclein appears to underlie the beneficial effects of a-synuclein immunotherapy (Baë et al., 2012). Additionally, strategies aimed at eliminating the interaction between a-synuclein and microglia by modulating the activity of specific targets involved in the respective pathways could dampen the inflammatory responses.
in the brain and could thus be applicable to therapy. Interestingly, although neuronal uptake rates of exosomes are much lower, they are more efficiently internalized by microglia (Yuyama et al., 2012), indicating that pathogenic α-synuclein released through exosomes could subsequently be degraded by microglia. However, in the absence of optimal regulation of microglia activity, for reasons discussed above, these exosomes could still pave the way for interneuronal propagation of pathogenic α-synuclein and/or neuroinflammatory...
episodes. On a more speculative basis, it is likely that the presence of α-synuclein containing extracellular exosomes is indicative of a clearance deficit for α-synuclein in neurons. During disease progression, as neurons accumulate pathologic α-synuclein aggregates, they could be released through exosomes. As such, higher levels of exosomal α-synuclein at progressed stages of the disease could be the result of increased disease activity. During disease progression, exosomes could promote release of pathologic α-synuclein aggregates that accumulate in neurons. During progressed stages of the disease, as more and more neurons become affected, a rise in exosomal α-synuclein levels in body fluids like the CSF could serve as a potential biomarker to assess α-synuclein levels and related pathology (Stuendl et al., 2016).

Extracellular α-synuclein could gain access to recipient cells through endocytosis and trafficking through endosomal pathways (Lee et al., 2008; Hansen et al., 2011). Receptor-mediated internalization has also been shown to enable entry of α-synuclein fibrils in a clathrin-dependent manner (Ben Gedalya et al., 2009; Cheng et al., 2011; Oh et al., 2016). Recent work indicates that the neuronal receptor lymphocyte activation gene 3 protein (LAG3/CD233) mediates endocytosis of exogenous α-synuclein PFFs. As such, interneuronal transmission of α-synuclein PFFs and the associated neurotoxicity toward DA neurons is attenuated by genetic deletion of LAG3 as well as by LAG3-specific antibodies in mouse models and primary neuronal cultures (Mao et al., 2016). Additionally, α-synuclein PFFs also bind the transmembrane proteins APLP1 and neurexins (Shrivastava et al., 2015; Mao et al., 2016). Although LAG3 binding is rather specific for α-synuclein PFFs (Mao et al., 2016), Amyloid Beta Precursor Like Protein 1 (APLP1) is also known to bind oligomeric amyloid-β 42 (Laurén et al., 2009) and thus could be a general binding protein for amyloid structures. It is currently unclear which stage of the neuron-to-neuron transmission cycle mediates α-synuclein neurotoxicity. However, interfering with any stage of the cycle has the potential to block α-synuclein transmission and ameliorate the associated neurotoxicity (Jucker and Heikkenwalder, 2016). Clinical trials targeting LAG3 using antibody-mediated blockade are currently underway in patients with cancer to augment apoptosis of tumor cells as well as to promote T-cell regulatory functions [reviewed in Nguyen and Ohashi (2015)]. In this regard, the interaction between LAG3 and α-synuclein PFFs as well as the neuroprotective effects observed with LAG3 antibodies in α-synuclein mouse models raise the possibility that specific suppression of LAG3 in the brain could be efficiently translated into PD therapeutics (Fig. 3). How the other transmembrane interactors of α-synuclein PFFs modulate transmission and pathogenesis are also worth exploring, as these represent additional nodal points for therapeutic interventions aimed at blocking α-synuclein transmission. Similarly, the identity of α-synuclein receptors on other cell types, such as astrocytes and microglia, will help delineate their relative contribution to pathogenesis resulting from α-synuclein transmission.

Besides endocytosis, macropinocytosis of α-synuclein fibrils through engagement of cell surface heparan sulfate proteoglycans is also observed to facilitate cellular uptake of α-synuclein (Holmes et al., 2013). Regardless of the mode of entry, once the extraneous α-synuclein gains access to the recipient cell, a potential hurdle for the protein aggregates would be to exit vesicles (if endocytosis was the mode of entry) and, importantly, gain access to soluble cellular proteins to seed the aggregation process (Guo and Lee, 2014). Since α-synuclein is degraded via autophagy (Webb et al., 2003; Xilouri et al., 2008), endosomes packed with α-synuclein seeds may fuse with autophagosomes containing endogenous α-synuclein in route for degradation. Chaperone-mediated autophagy could also mediate delivery of endogenous α-synuclein to lysosomes containing partially digested α-synuclein of exogenous origin (Lee et al., 2014a). Of note, several lines of evidence now suggest that lysosomal function impacts initiation of aggregation from seeds of α-synuclein fibrils. In in vitro settings, multiplication of aggregates from PFFs is more rapid at pH values lower than 6 than at physiologic pH (Buell et al., 2014), suggesting that acidic environments in lysosomal compartments may favor aggregate formation. The lysosomal enzyme cathepsin B was recently shown to enhance seeding activity of exogenous α-synuclein fibrils and form early aggregates in the lysosomes (Tsuchimura et al., 2015). In neuronal cell lines, synthetic α-synuclein aggregates induce rupture of lysosomes after their endocytosis (Freeman et al., 2013). These studies indicate that, after endocytosis, macroautophagy could mediate the trafficking of α-synuclein fibrils to lysosomes that essentially serve as nucleation sites for aggregate formation. The eventual rupture of these lysosomes could plant α-synuclein aggregates in the cytosol, forming intracellular pathologic inclusions that grow in size through sequestration of soluble cytosolic proteins and/or continuous deposition of lysosome/autophagosome-derived undigested substrates as the disease progresses. Pathogenic mutations in PD-linked genes such as LRRK2, GBA, and ATP13A2 that impair lysosomal function could further facilitate α-synuclein aggregation and interneuronal propagation. In this regard, therapeutic strategies aimed at promoting late steps of ALP, for instance, improving the efficacy of autophagosome maturation and enhancing lysosomal activation and substrate digestion through increased processing of degradative lysosomal enzymes, are likely to have neuroprotective effects (Fig. 3).
The emergence of an α-synuclein transmission hypothesis has provided a viable explanation for the stereotypical spreading of neuropathology and progressive deterioration of multiple functional networks in PD. However, there are still a number of open-ended questions that should be carefully considered to be able to evaluate the applicability of a prion-like mechanism to the underlying disease process. One of the more elusive challenges for this transmission hypothesis is to define the seed. In PD research, the presence of α-synuclein containing seed has been empirically defined by showing that extracts from pathogenic tissues or preaggregated forms of in vitro–generated α-synuclein catalyze aggregate formation (Golde et al., 2013). However, in vitro–generated variants of synthetic α-synuclein fibrils may not necessarily be identical to fibrils formed in the human brain, which limits the establishment of a convincing link between aggregate forms with seeding potential and disease pathogenesis. At least two different morphologically distinct oligomeric α-synuclein aggregates are now known to exist in post-mortem PD brain tissues (Xin et al., 2015). Distinct α-synuclein strains that differ in their conformation and activity have also been observed to differ in their propensity to cross-seed tau aggregation (Guo et al., 2013), implying that conformational variants of α-synuclein can possess varied biologic activities; thus, tremendous heterogeneity likely underlies α-synucleinopathies. Future studies should therefore be aimed at isolating the pathogenic protein species originating from the diseased brain to identify the pathogenic species of α-synuclein. This requires the development of highly sensitive biochemical diagnostic procedures. In this regard, techniques like protein misfolding cyclic amplification (PMCA) have been successfully applied to the detection of α-synuclein oligomers in biologic fluids of patients with PD (Shahnawaz et al., 2017). PMCA has also been employed for detection of prions in biologic fluids (Saborio et al., 2001; Saá et al., 2006) and to gain mechanistic understanding of factors involved in prion transmission (Morales et al., 2012). Seeding-competent amyloid-β oligomers have also been detected with high precision in the CSF of patients with AD using protein misfolding cyclic amplification (PMCA) (Salvadores et al., 2014). The PMCA platform adapted for detection of α-synuclein appears to be rather specific in its detection (Shahnawaz et al., 2017), which raises the possibility that techniques like these could facilitate identification of the pathogenic α-synuclein species, which could then be subjected to detailed biochemical and biophysical analyses to characterize their transmission properties. Notably, the feasibility of detecting α-synuclein in PD biologic fluids highlights the utility of diagnostic procedures such as PMCA in monitoring disease progression and facilitates preclinical identification of patients likely to develop PD.

There are notable differences in the time course of disease manifestation in murine models of transcellular transmission and the human disease. Although the neurodegenerative changes in human PD typically take decades to manifest, neuropathological changes resulting from exogenously supplied α-synuclein aggregates are evident within months or even weeks in mouse models (Guo and Lee, 2014). Even in patients with PD with nigral implants, only 10%–15% of transplanted dopaminergic neurons exhibit Lewy pathology 15–20 years after grafting (Hallett et al., 2014). This raises doubts as to whether currently employed transmission paradigms accurately mirror the pathologic process underlying α-synuclein transmissibility. The transmission paradigm for the most part has been modeled in transgenic animals overexpressing α-synuclein and sometimes carrying disease mutations that promote protein aggregation. Even studies demonstrating cell-to-cell transmission of pathologic α-synuclein in wild-type mice (Lu et al., 2012) rely on intrastratial inoculation of α-synuclein fibrils to initiate the fibrillation and transmission process. Such direct delivery of synuclein aggregates into the brains of animal models dramatically accelerates the α-synuclein dissemination process and could obscure CNS mechanisms that facilitate cell-to-cell transmission of physiologic levels of α-synuclein in vivo over time, as would be expected under pathophysiological conditions. These caveats should be given careful consideration while gauging the applicability of the transmission hypothesis to sporadic forms of PD. It is also imperative to determine whether the transmissible species of α-synuclein is in fact the toxic species that directly causes neurodegeneration. Furthermore, we do not yet fully understand the targets of the abnormal α-synuclein spread in the brain. Based on the widespread but specific location of abnormal α-synuclein in autopsy tissues, spread may not solely be from neighboring neurons, nor can it be based solely on the neuronal network and the ensuing circuitry (Surmeier et al., 2017). Although the spread of pathologic α-synuclein is likely governed by synaptic connectivity (Henderson et al., 2019; Henrik et al., 2020), cell type and other factors determine the pattern and persistence of pathologic α-synuclein, consistent with findings in humans (Surmeier et al., 2017). Thus, there is clearly some neuronal vulnerability, perhaps based on some of the mechanisms discussed above, that leads to the spread of the abnormal α-synuclein and neuronal dysfunction and ultimately neuronal death. In light of all of these considerations, at the current juncture, it is not feasible to infer whether propagation of Lewy pathology is responsible for all neuronal dysfunction and degeneration in PD or whether the proposed mechanism could be generalized to all forms of PD. Nevertheless, research in this area has offered additional dimensions to understand the processes underlying the
onset and progression of PD and has helped identify commonalities with other CNS maladies characterized by propagation of the associated pathogenic protein, providing critical insights needed to accelerate therapeutic discovery.

Studies of the transmissible property of α-synuclein have important therapeutic implications. Cell-based replacement therapies to replace degenerating DA neurons are emerging as strategies to restore DA delivery and abate the debilitating symptoms in PD. However, findings from the transmission studies indicate that these approaches, when used alone, may not be successful, as the implanted tissues eventually develop Lewy pathology owing to α-synuclein transmission from host tissues. The prevalence of extracellular α-synuclein raises the possibility that strategies that promote enhanced clearance of extracellular α-synuclein could be beneficial. In fact, the neuroprotective effects observed with α-synuclein immunotherapy (Lee and Lee, 2016) suggest that this would be a viable option to curb the intercellular transmission of α-synuclein. However, careful consideration is required to determine whether diverse pathologic strains of α-synuclein exist, which would necessitate extensive screening for antibodies with higher binding affinity and specificity for distinct pathogenic strains. In this regard, broader applicability can be achieved with antibodies that recognize shared conformations of multiple strains, especially those with cross-seeding potential (Guo and Lee, 2014). Studies on amyloid-β passive immunotherapy show that only 0.1% of circulating antibodies manage to cross the blood-brain barrier (BBB) and reach the brain (Yu and Watts, 2013). Therefore, considerable efforts should be directed at devising strategies that promote uptake of antibodies across the BBB to maximize therapeutic effects. Work is already being done toward use of antibodies as potential therapeutic agents, with a number of pharmaceutical companies putting forth their antibody for phase I or II testing (Dawson and Dawson, 2019). Differences between the molecules include the specific peptide target within α-synuclein as well as the delivery system, with some of the molecules relying on IV infusion and some relying on subcutaneous injections. Four α-synuclein antibodies are in clinical trials—namely, PRX002, PD01a, PD03a, and BIIB054. PRX002 is an α-synuclein–specific monoclonal antibody that was found to be safe and effective in a phase I ascending-dose study of healthy control participants and led to significant reduction of free serum α-synuclein levels, reaching its desired therapeutic effect. Subsequent completion of an ascending-dose study of PRX002 in participants with PD along with a reduction in free serum α-synuclein (Jankovic et al., 2018). PD01a (Schneeberger et al., 2012) and PD03a, vaccines that target the C terminus of the α-synuclein protein, have successfully completed a phase I study that demonstrated safety and tolerability of PD01a in participants without PD (Volc et al., 2020) and PD03a in individuals with PD (Zella et al., 2019). Biogen’s molecule, BIIB054, successfully completed an ascending-dose study in healthy volunteers (Brys et al., 2017), and the subsequent phase I investigation in individuals with PD and controls found a favorable safety, tolerability, and pharmacokinetic profile to warrant further investigation (Brys et al., 2019). Biogen recently announced discontinuation of the studies of the clinical efficacy of BIIB054, as it was found not to be effective (Table 2). Other monoclonal antibodies are seeking to treat the cognitive impairment in PD. The monoclonal antibody BMS-986168 against extracellular tau was shown to be safe and reduce CSF concentrations of free extracellular tau in healthy controls (Qureshi et al., 2018). Other potential immunologic molecules are being developed in models, including an α-synuclein antigen-sensitized dendritic cell vaccine (Ugen et al., 2015) and epitope-based vaccines that target B cell antigen determinants of α-synuclein (Ghochikyan et al., 2014). Identification of other small molecules that could tightly regulate microglial activation to enhance clearance of extracellular α-synuclein also represents attractive drug candidates. Small molecules that enhance proteolytic clearance of existing aggregates and thereby interfere with their propensity to promote templated recruitment of cellular proteins are key to blocking pathogenic transcellular transmission. Since several PD-causing gene mutations and genetic risk factors are now known to directly impact lysosomal function it would be an interesting topic of investigation to elucidate how these genetic factors fit into this cell-to-cell α-synuclein transmission model and facilitate disease propagation.

Drug repurposing is yet another therapeutic strategy that can be cost-effective and potentially circumvent the safety failures associated with the development of new drugs (Olsen and Feany, 2019). Recently, a new mechanism involving poly(adenosine 5′-diphosphate-ribose) (PAR) and PARP1 (P oly ADP-ribosyltransferase 1) was described to drive α-synuclein toxicity (Kam et al., 2018). PARP1 typically modifies nuclear proteins in response to genomic stress by appending units of PAR to them. This leads to the activation of a programmed cell death pathway termed parthanatos (Fig. 4). In mouse models of sporadic PD, α-syn PFF injections activate PARP1, generating PAR and causing dopaminergic cell death through the parthanatos pathway. PAR also directly interacts with α-synuclein and accelerates its fibrillation, thereby promoting α-synuclein toxicity and spread in vivo. Knockout of PARP1 or administration of PARP1 inhibitors abrogate α-synuclein propagation and toxicity in vivo and in vivo studies (Kam et al., 2018). These observations identify PARP1 as an attractive neuroprotective target for drug repurposing because PARP1 inhibitors are
already in use for the treatment of different forms of cancer, including breast cancer gene-mutated breast cancer and chronic lymphocytic leukemia. Several PARP inhibitors, such as olaparib, ABT-888, BMN-673, rucaparib, and niraparib, have also emerged as potential candidates for PD therapeutics (Brundin and Wyse, 2018). But uncertainties about their safety profile and tolerability, especially during chronic administrations, as would be necessary for PD treatment, have hindered their progress to clinical trials. However, elevated PAR levels are detectable in CSF from patients with PD (Kam et al., 2018), which raises the possibility that PAR could serve as a biomarker to guide appropriate dosing of potential PARP inhibitors and also assess target engagement. Additional future work will likely establish the validity of CSF PAR as a potential biomarker to monitor disease progression and assess the efficacy of PARP inhibitors to prevent PD progression. Parthanatos involves translocation of nuclear PAR to the cytosol, where it binds to mitochondrial apoptosis-inducing factor (AIF), causing its release from the outer surface of mitochondria (Wang et al., 2011b). AIF then binds macrophage migration inhibitory factor (MIF), a DNA nuclease that translocates to the nucleus and fragments genomic DNA, leading to cellular demise (Wang et al., 2016). Cleavage of genomic DNA could further activate PARP1 and drive parthanatos in a feed-forward process. Accumulation of the parkin

**Fig. 4.** PAR-mediated dopaminergic cell death pathways. Hyperactivation of PARP1 and cellular accumulation of PAR lead to neuronal cell death via parthanatos. PARP1 can be activated by the accumulation of the parkin substrate AIMP2 under conditions of parkin inactivation and pathologic α-syn (α-syn PFF)–induced oxidative and/or nitrosative stress. This leads to translocation of excess PAR to the cytosol, which promotes the mitochondrial release of AIF and its interaction with MIF. Nuclear translocation of MIF causes genomic stress, which in turn may further activate PARP1 and the ensuing parthanatos-mediated cell death. In a feed-forward loop, PAR interacts with α-syn, increasing its aggregation potential and interneuronal transmission, resulting in further cellular toxicity. This figure was drawn by Noelle Burgess. Copyright JHU/ICE. Permission granted by JHU/ICE.
substrate AIMP2 also leads to PARP1 activation and dopaminergic toxicity through direct association of these proteins in the nucleus. Intriguingly, PARP1 deletion protects against dopamine neuron loss and behavioral deficits in AIMP2 transgenic mice (Lee et al., 2013b). These studies point to a pivotal role for parthanatos in dopaminergic cell death and indicate that inhibitors of PARP1 or other key regulators of parthanatos could have potential therapeutic utility. From a translation point of view, a detailed dissection of the molecular pathways mediating the release and uptake of α-synuclein, factors that promote misfolding of α-synuclein thereby increasing its transmission and aggregation probability, and identification of the neurotoxic transmissible species of α-synuclein will provide more definitive answers and aid the development of effective, preventive neurotherapeutics for PD associated with α-synuclein pathology.

XI. Therapeutic Avenues from Neuroinflammatory Pathways

A wealth of new information has emerged implicating neuroinflammation within specific brain regions in the cascade of events contributing to nigrostriatal neurodegeneration. Some of the data are based on epidemiologic studies that found a possible effect of anti-inflammatory medications toward risk of developing PD (Gagne and Power, 2010). The persistence of inflammatory processes in PD is evidenced by the presence of activated microglia, accumulation of proinflammatory cytokines (Mogi et al., 1996; Hirsch et al., 2003), and oxidative damage to proteins in the CSF and SNpc from post-mortem PD brain tissues and most experimental models of PD (McNaught and Jenner, 1999; Herrera et al., 2000; Irvani et al., 2005). Genome-wide association studies also link polymorphisms in the human leukocyte antigen (HLA) region harboring immune response genes to sporadic and late-onset PD (Hamza et al., 2010; Simón-Sánchez et al., 2011; Ahmed et al., 2012; Wissmann et al., 2013). Population-specific noncoding variants in the HLA locus (Ran et al., 2013; Ma et al., 2015) influence the expression of Human Leukocyte Antigen – DR isotype (HLA-DR) and Human Leukocyte Antigen – DQ isotype (HLA-DQ), major histocompatibility class (MHC)-II molecules that mediate microglial antigen presentation to elicit T-cell responses (Hamza et al., 2010). Elevated HLA-DR levels are observed in the brain (McGeer et al., 1988) and CSF of individuals with PD (Fiszer et al., 1994). The sustained presence of reactive HLA-DR-positive microglia has also been observed in the SN of patients with PD (McGeer et al., 1988) as well as in animals (McGeer et al., 2003) and humans (Langston et al., 1999) affected with MPTP parkinsonism. In vivo imaging using the tracer [11C]DPA713 in positron emission tomography scans indicates global microglial activation in the PD brain even during the early stages of the disease (Terada et al., 2016). Widespread microglial activation has also been observed in patients with idiopathic PD in early and late stages of the disease, and growing evidence suggests that this activation does not correlate with clinical disease severity (Gerhard et al., 2006). Rather, these studies suggest that activation of microglia could be an early event in the disease process occurring in parallel with the loss of dopaminergic terminals. Once stimulated, activated microglia could remain primed and poised to robustly respond to subsequent stimuli (Tansey and Goldberg, 2010). However, excessive activation of microglia can itself contribute to oxidative stress through continued production of cytokines and other inflammatory mediators that increase ROS with detrimental effects in vulnerable neuronal populations. Infiltration of activated microglia could also be promoted by chemoattractants released by dying neurons to remove neuronal debris. The respiratory bursts that accompany microglial phagocytic activities could nevertheless increase oxidative stress for the remaining neuron populations (Tansey and Goldberg, 2010). In support of this notion, a microarray study of PD and control brains revealed that genes encoding proinflammatory cytokines and subunits of the mitochondrial electron transport chain are induced in the lateral tier region of PD SN, which also exhibits decreased expression of several glutathione-related genes. A direct inference of such differential mRNA profiles is that inflammation-induced oxidative stress could potenti ate mitochondrial dysfunction and thereby increase the vulnerability of the SN lateral tier region, which is observed to be more prone to neurodegeneration (Duke et al., 2007). Furthermore, activated microglia positive for the proinflammatory cytokines TNF-α and IL-6 are detectable in end-stage PD at post mortem (Imamura et al., 2003), indicating that, once activated, microglia could continue to promote disease progression.

Among factors that could potentially trigger microglial activation in PD are damaged neurons, environmental toxins, and extracellular α-synuclein. Substantial evidence indicates a bidirectional relationship between α-synuclein oligomerization and ROS generation (Esteves et al., 2009; Deas et al., 2016), and a potential route by which α-synuclein accumulation leads to oxidative stress is through microglial activation (Fig. 5). In fact, α-synuclein–mediated microglial activation has been demonstrated in rat primary cultures (Lee et al., 2010a), primary microglial cultures (Su et al., 2008; Hu et al., 2016), and the monocytic THP-1 cell line after treatment with exogenous α-synuclein (Klegeris et al., 2008). In vivo, α-synuclein released from nigrostriatal DA neurons could activate microglia via direct interaction, resulting in phagocytosis (Lee et al., 2008; Park et al., 2008) and/or receptor-mediated activation (Stefanova et al., 2011; Fellner et al., 2013; Kim et al., 2013).
Fig. 5. Neuroinflammatory mechanisms underlying dopaminergic toxicity. Toxic forms of α-synuclein released from nigrostriatal dopamine and other susceptible neurons activate microglia that can be detrimental to dopaminergic neurons through various pathways. 1) Activated microglia can phagocytose and degrade neurons. 2) Cytokine release from activated microglia promotes microglial clustering around dopamine neurons, triggering self-perpetuating cycles of chronic neuroinflammation and eventually resulting in cell death. 3) Activation of the NLRP3 inflammasome by α-synuclein, mitochondrial damage, and ROS also leads to neurotoxic inflammatory responses. 4) Activated microglia can also elicit CD8+ cytotoxic T-cell response by inducing dopaminergic expression of MHC-I molecules that aid in antigen presentation. 5) Inflammatory activation of microglia can subsequently activate astrocytes and their upregulation of NF-κB responsive proinflammatory genes that lead to NO and ROS production. 6) Activated microglia also induce a subtype of reactive neurotoxic astrocytes, termed A1 astrocytes, by secreting IL-1α, TNF-α, and C1q. Transfer of α-synuclein from diseased neurons to surrounding astrocytes can also lead to induction of proinflammatory gene expression. Signaling pathways within reactive microglia and astrocytes thus hold therapeutic potential. These include blocking the formation of activated A1 astrocytes via microglia formation of IL-1α, TNF-α, and C1q. Targeting the orphan nuclear receptor Nurr1 (NR4A2) to promote anti-inflammatory effects in microglia and astrocytes may be beneficial. Blockage of IL-1β, soluble TNF, and other cytokines or iNOS production that stem from microglial activation also holds promise as therapy. Regulation of NLRP3 inflammasome activity could counter neuroinflammatory responses. TCR, T-cell receptor. This figure was drawn by I-Hsun Wu. Copyright JHU/ICE. Permission granted by JHU/ICE.
Increased α-synuclein release in response to neural activity (Fortin et al., 2005; Emmanouilidou et al., 2010) and via endoplasmic reticulum/Golgi-independent exocytosis has been observed (Lee et al., 2005), making extracellular α-synuclein available for direct interactions with microglia. In support of this view, early microglial activation has been demonstrated in murine models of α-synuclein overexpression (Su et al., 2008; Theodore et al., 2008; Watson et al., 2012). Microglial activation also appears to depend on the specific aggregation state of α-synuclein, with fibrillar forms more prone to induce the production and secretion of proinflammatory cytokines in microglial-like BV2 cells (Hoffmann et al., 2016) and enhance dopaminergic neurodegeneration in primary mesencephalic neuron-glia cultures (Zhang et al., 2007). Although both WT and mutant α-synuclein are implicated in receptor-mediated activation of microglia, the A30P and A53T forms induce a more robust and sustained oxidative stress environment in mixed neuron-glia cultures (Zhang et al., 2007; Jiang et al., 2015), suggesting that the enhanced dopaminergic neurotoxicity induced by mutant α-synucleins could, in part, be attributed to their differential activation of microglial responses. These studies show that microglial activation represents a non–cell-autonomous mechanism by which neuronal α-synuclein could cause neurodegeneration wherein microglia serve to amplify the detrimental effects of extracellular α-synuclein by triggering neurotoxic inflammatory responses. ROS emanating from α-synuclein–mediated microglial activation are especially consequential to DA neurons, given their susceptibility to mitochondrial dysfunctions, and may well tip the balance between life and death for these neurons. The microglial response to α-synuclein accumulation could also trigger self-perpetuating cycles of chronic neuroinflammation that promote further microglial clustering around DA neurons, resulting in irreversible neuronal dysfunction and eventual cell death through cytokine release and ROS production. Indeed, α-synuclein aggregates have been observed to serve as chemoattractants and promote directional migration of microglia in rat primary neuron enriched cultures by activating integrin CD11b-dependent signaling events, leading to the production of H2O2, which in turn drives microglial migration, likely toward neurons releasing α-synuclein (Wang et al., 2015). Furthermore, β1-integrin signaling also promotes α-synuclein–induced microglial recruitment to the affected regions of the brain, where high levels of extracellular α-synuclein and its oligomeric forms likely persist (Kim et al., 2014). Thus, pathologic potentiation between α-synuclein and activated microglia could lead to loss of dopaminergic neurons in PD.

Extracellular α-synuclein has also been observed to induce expression of the proinflammatory cytokine IL-1β in peripheral immune cells (Codolo et al., 2013) through activation of the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome. This cytosolic protein complex responds to infectious microbes and molecules derived from host proteins and induces inflammation through regulated activation of caspase-1 (Guo et al., 2015) (Fig. 5). Chronic expression of exogenous IL-1β in the SN leads to DA neuron loss in a rat model of PD (Ferrari et al., 2006). Although both monomeric and fibrillar forms of α-synuclein induce pro–IL-1β expression via Toll-like receptor 2 signaling in human primary monocytes, only fibrillar α-synuclein fully activates monocyte NLRP3 inflammasome and leads to induction of caspase-1 activation and mature IL-1β production (Codolo et al., 2013). MPTP mouse models of PD deficient for NLRP3 do not develop PD phenotypes (Yan et al., 2015), and caspase-1 inhibition is also neuroprotective in lipopolysaccharide (LPS) and 6-OHDA rat models of PD (Mao et al., 2017), indicative of a link between the NLRP3 inflammasome and PD. Besides α-synuclein, increased mitochondrial ROS generated from varied sources, including hypoxia, increased metabolic activity, or mitochondrial membrane damage, also activate the NLRP3 inflammasome. Indeed, ROS resulting from inhibition of complex I or III of the mitochondrial respiratory chain, as well as the presence of damaged, ROS-generating mitochondria under conditions of mitophagy inhibition, cause unprompted NLRP3 inflammasome activation and increased IL-1β in the human THP-1 macrophage cell line (Zhou et al., 2011). During inflammatory events, discrete populations of exogenous, blood-borne monocytes are recruited to the CNS by traversing the BBB and contribute to resolution of the inflammatory episode by clearing neuronal debris and extracellular material. In fact, increased permeability of the BBB is evident in PD, allowing peripheral immune cells to gain access to the brain parenchyma (Kortekaas et al., 2005). Indeed, MPTP intoxication in mice leads to transient and global increases in the infiltration of bone marrow–derived cells into the CNS parenchyma (Kokovay and Cunningham, 2005; Rodriguez et al., 2007). However, bone marrow–derived cells that localize to areas of nigrostriatal degeneration exhibit increased expression of the inducible form of nitric oxide synthase (iNOS) (Kokovay and Cunningham, 2005), indicating that the reparative process could in itself increase the oxidative burden to a point that is detrimental to the surrounding neurons. Peripheral monocytes from patients with PD also exhibit transcriptional upregulation of inflammatory pathways that correlate with disease severity (Grozdanov et al., 2014) and thus display pathologic hyperactivity. These studies exemplify a scenario wherein peripheral immune cells in PD are likely primed to be in a state of inflammatory predisposition that allows them to respond to “secondary hits” in the form of environmental stimulants or
CNS-derived factors by hyperactivity that nevertheless contributes to the inflammatory load in the CNS and neurotoxicity. As such, strategies to fine-tune proinflammatory pathways in peripheral immune cells could reduce the CNS disease burden.

Multiple lines of evidence indicate that proinflammatory/neurotoxic cytokines and chemokines secreted by activated microglia and monocytes in PD brains disrupt the BBB, attracting lymphocytes to the site of neuronal injury (Mosley et al., 2012). Aberrant brain proteins that leak into the lymphatic system as a consequence of nigrostriatal injury could also activate lymphocytes and mobilize them to infiltrate the CNS. Indeed, nitrotyrosine-modified α-synuclein draining into the lymphatics from the CNS induce macrophage activation and T-cell responses that elicit profound neurodegeneration in MPTP-intoxicated mice (Benner et al., 2008). T helper 1 or T helper 17 effector T cells specific for α-synuclein are also observed to enter the brain during MPTP toxicity and contribute to the inflammatory phenotype in the SN (Reynolds et al., 2010). Studies demonstrating the involvement of CD4+ T-cell population and microglial MHC-II signaling complex in exacerbating dopaminergic degeneration in MPTP (Brochard et al., 2009) and α-synuclein mouse models (Harms et al., 2013), respectively, further reinforce a role for infiltrating T cells and subsequent cytokine release in propagating the inflammatory process. Catecholamine neurons including the SN dopaminergic neurons can themselves elicit a CD8+ cytotoxic T-cell response by virtue of expressing MHC-I molecules that aid in antigen presentation by these cells. In fact, activation of microglia by neuromelanin, α-synuclein, elevated cytosolic DA, and/or oxidative stress can induce neuronal MHC-I in murine models. Chronic exposure to l-dihydroxyphenylalanine, the DA precursor, also induces MHC-I in murine SN dopaminergic neurons. leading to activation of cytotoxic T lymphocytes that target and kill the MHC-I–expressing SN dopaminergic neurons (Cebrian et al., 2014) (Fig. 5). Peptides derived from two regions of α-synuclein (Y39 and S129) also serve as antigenic peptides and elicit cytotoxic and helper T-cell responses in patients with PD through both MHC class I and class II restricted components (Sulzer et al., 2017). Proteolytic processing of α-synuclein has been observed to impact its aggregation potential and intracellular clearance (Dufy et al., 2007; Kasai et al., 2008). A proinflammatory component also underlies PD pathogenesis associated with loss of PINK1 or Parkin function. In the absence of these proteins, MHC class I molecules present high levels of mitochondrial antigens in both macrophages and dendritic cells and could trigger cytotoxic T-cell responses detrimental to dopaminergic neurons (Matheoud et al., 2016). Thus, altered degradation of α-synuclein and/or the presence of mitochondrially derived peptides could produce antigenic epitopes that subsequently induce effector T-cell–mediated responses and directly kill neurons. As the disease progresses, it is likely that T-cell responses lose their regulatory function and shift from a neuroprotective state to one with deleterious consequences. Regulatory T cells (Treg), an important subset of CD4+ T-cell populations, are known to regulate immune homeostasis by migrating to foci of inflammation and attenuating excessive inflammatory responses (Fig. 6) (Mosley et al., 2012). Adoptive transfer of Tregs in MPTP-intoxicated mice has neuroprotective effects on the nigrostriatal system (Reynolds et al., 2007). Decreased Treg levels and an increase in CD8+ T cells has also been observed in patients with PD (Baba et al., 2005). These studies raise the possibility that regulatory T-cell–based strategies could dampen the inflammatory and neurotoxic profiles in the PD brain and thereby counter the neurodegenerative process.

Sustained inflammatory activation of microglia is also implicated in the activation of adjacent astrocytes through the release of inflammatory mediators like TNF-α and IL-1β (Fig. 5). Astrocytes could in turn amplify the effect of such microglial mediators through further secretion of inflammatory neurotoxic factors. In fact, activated astrocytes can upregulate many nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) responsive proinflammatory genes upon IL-1β and TNF-α stimulation, including the iNOS and the neutrophil cytosol factor 1 genes, which are essential enzymes for NO and ROS production, respectively (Saijo et al., 2009). Activated neuroinflammatory microglia also induce a subtype of reactive astrocytes, termed activated A1 astrocytes, by secreting IL-1α, TNF-α, and complement component 1q (C1q). In contrast to normal astrocytic functions that promote synapse formation, debris clearance, and neuronal survival and outgrowth, A1 astrocytes are highly neurotoxic, substantially enriched in the SN of patients with PD, and rapidly kill neurons and mature oligodendrocytes, presumably through a secreted neurotoxin (Liddelow et al., 2017). Notably, supportive astrocytes are converted to A1 astrocytes by microglia activated by α-synuclein PFFs, and such neurotoxic conversion can be effectively blocked by glucagon-like peptide 1 receptor (GLP1R) agonism. In fact, a novel GLP1R agonist termed NLY01 was recently found to inhibit α-synuclein PFF–induced microglial activation and generation of A1 astrocytes. Importantly, NLY01 protects against dopaminergic neuronal loss and behavioral deficits in an α-synuclein PFF mouse model of sporadic PD primarily through inhibition of microglial activation, suggesting that NLY01 could have disease-modifying effects in PD (Yun et al., 2018). A large epidemiologic study of diabetic patients showed that patients on GLP1R agonists [and dipeptidyl peptidase (DPP4) inhibitors] for their diabetes had a lower rate of PD
than patients on other antidiabetic drugs lending credence to the use of GLP1R agonists (or DDP4 inhibitors) as potential disease modifying therapies in PD (Brauer et al., 2020). Neuron-derived α-synuclein aggregates can also be internalized by neighboring astrocytes, where they form glial inclusion bodies and trigger proinflammatory gene expression (Lee et al., 2010b). Accumulation of α-synuclein within astrocytes has been observed in the neocortex and striatum (Braak et al., 2007) as well as SN (Wakabayashi et al., 2000) of post-mortem PD tissue, indicative of α-synuclein transfer from diseased neurons to surrounding astrocytes. Astrocytic overexpression of A53T α-synuclein mutant in mouse models is associated with widespread astrocytosis and severe microglial activation, which contribute to midbrain dopaminergic and spinal cord motor neuron degeneration (Gu et al., 2010). Together, these studies indicate that factors that cause aberrant activation of astrocytes in the brain parenchyma could also facilitate non–cell-autonomous neurotoxicity in PD and raise the possibility that signaling pathways within reactive microglia and astrocytes could be targeted for therapeutic interventions. For instance, the orphan nuclear receptor Nurr1, Nuclear Receptor Subfamily 4 Group A Member 2 (NR4A2) has been observed to promote

![Diagram](image-url)
anti-inflammatory effects in both microglia and astrocytes by promoting clearance of NF-xB-p65 transcriptional complex from target inflammatory gene promoters in a signal-dependent manner, thereby promoting transcriptional repression of such targets (Saijo et al., 2009). Nurr1 plays a crucial role in the development and/or maintenance of dopaminergic neurons, and Nurr1 gene deletion in mice results in a severe reduction in dopaminergic neurons and perinatal lethality. Notably, mutations in the NR4A2 gene are associated with rare familial PD (Zetterström et al., 1997). These studies suggest that small-molecule agonists to promote Nurr1 activity could produce both anti-inflammatory effects and promote dopaminergic neuron function (Fig. 5).

Chronic inflammatory conditions could also exacerbate pathogenesis associated with other PD-linked genes. Activated microglia from Parkin null mice display increased levels of TNF, IL-1b, and iNOS mRNA (Tran et al., 2011), and systemic endotoxin-mediated neuroinflammation in Parkin null mice promotes loss of nigral DA neurons (Frank-Cannon et al., 2008), indicating that the nigrostriatal pathway is more vulnerable to inflammation-mediated neurodegeneration in the absence of Parkin. Given the central role Parkin plays in regulating mitochondrial homeostasis, Parkin inactivation or its reduced expression could lead to inflammasome-mediated block of mitophagy and presumably other Parkin-dependent mitochondrial quality control processes. Mitochondria themselves appear to be targets of aberrant inflammasome signaling events that lead to disassembly of the organelle and fragmentation of the mitochondrial network (Yu et al., 2014). Such mitochondrial stress can in turn activate innate immunity by releasing damage-associated molecular patterns. Intriguingly, biallelic loss of PINK1 or parkin in mice triggers aberrant inflammatory response, leading to increased IL-6 levels, and is mediated by cyclic guanosine monophosphate–adenosine monophosphate synthase/stimulator of interferon genes (STING) pathway, presumably through the release of mtDNA into the cytosol. Loss of STING prevents the inflammatory phenotype and the resulting neurodegeneration and locomotor defects (Sliter et al., 2018). Patients with PD with biallelic PINK1 or parkin mutations also exhibit elevated IL-6 levels in serum that results from STING signaling triggered by circulating cell-free mtDNA (Borsche et al., 2020). Although these studies implicate STING-mediated inflammation as a pathogenic cause in PD, they also indicate that mitochondrial insults incurred in the absence of PINK1 or Parkin, particularly in peripheral immune cells, could perpetuate feed-forward cycles of inflammasome activation and mitochondrial dysfunctions. Intranigral endotoxin-mediated activation of microglia also leads to increased microglial expression and LRRK2 activity, and LRRK2 knockdown or inhibition of its kinase activity reduces TNF-2 secretion and iNOS induction (Moehle et al., 2012). Similarly, dopaminergic neurodegeneration elicited by 2-synuclein overexpression or endotoxin exposure are abrogated by LRRK2 KO in rats. Neuroprotection in this scenario is also accompanied by reductions in CD68/iNOS-positive myeloid cells (Daher et al., 2014). Increased LRRK2 expression is also observed in T cells, B cells, and CD16+ monocytes of patients with PD compared with healthy controls (Cook et al., 2017). These studies underscore a possible modifying role for LRRK2 in the progression of neuroinflammatory responses and raise the possibility that LRRK2 inhibition could be beneficial in PD cases in which LRRK2 mutations are causal for the typical late-onset PD. In support of this view, chronic administration of a potent LRRK2 kinase inhibitor (PF-06447475) was recently shown to be effective in abating the exacerbated neuroinflammation and dopaminergic neurodegeneration caused by the G2019S LRRK2 mutation in 2-synuclein-overexpressing rats. Dopaminergic neurodegeneration induced by 2-synuclein in wild-type rats was also attenuated by the LRRK2 inhibitor PF-06447475 (Daher et al., 2015), indicating that LRRK2 inhibition could be of benefit even in PD cases without LRRK2 mutations. Thus, small-molecule kinase inhibitors that can evoke LRRK2-based pharmacodynamic profiles will have tremendous therapeutic impact and, as such, need to be evaluated for their efficacy and mechanism of action to gauge the tolerability and safety of LRRK2 kinase inhibitors in PD. Elevated peripheral inflammation is also observed in idiopathic PD (Brockmann et al., 2016) and LRRK2 G2019S mutation carriers (Dzamko et al., 2016), suggesting that the associated proinflammatory cytokines could further serve as early biomarkers of prodromal PD and/or be amenable to quantify the risk of conversion to clinical PD.

In light of the above studies, strategies that modulate the glial reaction and/or target inflammatory cytokines are emerging as a crucial area of preclinical investigation. For instance, antibody-based therapeutics with the capacity to restrain inflammation could confer neuroprotection. Toward this end, rapid increases in IL-1b levels resulting from aberrant microglial activation can be countered with delivery of anti-IL-1b–neutralizing antibodies, which have recently been reported to exhibit in vivo efficacy in multiple murine disease models, including multiple myeloma and rheumatoid arthritis (Goh et al., 2014). In fact, blockade of IL-1b biologic activity in the SN using an IL-1 receptor antagonist leads to decreased neurodegeneration in 6-OHDA/LPS rat models, and the anti-inflammatory steroid dexamethasone mitigates IL-1b synthesis in these animal models (Pott Godoy et al., 2008), indicating that inhibition of IL-1b–mediated inflammatory responses could have therapeutic potential in PD. Similarly, retrograde nigral degeneration induced by a striatal injection of oxidative neurotoxin or endotoxin is dramatically reduced by XENP345, an engineered dominant-negative TNF...
compound that neutralizes soluble TNF in vivo (McCoy et al., 2006), raising the possibility that therapeutic blockage of soluble TNF in the early stages of PD could delay the progressive degeneration of nigrostriatal pathway. Inflammatory mediators such as cyclooxygenase 2, free radicals, and NO are also investigated as targets in the development of PD therapeutics. In this regard, minocycline, a broad-spectrum tetracycline antibiotic with anti-inflammatory properties, has been observed to promote survival of nigral dopaminergic neurons in MPTP and 6-OHDA rodent models by reducing microglial activation and production of iNOS and IL-1β (Du et al., 2001; He et al., 2001; Tikka et al., 2001), although a small phase II study of minocycline was disappointing regarding its efficacy (NINDS NET-PD Investigators, 2008), and a large phase III study looking at the clinical impact of minocycline was never undertaken. Similarly, the nonselective cyclooxygenase 2 inhibitor sodium salicylate is observed to confer neuroprotection against MPTP-induced nigral injury (Liu and Hong, 2003) as well as 6-OHDA rat models of PD (Sánchez-Pernaute et al., 2004). The iNOS selective inhibitors S-methylisothiourea and l-N(G)-nitroarginine have neuroprotective effects in LPS rat models and highlight the therapeutic potential in free radical scavengers or iNOS inhibitors (Iravani et al., 2002; Arimoto and Bing, 2003). Moreover, iNOS knockout mice are also resistant to MPTP intoxication (Libratore et al., 1999).

Recent evidence indicates that immune responses can be modulated by neurotransmitters, most notably DA itself. In primary microglia and astrocytes, DA acts as an endogenous inhibitor of NLRP3 inflammasome activation through induction of a DA D1 receptor (DRD1)-cAMP signaling pathway. DRD1-deficient mice are more susceptible to MPTP parkinsonism and exhibit increased loss of dopaminergic neurons as a result of the enhanced NLRP3 activation–dependent IL-1β and IL-18 production. Furthermore, cAMP directly binds NLRP3 and promotes its ubiquitination–dependent degradation via the E3 ubiquitin ligase, membrane associated ring-CH-type 7 (Yan et al., 2015). Thus, DRD1-based therapeutic interventions could represent an endogenous mechanism of inflammasome regulation for the treatment of NLRP3-mediated neuronal inflammation in PD. Reversal of ubiquitin modifications of proteins, mediated by a large class of deubiquitinating enzymes (DUBs), impacts protein function, subcellular localization, and protein-protein interactions (Komander et al., 2009). In this regard, G5, a small-molecule DUB inhibitor, has been shown to inhibit NLRP3 deubiquitination and its subsequent activation. Furthermore, deubiquitination of NLRP3 by the DUB BRCA1/BRCA2-Containing Complex Subunit 3, has also been observed to critically regulate inflammasome activity (Py et al., 2013), indicating that small-molecule modulators or specific activators of BRCA1/BRCA2-Containing Complex Subunit or the E3 ligase membrane associated ring-CH-type 7, could serve as therapeutic modalities to counter neuroinflammatory responses associated with inappropriate activation of the NLRP3 inflammasome. Administration of an anti-α-synuclein antibody in mice overexpressing human α-synuclein is observed to improve behavioral performance and promote degradation of accumulated α-synuclein (Maslia et al., 2005; Maslia et al., 2011), suggesting that passive immunization strategies targeting α-synuclein could be of therapeutic relevance in PD by preventing downstream inflammatory signaling events that would otherwise be triggered by α-synuclein.

Although the above studies highlight the efficacy of targeting specific inflammatory mediators, successful interventions to protect the nigrostriatal pathway nevertheless might require a multidrug approach to counter inflammatory insults, especially during the early stages of the disease. However, inhibiting proinflammatory responses globally may not be effective in the long term. In fact, the challenge ahead lies in distinguishing the beneficial mediators of inflammation from the neurotoxic ones within brain regions that are particularly susceptible to neurodegeneration in PD. Moreover, cell type–specific roles of proteins that mediate complex signaling cascades and the trophic or toxic effects they have toward neuronal survival warrant further investigation before the respective pathways could be pharmacologically targeted. Furthermore, techniques to identify the specific areas of neuroinflammation that occur in vivo within patients with PD have not been well established. One potential imaging mechanism would be through the positron emission tomography ligand to the translocator protein (TSPO). The TSPO protein is located within steroid-synthesizing cells on the outer mitochondrial membrane and therefore may serve as a marker of inflammation (Rupprecht et al., 2010). The TSPO pet tracer has been shown in small studies to differentiate patients with PD from controls (Terada et al., 2016; Ghadery et al., 2017), but it is not specific, since TSPO is present in more than just microglia (Rupprecht et al., 2010). More specific tracers of neuroinflammation, such as colony stimulating factor 1 receptor ligands are under investigation (Horti et al., 2019). Another caveat to be considered is that much of the signaling pathways pertaining to inflammatory responses have been primarily studied in peripheral cellular models. Whether such signaling modalities are also conserved in the CNS is worth exploring to fully gauge their potential in PD therapeutics. These considerations will enable rational design and selection of successful anti-inflammatory therapy targeting specific brain regions that are susceptible to neurotoxic inflammatory episodes.
XII. Targeting Altered Protein Translation

Protein translation is one of the most fundamental, energetically demanding, and tightly controlled cellular processes. Mutations that impact the protein translation machinery are linked to a wide spectrum of diseases, indicating that dysregulation of translation regulation can be deleterious [reviewed in Scheper et al. (2007) and Taymans et al. (2015)]. Evidence pointing to a role for altered protein translation in PD pathogenesis is also emerging. In a postmortem transcriptome analysis of SN (SN) from patients with PD, age-matched controls and individuals with incidental Lewy body disease, pathways related to the translation initiator eukaryotic Initiation Factor 2 (eIF2), were observed to be among the most severely deregulated pathways (Mutez et al., 2014). eIF2 regulates global protein synthesis in response to varied stress conditions, in particular, the unfolded protein response (UPR) in the endoplasmic reticulum (ER) to promote protein refolding and degradation (Wek and Cavener, 2007). Phosphorylated forms of eIF2, which serve as indicators of UPR activation, have been observed in PD SN and correlate with increased α-synuclein immunoreactivity (Hoozemans et al., 2007; Hoozemans et al., 2012), implying a role for eIF2 signaling in neuronal death caused by ER stress and prolonged activation of UPR. Of note, sustained repression of translation resulting from eIF2 activation has detrimental effects in Alzheimer disease (Ma et al., 2013), prion disorders (Moreno et al., 2012), and other neurodegenerative diseases caused by protein misfolding (Leitman et al., 2014), underscoring the importance of translation homeostasis in neuronal viability. A combined transcriptome and proteomics analysis of brain regions functionally impacted in PD has also identified changes in protein abundance in the cortex and striatum that are largely independent of transcriptional changes, indicating dysregulation of translation regulatory mechanisms in the PD brain (Riley et al., 2014). Recent studies also suggest that translational control could rely on the preferential usage of specific transcripts for protein synthesis, as is evident in the case of α-synuclein. A differential coexpression analysis of PD brain tissue recently identified RNA transcript isoforms of α-synuclein with an extended 3′ UTR, termed aSynL. Diverse PD risk factors, such as common variants in SynL 3′ UTR, age, DA, and toxin exposure, all increase the aSynL ratio relative to the known shorter α-synuclein transcript. Expression of the aSynL transcript in turn leads to preferential accumulation of α-synuclein and its redistribution closer to somatic organelles such as the mitochondria, where it could exert detrimental effects (Rhinn et al., 2012). Whether similar transcript preferences exist for other PD-linked proteins is unclear. Nevertheless, the observations made with α-synuclein indicate that differential transcript usage could be a translation-dependent mechanism upon which different PD risk factors converge.

In terms of the PD-associated proteins, the LRRK2 signaling pathway exemplifies a seminal link between PD and protein translation. LRRK2 is enriched in the ribosomal subcellular fraction (Martin et al., 2014b) and interacts with proteins of the translation machinery that include translation initiation factors (Dachsel et al., 2007), elongation factors (Gillardon, 2009), and ribosomal proteins (Martin et al., 2014b). In fact, LRRK2 has been found to phosphorylate several ribosomal proteins, many of which exhibit increased phosphorylation in the presence of LRRK2 G2019S and I2020T mutants (Martin et al., 2014b). The LRRK2 G2019S mutant, which exhibits increased kinase activity, has also been reported to increase translation activity in Drosophila by stimulating cap-dependent and cap-independent mRNA translation, resulting in a net increase in protein synthesis. Of note, such effects on bulk translation are mediated via the ribosomal protein s15, which was recently demonstrated to be a phospho substrate of LRRK2 and mechanistically link LRRK2 kinase activity to neurotoxicity (Martin et al., 2014b). A number of other LRRK2 substrates with proposed roles in translation regulation have also been identified. Drosophila studies indicate that increased kinase activity of LRRK2 mutants can stimulate phosphorylation of eukaryotic initiation factor 4E–binding protein (4E-BP), causing its release from eukaryotic initiation factor 4E (eIF4E) and relieving its repression on translation. As such, chronic inactivation of 4E-BP by pathogenic LRRK2 mutants has been proposed to deregulate protein translation and result in dopaminergic neurodegeneration (Imai et al., 2008). LRRK2 phosphorylation of 4E-BP has also been shown to antagonize microRNA (miRNA) activity and promote increased translation of a small number of mRNAs that are regulated by the miRNA pathway (Gehrke et al., 2010). However, mechanistic evidence showing 4E-BP to be a direct phospho target of LRRK2 or a direct role for 4E-BP in mediating altered translation of the said mRNAs through the miRNA pathway is lacking. Importantly, subsequent studies have failed to detect 4E-BP phosphorylation in cells, mouse brains under conditions of increased LRRK2 kinase activity, or G2019S LRRK2 transgenic fly heads (Kumar et al., 2010; Trancikova et al., 2012; Martin et al., 2014b). Consequently, it is not clear whether 4E-BP phosphorylation–induced stimulation of protein translation or impairment of the miRNA pathway plays a major role in LRRK2 pathogenesis. On the contrary, phosphorylation of the LRRK2 interacting s15 appears to be central to G2019S neurotoxicity, as the s15 phosphomutant (T136A) is neuroprotective in the G2019S Drosophila PD model and also blocks
G2019S LRRK2 toxicity in human DA neurons (Martin et al., 2014b).

Nevertheless, the precise mechanisms by which s15 stimulates protein synthesis and how increased protein synthesis impacts neuronal survival are open areas for investigation. Given that protein synthesis is an energy-dependent process, it is likely that conditions that stimulate bulk protein translation would impose an enormous drain on neuronal energy reserves and also increase the burden on protein folding/degradation machineries, eventually compromising overall cellular protein quality control. Furthermore, increased bulk protein synthesis could alter gene-specific translational profiles, effectively disrupting their translation output, which would otherwise be tightly regulated under basal conditions. Disruption of such fine-tuning of translation control becomes even more crucial under stress conditions that would require translation of specific stress-responsive proteins rather than bulk induction in protein synthesis. The selective vulnerability of DA neurons to LRRK2-dependent translation changes is also an intriguing question to explore. Examining whether cell type–specific translational profiles are induced by LRRK2 pathogenic mutants and identifying downstream factors that mediate such effects in different cell types and importantly in the DA neurons would provide tremendous insight in this regard (Martin et al., 2014a).

Further reinforcing the importance of translational control in PD is the identification of PD-linked missense mutations in translation initiation factor eIF4G1, which serves as a scaffold in the eIF4F translation initiation complex that recruits ribosomes and tRNAs to the 5' cap structure of mRNA (Sonenberg and Dever, 2003). Genome-wide linkage analysis had initially identified, among others, two frequent eIF4G1 mutations in familial patients with PD. These two mutations—eIF4G1 p.R1205H and p.A502V—impair eIF4F complex formation, indicative of a dominant-negative loss of function, and were shown to be associated with mitochondrial dysfunctions under stress conditions (Chartier-Harlin et al., 2011). However, subsequent studies in different populations have failed to provide strong evidence implicating eIF4G1 mutations to be causal to PD (Lesage et al., 2012; Sudhaman et al., 2013; Blanckenberg et al., 2014; Nishioka et al., 2014; Huttenlocher et al., 2015; Nichols et al., 2015), suggesting incomplete penetrance or possible population heterogeneity of these mutations. Nevertheless, eIF4G1 was recently shown to genetically and functionally interact with the retromer complex protein Vps35. As such, upregulation of the yeast homolog of eIF4G1 under conditions of Vps35 deficiency have been observed to cause proteotoxic stress resulting from the accumulation of misfolded and unfolded proteins in the absence of retromer function. Notably, such defects are ameliorated by WT Vps35 (Dhungel et al., 2015). Although the validity of eIF4G1 as a bona fide PD gene warrants further verification, the synthetic effect observed between eIF4G1 and Vps35 suggest that stoichiometric imbalances in translation control could amplify the detrimental effects of underlying protein quality control defects and thus could have implications for sporadic PD. In this regard, identifying eIF4G1 interacting genes could provide insight into its potential impact on other PD genes and identify disease-relevant pathways that are impacted. Indeed, eIF4G1 was recently identified as a suppressor of α-synuclein toxicity in a yeast overexpression screen. In fact, perturbations in protein translation are observed in cellular α-synucleinopathy models as well as in iPSC neurons harboring an α-synuclein A53T mutation, implying an association between α-synuclein toxicity and bulk mRNA translation (Khurana et al., 2017). Moreover, spatial mapping studies of α-synuclein in neurons localizes it to the immediate vicinity of proteins involved in mRNA translation and protein trafficking, many of which are in complex with α-synuclein (Chung et al., 2017). This suggests that spatial localizations of α-synuclein within the cell could in part explain the susceptibility of particular pathways to α-synuclein toxicity.

Interestingly, recessive PD genes such as PINK1, Parkin, and DJ-1 have also been observed to influence protein translation. DJ-1 has been found to interact with mRNA transcripts of specific mitochondrial genes, genes involved in glutathione metabolism, and members of the phosphatase and tensin/phosphoinositide 3-kinase cascade (van der Brug et al., 2008). Although DJ-1 binding to such mRNA slows translation under basal conditions, it dissociates from these mRNA under oxidative stress, thereby facilitating rapid translation in response to stress. Such a translation-dependent mechanism appears to underlie DJ-1's protective effects as a redox sensor (van der Brug et al., 2008). PINK1 has also been reported to mediate adaptive responses under conditions of oxidative stress by promoting a translation switch from cap-dependent to cap-independent translation in a 4E-BP1–dependent manner (Lin et al., 2014). PINK1 and Parkin also promote localized translation of nuclear encoded respiratory chain complex mRNAs on the mitochondrial surface through displacement of translation repressors and recruitment of translational activators such as eIF4G (Gehrke et al., 2015). Furthermore, Parkin has been found to interact with eIF4E in a common pathway to modulate cap-dependent translation initiation events that influence the severity of several Parkin mutant phenotypes in Drosophila (Ottone et al., 2011).

The above studies collectively suggest that a subset of proteins whose expression is regulated at the translational level by PD-linked genes are part of a functional network that is crucial to the survival of DA neurons. However, the precise mechanisms that link
PD genes to translation regulation of specific key proteins are incompletely understood at present (Taymans et al., 2015). Furthermore, whether and how deregulation of translational control contributes to the demise of nigral neurons in sporadic PD requires extensive investigation, as does the identification of extraneous factors that could potentially impinge on translation homeostasis. In-depth mechanistic studies on the PD-linked proteins currently known to impact protein translation could essentially serve as starting points to establish a reinforcing role for aberrant translation in the disease process and identify therapeutically viable targets. For instance, G2019S LRRK2 has been reported to upregulate ribosomal functions in mouse brain compared with LRRK2 KO brain tissues (Nikonoa et al., 2012), raising the possibility that disease-causing LRRK2 mutations could impact protein translation by altering the levels of ribosomal proteins and/or the ribosomes. Given the overlap between LRRK2-linked PD and sporadic cases, it would be informative to investigate whether increased mRNA translation paves the way for pathogenesis in sporadic PD by examining the phosphorylation status of LRRK2 substrates such as s15 and 4E-BP1 in brain tissues from patients with PD (Martin, 2016). In the context of PINK1 and Parkin, genetic interaction studies in Drosophila indicate that overexpression of 4E-BP1 suppresses dopaminergic degeneration in PINK1 and Parkin mutant flies, suggesting that translational suppression could be beneficial in the case of some PD mutations. Upregulation of unphosphorylated 4E-BP can be achieved in vivo by the target of rapamycin (TOR) inhibitor rapamycin. Indeed, rapamycin treatment that prevents TOR-mediated 4E-BP phosphorylation phenocopies the neuroprotective effects of 4E-BP activation in PINK1 and Parkin mutant flies, suggesting that translational suppression could be beneficial in the case of some PD mutations. Upregulation of unphosphorylated 4E-BP can be achieved in vivo by the target of rapamycin (TOR) inhibitor rapamycin. Indeed, rapamycin treatment that prevents TOR-mediated 4E-BP phosphorylation phenocopies the neuroprotective effects of 4E-BP activation in PINK1 and Parkin mutant flies (Tain et al., 2009). Another downstream effector of TOR signaling is the ribosomal protein S6 kinase (S6K), and overexpression of a constitutively active S6K in PINK1 mutant flies exacerbates the muscle degeneration and DA neuron loss caused by loss of PINK1. Interestingly, S6K activity is attenuated in the PINK1 mutants likely as a cellular compensatory response to the energy deficit caused by mitochondrial dysfunctions in the PINK1 mutants (Liu and Lu, 2010). This suggests that for some forms of PD, deregulated translation impacts disease pathogenesis by impinging on energy metabolism. Because ribosome biogenesis is a highly energy-consuming process, upregulation of ribosome biogenesis induced by S6K activation when mitochondrial functions are already compromised could impose serious energy constraints and disrupt other energy-dependent cellular processes, eventually culminating in tissue degeneration. In fact, knockdown of positive regulators of translation such as S6K and the ribosomal proteins S6 and S9 is beneficial in the PINK1 mutant flies (Liu and Lu, 2010), further supporting the energy metabolism model. An emerging theme from these studies is that decreasing bulk protein translation is neuroprotective in vivo under pathologic conditions. Mechanistically, the link between bulk translation and neurodegeneration could be exemplified by translational upregulation of pathogenic targets or downregulation of neuroprotective proteins due to loss of precise control over translation (Martin, 2016). At present, the identity of specific proteins whose translation dysregulation contributes to the disruptive global translation effect remains obscure. Future studies should therefore be aimed at identifying a specific subset of proteins that are subject to translation alteration by PD-linked pathogenic proteins. A combination of transcriptomics and proteomics approaches could be employed to identify such targets, which can then be further validated in unbiased system-level screens. More recently, ribosome profiling was successfully adapted to characterize changes in mitochondrial translation caused by disease-causing tRNA mutations (Rooijers et al., 2013) and highlight the applicability of ribosome profiling techniques to identify distinct translation profiles in different PD models that could be of relevance to the disease process. Along these lines, RNA footprinting in human DA G2019S LRRK2 neurons revealed that enhanced phosphorylation of s15 leads to enhanced calcium influx and elevated intracellular calcium levels due to translation of genes with complex secondary structure in the 5′-untranslated region. This enhanced Ca\(^{2+}\) signaling likely contributes to the progressive and selective dopaminergic neurotoxicity in PD (Kim et al., 2020) (also see the following section).

Furthermore, chemical screens to identify small-molecule modulators of protein translation will help test the possibility of rectifying protein translation dysregulation using small molecules. In this regard, small molecules targeting eIF4G1 have been found to be effective as potential therapeutics in multiple myeloma (Attar-Schneider et al., 2014), and pharmacological inhibition of eIF4G1 has been found to negatively impacts its downstream molecular targets, resulting in increased cytotoxicity in breast cancers (Yi et al., 2014). Similarly, pharmacological modulation of phosphorylated eIF2α-mediated translational inhibition has been found to have neuroprotective effects in a prion disease mouse model (Halliday et al., 2015). These studies support the pursuit of small-molecule modulators to therapeutically target mediators of translation dysregulation in PD. Although the studies discussed here indicate a significant role for translational regulation in PD pathogenesis, it is important to note that these studies are in the early stages of establishing a compelling relationship and thus await
extensive independent verification (Martin, 2016). It is therefore imperative to identify specific mechanisms that contribute to translation defects and verify findings from experimental models in patient samples. Such studies have the potential to identify novel biomarkers that reflect alterations in translation during the early stages of the disease and would facilitate development of diagnostic tools for early detection of the disease or monitoring disease progression.

XIII. Maintenance of Neural Calcium Homeostasis for Neuroprotection

Calcium is central to the normal functioning of neurons and is also involved in many cellular processes that are implicated in neurodegeneration in PD. Of relevance to the disease pathology, calcium appears to play a role in determining the susceptibility of neurons within brain regions where degeneration is more pronounced in PD. For instance, the degenerating neurons in the SN are observed to be mainly in areas with low levels of the calcium binding protein calbindin-D28K, which buffers toxic fluctuations in intracellular calcium levels (Yamada et al., 1990; German et al., 1992; Damier et al., 1999). The distinctive physiology of the SNpc DA neurons likely plays a contributing role in this regard in that they are autonomously active, generating continuous low-frequency activity that is dependent on L-type calcium channels (Surmeier, 2007). Such a pacemaking activity is nevertheless crucial to maintaining ambient levels of DA in regions that are innervated by these neurons, especially the striatum (Romo and Schultz, 1990). Although the pacemaking activity of SN DA neurons relies on the calcium channel, voltage-dependent, L type, alpha 1D subunit (CaV1.3) subtype of calcium channels, DA neurons in the adjacent ventral tegmental area (VTA), which are less impacted by the neurodegenerative process in PD, maintain conductance oscillations by utilizing sodium ion entry (Chan et al., 2007; Khaliq and Bean, 2010). The reliance on calcium rather than sodium ions for pacemaking comes at a significant bioenergetic cost, as the extrusion of calcium across the steep plasma membrane concentration gradient, as well as its sequestration into intracellular stores, requires energy from mitochondrial oxidative phosphorylation (Hurley and Dexter, 2012). Distribution of the CaV1 subtypes that exhibit regional differences in the human brain are also significantly altered in the PD brain. In particular, an increase in expression of CaV1 subtypes was observed to precede PD pathology, with a trend toward increased expression of CaV1.3 channels throughout the PD brain (Hurley et al., 2013). Such increased expression could favor greater utilization of these channels and render neurons more susceptible to metabolic stress and contribute to or cause cell death. So, how might the increased reliance of DA neurons on CaV1.3 channels increase their risk for degeneration? Although calcium cycling is episodic in most neurons, the pacemaking activity of the SN DA neurons makes this a rather continuous event in these neurons, as the spatial magnitude of calcium influx is much larger (Wilson and Callaway, 2000). The energy dependence of intracellular calcium handling mechanisms means vulnerable populations of DA neurons in essence have less bioenergetics/respiratory reserves that would in turn impose enormous burden on these neurons when metabolic demands increase (Nicholls, 2002). Indeed, under conditions of insufficient ATP levels to meet cellular energy demands, depletion of the cell membrane potential would initiate massive calcium influx and cell death (Choi et al., 2011). Since ROS production is an inevitable consequence of increased metabolic activity, such increased metabolic demand could also increase the basal levels of mitochondrial oxidant stress and set in motion feed-forward cycles of bioenergetics deficiency and further ROS generation. In fact, in transgenic mice expressing a mitochondria-targeted redox variant of green fluorescent protein, engagement of the L-type calcium channel during autonomous pacemaking generates mitochondrial oxidant stress in the vulnerable SNpc DA neurons while sparing VTA DA neurons (Guzman et al., 2010). In PD in which impairment of mitochondrial function can be incurred from various sources, the reliance on CaV1.3 channels could thus make the SN DA neurons even more prone to calcium-mediated cytotoxicity and/or oxidative stress.

Intracellular calcium levels are under tight homeostatic control, and altered calcium handling by intracellular organelles also threatens neuronal viability. Intriguingly, the two organelles responsible for regulating intracellular calcium levels—the ER and the mitochondria—are also the organelles most affected in PD (Chan et al., 2009). Sequestration of cytosolic calcium into the ER is facilitated by high-affinity ATP-dependent transporters, and luminal ER calcium can be either used locally to modulate various cellular functions or transported back across the plasma membrane (Rivero-Rios et al., 2014). Calcium is inherent to the functioning of ER, as it is an allosteric regulator of protein processing and folding. Depletion of ER calcium stores as such induces ER stress and UPR (Mekahli et al., 2011). Aberrant regulation of ER calcium is also observed in different PD models. For instance, impaired replenishment of ER calcium has been observed to activate UPR and increase vulnerability of dopaminergic neurons in an MPTP PD mouse model (Selvaraj et al., 2012). Specifically, these effects result from MPTP-induced decrease in the levels of transient receptor potential channel 1 (TRPC1), which replenishes ER calcium levels by triggering calcium entry through the plasma membrane. Notably, TRPC1 levels are also reduced in
the SN of patients with PD, and restoring ER calcium reserves by increasing TRPC1 expression is neuroprotective in the MPTP mouse model through an as yet un
clear mechanism involving activation of the protein kinase B/mechanistic target of rapamycin kinase pathway (Selvaraj et al., 2012). Induced pluripotent stem cell–derived dopaminergic neurons from patients with PD harboring GBA mutation exhibit increased basal lev
els of calcium and marked upregulation of the neuronal calcium binding protein 2, likely as a compensatory re
response. Moreover, agonist-stimulated release of calcium from ER or knockdown of neuronal calcium binding pro
tein 2 increases the vulnerability of GBA mutant neu
rons to ER stress responses and calcium-mediated neurotoxicity (Schöndorf et al., 2014). Similarly, lysoso
mal dysfunctions that lead to accumulation of glucosylceramide in Gaucher disease have been reported to stimu
late efflux of ER calcium stores, resulting in neuronal cell death (Pellet et al., 2005). The A53T mutant α-synuclein–induced cell death has also been observed to result from ER stress evoked by aberrant ER calcium re
lease. As such, pharmacological inhibition of the ER res
ident calcium release channels ameliorates ER stress and α-synuclein aggregation, as does the upregulation of homocysteine-induced ER protein (Herp), an ER stress
inducible protein that interacts with and promotes deg
radation of ER calcium release channel proteins (Belal et al., 2012). Herp has also been observed to counteract MPP⁺-induced perturbations of ER calcium homeostasis and promote adaptive responses to MPP⁺-induced stress response (Chigurupati et al., 2009). Coexpression of calbindin-D28K or cotreatment with the calcium chelator 1, 2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid also inhibits MPP⁺-induced cell death, further implying a crucial role for intracellular calcium load in MPP⁺
induced toxicity (Choi et al., 2008).

Intracellular calcium buffering is also provided by shuttling of calcium to the mitochondria at points of apposition between the ER and mitochondria, which form functional calcium microdomains (Csordas et al., 2006). The mitochondrial calcium uniporters drive calcium entry into the matrix, where the activity of calcium-regulated dehydrogenases involved in the tri
carboxylic acid cycle stimulate mitochondrial metabo
lism (Kirichok et al., 2004; Kamer and Mootha, 2015). Although mitochondrial calcium has an effect toward augmenting mitochondrial function, pacemaking in the SN DA neurons could nevertheless impose a large calcium buffering burden and increase the calcium load in the mitochondria. Furthermore, under condi
tions of ER stress, calcium transfer from ER to mito
chondria is increased, and depletion of stress
inducible ER proteins such as Herp together could further increase ER calcium leakage and mitochondrial calcium overload (Chigurupati et al., 2009). During the initial phases of calcium influx due to ER stress, the increased activity of the mitochondrial dehydrogenases could presumably promote a surge in bioenergetics capacity sufficient to mount an adaptive response and alleviate ER stress. However, chronic exposure to ER stress and the resultant overload of calcium in the mitochondria can collapse the mito
ochondrial membrane potential, compromise ATP syn
thesis, and enhance free radical generation, all of which are detrimental to neuronal survival. Indeed, all of these mechanisms have been observed to underlie the mitochondrial pathophysiology triggered by loss of PINK1 function. In primary human and mouse midbrain neurons lacking PINK1, dysfunctions of the mitochondrial Na⁺/Ca²⁺ exchanger impair calcium ef
lux and lead to mitochondrial calcium overload (Gan
dhi et al., 2009). As an epiphenomenon to elevated mitochondrial calcium load, the PINK1-deficient neu
rons also exhibit increased ROS production, which triggers opening of the mitochondrial permeability transition pore, thereby compromising mitochondrial membrane permeability and membrane potential (Gandhi et al., 2009). Increased oxidative stress trig
gered by mitochondria-derived ROS could also recipro
cally impact the ER and disrupt protein folding, thereby potentiating further ER calcium release through a positive feed-forward mechanism. The low intrinsic calcium buffering capacity of SN dopaminer
gic neurons also imposes an increase in basal mito
chondrial oxidant stress that is further augmented in the absence of effective oxidant defense mechanisms, as has been observed in the case of DJ-1 depletion. Under conditions of calcium-induced stress, DJ-1 de
ficiency in the SN dopaminergic neurons leads to di
minished expression of uncoupling (UCP) proteins UCP4 and UCP5 in the redox-sensitive green fluores
cent protein mouse model (Guzman et al., 2010). UCP activation enables mild dissipation of the hyperpolarized MMP during oxidative phosphorylation and thus serves as a protective mechanism to minimize ROS generation (Ho et al., 2012). Lack of the uncoupling protein UCP2 in mice has also been reported to in
crease ROS generation and increase the sensitivity of nigral DA neurons to MPTP-induced neurotoxicity (Andrews et al., 2005). Accordingly, failure to launch UCP response could manifest in disease pathology in the form of reduced mitochondrial competence and sustained superoxide exposure, which impair proteo
stasis over time, contributing to phenotypic decline and ultimately neuronal loss. Pathogenic LRRK2 G2019S or R1441C mutations have also been reported to cause calcium imbalance and enhance mitophagy in neurons, presumably leading to the dendrite-short
ening phenotype observed with these mutants (Cherr
a et al., 2013). Interestingly, decreased mitochondrial membrane potential and the consequent decrease in ATP generation induced by the pathogenic G2019S
LRRK2 mutant in PD fibroblasts and SH-SY5Y cells lead to upregulation of UCP2 and UCP4 proteins (Papkovskaia et al., 2012). UCP2 mRNA levels have also been observed to be upregulated in response to elevated ROS generation in fibroblasts from carriers of G2019S LRRK2 mutation (Grunewald et al., 2014). Although the molecular mechanisms that contribute to the induction of UCP gene expression are unclear, these observations raise the possibility that UCP proteins might serve as markers for early diagnosis of mitochondrial dysfunctions and/or to monitor the disease status, at least in PD caused by LRRK2 mutations.

The unusual reliance of SNpc dopaminergic neurons on a metabolically expensive strategy that taxes the mitochondria and imposes a burden on both the mitochondria and ER to maintain homeostatic calcium control likely renders these neurons particularly vulnerable to other metabolic stressors and PD triggers. This notion is also consistent with the central role of ER stress and mitochondrial dysfunctions in existing models of PD pathogenesis (Chan et al., 2009). If the neurodegenerative process in PD is in fact potentiated by calcium dyshomeostasis, then reducing the dependence on the L-type calcium channels should delay the clinical appearance and/or slow the disease progression. The L-type calcium channels are dispensable for SNpc DA neuron pacemaker activity (Dragicevic et al., 2014; Poetschke et al., 2015) and therefore are amenable to pharmacological manipulations. Retrospective epidemiologic studies indicate that treatment with the Cav1 subtype antagonist isradipine at the treatment dose hypothesized that isradipine at the treatment dose may not have adequately engaged the calcium channels (Parkinson Study Group STEADY-PD III Investigators, 2020). To that end, novel Ca$_{v}$1.3 selective L-type calcium channel blockers are also in preclinical development (Ortner and Striessnig, 2016).

However, there are caveats to be considered about the potential applicability of therapeutics targeting the L-type calcium channels. Recent reports indicate that the L-type calcium channel activity could be coupled to DA-dependent adaptive signaling in the SNpc dopaminergic neurons. As such, DA sensing somatodendritic D2-autoreceptors (D2-AR) are coupled to inhibitory G-protein-regulated inward-rectifier potassium channel 2 potassium channels and regulate excitability of SN DA neurons in response to DA released locally in the midbrain (Beckstead et al., 2004; Ford, 2014). Notably, such feedback regulation is more pronounced in the vulnerable SN DA neurons than the VTA DA neurons that are resistant to degeneration (Brichta and Greengard, 2014; Dragicevic et al., 2014). A sensitized D2-autoreceptor response could thus counteract neurotoxic intracellular calcium loads. Moreover, homeostatic regulation of this sensitized D2-AR phenotype is functionally coupled to the neuronal calcium sensor neuronal calcium sensor-1, which interacts with D2-AR and modulates receptor desensitization in a calcium-dependent manner. Of note, this D2-AR sensitization relies on the Ca$_{v}$1.3 calcium channels (Dragicevic et al., 2014; Poetschke et al., 2015). These observations raise caution about the potential side effects of selective L-type calcium channel blockers, as failure to prevent D2-autoreceptor desensitization could in effect enhance excitability of SNpe DA neurons and counteract the metabolic stress relief provided by the calcium channel antagonists. Moreover, L-type calcium channels appear to have bidirectional and context-dependent functions in the SN. Although not essential for generation of pacemaker activity in the SN, the L-type calcium channels stabilize pacemaker activity and its precision (Poetschke et al., 2015). Such L-type calcium channel–dependent maintenance of neuronal electrical activity is particularly crucial under conditions of increased metabolic demand, as neural activity–dependent influx of calcium stimulates the tricarboxylic acid cycle and ETC and thus facilitates ATP production crucial for neuronal functions (Duda et al., 2016). L-type calcium channels are also known to regulate gene expression. For instance, cAMP-response element binding protein -dependent transcription is coupled to Ca$_{v}$1.3 channels dependent neural activity in the hippocampus and striatal medium spiny neurons (Zhang et al., 2005a, 2006) and can be perturbed by calcium channel blockers (Wheeler et al., 2012). L-type calcium channels have also been observed to regulate activity of transcription factors like nuclear factor activated T cell (Graef et al., 1999), myocyte enhancer factor-2 (Mao et al., 1999), and transcriptional repressors such as potassium channel interacting protein downstream regulatory element antagonist modulator (Carrión et al., 1999). Furthermore, the C terminus of Ca$_{v}$1.3 has been observed to be cleaved in neurons and can translocate to the nucleus and function as a transcriptional regulator (Lu et al., 2015). Together, these studies indicate that the relationship...
between calcium channel activity, calcium homeostasis, and physiologic functions in neurons is rather complex, context-dependent, and intricate to signaling events in the SNc DA neurons. As such, pharmacological modulation of L-type calcium channels could have unforeseen consequences on the viability and/or physiologic functioning of the SNc DA neurons in the long term, with unwarranted side effects beyond the desired neuroprotection. It is therefore essential to not only develop calcium channel modifiers that are selective for specific subtypes but also characterize the acute and chronic effects of such modifiers on the SNpc DA neurons as well as other neuronal subtypes, which would help gauge the applicability of calcium channel modifiers in PD therapeutics.

XIV. Regulation of Neuronal Oxidative and Nitrosative Stress

ROS and reactive nitrogen species (RNS) are produced in most mammalian cells at low levels and act as important physiologic regulators of intracellular signaling events (Finkel, 2011). However, an imbalance between the production of ROS/RNS and antioxidative capacity in cells accrued during the normal process of aging, triggered by exposure to environmental toxins or disease-associated mutations, can result in an excess of ROS/RNS and lead to neuronal oxidative and nitrosative stress (Bisaglia et al., 2014). Modification of proteins by ROS promotes protein aggregation and loss of function (Danielson and Andersen, 2008). Free radicals can also peroxidize unsaturated fatty acids, resulting in lipid degradation and cell membrane damage (Pratt et al., 2011). As such, the involvement of ROS/RNS in PD pathogenesis is supported by observations of high levels of lipid peroxidation, glutathione depletion, and increased DNA, RNA, and protein oxidation in the brain tissues of patients with PD (Dias et al., 2013). ROS in the brain can be derived from several sources, with the bulk generated as a consequence of mitochondrial respiration (Murphy, 2009). Mitochondrial toxins that recapitulate aspects of nigral degeneration and motor deficits in animal models also induce ROS generation (Sherer et al., 2003; Wu et al., 2003; Elkou et al., 2003; Somayajulu-Nitu et al., 2009), indicating that oxidative damage could be an important mechanism underlying dopaminergic neurotoxicity. Nitration a-synuclein is a major filamentous component of proteinaceous inclusions in PD and is present in the insoluble fractions of affected brain regions in different a-synucleinopathies, providing evidence of oxidative and nitrosative damage in neuregenerative a-synucleinopathies (Giasson et al., 2000). Increased S-nitrosylation of Parkin that inhibits its E3 ligase activity and protective function is also observed in the affected brain regions of PD and dementia with Lewy bodies patients as well as in MPTP-intoxicated mouse models (Chung et al., 2004).

Although the reasons for preferential degeneration of SNpc dopaminergic neurons are still unclear, one hypothesis hinges on a role for DA and its oxidative chemistry. Intraneuronal accumulation and/or impaired detoxification of the toxic DA metabolite 3,4-dihydroxyphenylethylaldehyde (DOPAL) has been linked to PD pathogenesis, and post-mortem analysis of PD brains revealed elevated DOPAL levels that are also observable in the caudate and putamen of PD brains (Goldstein et al., 2013). DOPAL is a reactive aldehyde, and in the presence of hydrogen peroxide can generate toxic hydroxyl radicals (Li et al., 2001b). DOPAL also stimulates the formation of potentially toxic a-synuclein oligomers and aggregates (Burke et al., 2008). Furthermore, at physiologic pH, DA is unstable and can self-oxidize to form ROS and generate dopamine-derived quinones (DAQs), which are highly reactive toward cellular nucleophiles (Bisaglia et al., 2014). At neutral pH, DAQs have been observed to react with DNA to form depurinating adducts, and accumulation of such apurinic sites on DNA could generate mutations that lead to neurodegeneration (Zahid et al., 2011). DAQs are also highly reactive toward cysteine residues, which are often located at the active sites in proteins. Consequently, covalent modification of proteins by DAQs could impair protein function with deleterious consequences (LaVoie and Hastings, 1999). For instance, covalent modification of sulfhydryl groups of tyrosine hydroxylase, the rate limiting enzyme in DA biosynthesis, leads to loss of its enzymatic activity (Kuhn et al., 1999; Xu et al., 1998). DAQs have also been observed to cause proteasomal dysfunctions in different cell types (Zafar et al., 2006; Zhou and Lim, 2009). In dopaminergic-like MES23.5 and SH-SY5Y neuronal cells, DAQ binding to Parkin promotes Parkin aggregation and inactivation of its E3 ligase activity (LaVoie et al., 2005). DAQ-modified a-synuclein is a poor substrate for CMA and also blocks degradation of other substrates by this pathway through tight binding of the lysosomal membrane (Martinez-Vicente et al., 2008). Additionally, covalent modification of DJ-1 by DAQ leads to the formation of DAQ-modified covalent dimers or induces severe structural perturbations, both of which could reduce the bioavailability and/or hamper the cytoprotective effects of DJ-1 (Girotto et al., 2012). Furthermore, interaction between DAQ and the mitochondrial detoxifying enzyme superoxide dismutase 2 induces loss of superoxide dismutase 2 enzymatic activity and promotes formation of protein aggregates (Belluzzi et al., 2012). Together, these studies indicate that DAQ-mediated modifications of PD-linked proteins could impair their normal function and, as such, exacerbate the oxidative effects of DA, leading to neuronal dysfunction.
and eventual cell death. DAQs also inhibit mitochondrial respiration through inactivation of complex I and IV activities (Khan et al., 2005), which triggers opening of the mitochondrial permeability transition pore. This in turn depolarizing the transmembrane potential, and causes osmotic swelling and loss of oxidative phosphorylation (Bisaglia et al., 2010). Inhibition of complex I activity could in itself cause electrons to be leaked from the ETC, and the resultant reduction of oxygen to superoxide anions could initiate deleterious feed-forward mechanisms that further generate ROS and reduce ATP biosynthesis (Cadenas and Davies, 2000). Low ATP levels could in turn inhibit ATP-dependent neuroprotective pathways as well as the expression of detoxifying enzymes that would otherwise help cope with oxidative challenges.

DA can also form neurotoxic intermediates in reactions facilitated by iron. Intriguingly, a marked increase in iron levels has been observed in both living and post-mortem PD brains (Barbosa et al., 2015) and is thought to result from a multifaceted failure of iron metabolism that contributes to a hazardously pro-oxidant environment [reviewed in Hare and Double (2016)]. Nonenzymatic oxidation of DA by iron forms both DAQ and 6-OHDA, a commonly used neurotoxin in animal models of PD. In addition to being a potent inhibitor of mitochondrial complexes I and IV, 6-OHDA can be oxidized by iron to reactive semiquinones (Rodriguez-Pallares et al., 2007). Unilateral injections of exogenous 6-OHDA in a mouse PD model causes loss of SN dopaminergic neurons with concomitant increases in nigral iron pool (Hare et al., 2009), which likely promotes additional cycles of DA oxidative reactions and eventually overwhelms neuronal antioxidant mechanisms. Dopaminergic neurons in the adjacent VTA that contain relatively less iron than the SNpc are nevertheless unaffected by 6-OHDA (Hare et al., 2014). Although 6-OHDA production is not impacted by antioxidant enzymes and radical scavengers, chelation of iron reduces its production (Monteiro and Winterbourn, 1989; Double et al., 1998; Workman et al., 2015), further reinforcing a role for iron in mediating oxidative stress. Furthermore, hydroxyl radicals derived from 6-OHDA metabolism react indiscriminately with cellular components, generating DNA adducts and promoting lipid peroxidation and loss of membrane integrity, culminating in cell death (Blum et al., 2001). DA metabolite–mediated toxicity can also be exacerbated by defects in iron regulation. Increasing levels of transition metals in the brain target the divalent metal transporter (DMT) 1 for ubiquitination and degradation to limit metal ion entry and protect neurons from metal toxicity. This process is regulated by Neuronal precursor cell-expressed developmentally downregulated 4 (Nedd4) Family Interacting Protein 1 (Ndfip1), an adaptor for the Nedd4 family of E3 ligases (Foot et al., 2008). DMT1 transports iron into cells (Garrick et al., 2003), and DMT1 levels are also elevated in post-mortem PD brains, implicating DMT1 in iron misregulation (Salazar et al., 2008). In PD brains, iron accumulation correlates with upregulation of Ndfip1 in a subset of dopaminergic neurons containing α-synuclein deposits (Howitt et al., 2014). In neurons exposed to excess iron, α-synuclein overexpression has been observed to further increase intracellular levels of iron and also cause redistribution of iron from cytoplasm to the perinuclear region within α-synuclein-rich inclusions (Ortega et al., 2016). α-Synuclein expression itself appears to be reciprocally regulated by iron and redox events via iron regulatory elements located at the 5’UTR of its mRNA (Friedlich et al., 2007). It is therefore likely that under conditions of stress imposed by α-synuclein accumulations, surviving dopaminergic neurons upregulate the Ndfip1-mediated pathway as a reactive response to promote DMT1 degradation and prevent further entry of iron into neurons that could otherwise have catastrophic effects. Quinones and semiquinones that are potentially toxic could be converted to more stable and inactive polymers by neuromelanin, the end product of DA metabolism in SN (Zecca et al., 2008a). Neuromelanin also exhibits strong chelating ability for redox reactive metal ions, such as iron, copper, and manganese (Zecca et al., 2001). Consequently, neuromelanin formation is thought to be neuroprotective. However, neuromelanin released from dying neurons can lead to microglia activation (Zecca et al., 2008b), and the resulting immunologic response could incidentally damage neighboring neurons and initiate a vicious cycle of neuroinflammation that contributes to disease progression. In fact, the SNpc contains a higher density of microglia than other brain regions, exposing this region to such inflammatory insults (Kim et al., 2000). DAQs themselves have also been shown to promote microglia-mediated inflammatory responses in cellular models and stimulate the release of NO and hydrogen peroxide, which causes dopaminergic neuronal injury (Le et al., 2001).

With regard to RNS, NO-related species play a critical role in PD pathogenesis. Knockout of neuronal NO synthase (Przedborski et al., 1996) or iNOS (Libratore et al., 1999) protects against the loss of DA neurons in the MPTP model of PD. In particular, protein S-nitrosylation, a redox-mediated post-translational modification that involves addition of NO to a cysteine thiol group on the target protein, serves as a ubiquitous mediator of NO signaling (Sun et al., 2006). Although S-nitrosylation can be an important modulator of signal transduction pathways under physiologic conditions, excessive NO generation and aberrant S-nitrosylation of specific proteins can contribute to an array of degenerative processes, such as proteasomal impairment, ER stress, mitochondrial dysfunction, and neuronal cell death (Nakamura
et al., 2013). A number of proteins critically linked to PD are also subject to S-nitrosylation–mediated misregulation and provide a pathogenic basis for neurodegeneration in PD. For instance, Parkin has multiple cysteine residues that predispose it for S-nitrosylation (Chung et al., 2004). In fact, neurotoxins such as rotenone and MPTP promote S-nitrosylation of Parkin, which leads to an initial increase in Parkin E3 ligase activity that is subsequently inhibited through sequential cysteine S-nitrosylation (Chung et al., 2004; Yao et al., 2004). Post-mortem analysis of sporadic PD brains exhibits marked upregulation of S-nitrosylated Parkin, which is also evident in the brains of rodent models of PD, indicative of a role for S-nitrosylated Parkin species in disease pathogenesis (Chung et al., 2004). A similar effect on Parkin’s catalytic function has been observed under conditions of oxidative stress that promote sulfonation of Parkin, decrease its solubility, and induce its aggregation (Meng et al., 2011). Since the loss of Parkin enzymatic activity could consequently impair proteasomal degradation of its substrates, the resultant accumulation of pathogenic Parkin substrates could contribute to neuronal injury or cell death. Sequestration of Parkin in aggregates can also reduce its bioavailability in the cell and limit its intrinsic neuroprotective effects. Oxidation/nitration-induced post-translational modifications of Parkin could further alter the protein structure akin to hereditary cysteine substitutions linked to parkinsonism. Thus, modifications of cysteine residues that are critical to maintaining the structure and/or function of Parkin could be a molecular point of convergence that links hereditary mutations impacting Parkin structure and solubility with adverse environmental insults that have similar detrimental effects on Parkin function (Meng et al., 2011). S-nitrosylation of the antioxidant peroxiredoxin 2 also inhibits its enzymatic activity, resulting in an accumulation of cellular peroxides. Given the neuronal enrichment of peroxiredoxin 2, its inactivation could increase the vulnerability of dopaminergic neurons to oxidative stress (Fang et al., 2007). Furthermore, S-nitrosylation of stress-responsive proteins such as protein-disulphide isomerase also inhibits its enzyme activity and attenuates protein-disulphide isomerase–dependent cytoprotective effects against ER stress, misfolded proteins, or proteasome inhibition (Uehara et al., 2006). Moreover, S-nitrosylation of ER stress sensors such as inositol-requiring transmembrane kinase/endoribonuclease 1z and protein kinase R-like endoplasmic reticulum kinase inhibit the ribonuclease and kinase activity, respectively, of these proteins, with an effect toward reduction of the UPR that contributes to neuronal death (Nakato et al., 2015). In addition to the few examples cited here, S-nitrosylation has been identified on a growing number of protein targets and thus represents a common pathologic feature that contributes to the onset and progression of neurodegeneration [reviewed in Nakamura et al. (2015)].

Since the maintenance of redox potential is crucial to neuronal survival, it is likely that imbalances in redox potential interface with other pathogenic mechanisms and in the process generate more ROS, ultimately culminating in neuronal demise. As such, excessive oxidative/nitrosative stress could play a causal role in the more common sporadic forms of PD and likely lower the threshold for the effects of rare genetic mutations to manifest. A crucial and integral part of PD therapy should therefore be focused on countering ROS- and RNS-mediated toxicity. In the brain, some sources of ROS generation are DA metabolism, mitochondrial dysfunction, and neuroinflammation (Chinta and Andersen, 2008; Hauser and Hastings, 2013). Therefore, protective mechanisms involved in regulating these processes could help design effective therapeutic interventions. It is also becoming clear that one or more mechanisms involved in regulating free iron levels are impaired in PD (Chinta and Andersen, 2008). Under these conditions, chelation of iron could represent a practicable means to reduce labile iron levels and attenuate the ensuing oxidative damage. In this regard, the iron chelator deferiprone has had promising effects in translational studies in mice and a small-scale clinical trial in patients with early-stage PD (Devos et al., 2014). Deferiprone treatment confers protection against oxidative insults on dopaminergic neurons and DA depletion in a MPTP mouse model. In human trials using the delayed-start treatment paradigm, deferiprone is associated with decreased nigral iron deposition and symptomatic improvement (Devos et al., 2014). Although the protective effects of iron chelating therapy on the integrity of the nigrostriatal system need further validation, studies like these highlight the potential to modulate neuronal iron accumulation and curb oxidative stress–associated toxicity with clinical benefits. Excessive nitrosative stress that increases the propensity for aberrant protein S-nitrosylation reactions can contribute to neurodegeneration in PD by disrupting a number of essential molecular pathways. As such, elucidation and functional characterization of the aberrant S-nitrosylated proteome in the PD brain will deepen our understanding of their pathologic roles and help identify viable mechanism-based therapeutic targets for drug discovery. Of note, S-nitrosylation of proteins can be neuroprotective or neurodegenerative depending on the context, the target protein, and the site of modification (Nakamura and Lipton, 2016). For instance, although S-nitrosylation of Parkin predominantly inhibits its function, S-nitrosylation of Parkin (at Cys323) has also been observed to increase its E3-
ligase activity to promote mitophagy upon mitochondrial depolarization (Ozawa et al., 2013). However, the potent oxidant peroxynitrite, which is formed when mitochondrial superoxide reacts with NO, denitrosylates Parkin and decreases mitophagy. This indicates that controlled activation of Parkin by S-nitrosylation under circumstances that compromise mitochondrial quality control could be a viable therapeutic modality (Ozawa et al., 2013). Another example involves S-nitrosylation of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which enhances binding of GAPDH with the ubiquitin E3 ligase Siah. This S-nitrosylated GAPDH/Siah complex then translocates to the nucleus and, by activating specific nuclear proteins, initiates apoptotic cell death (Hara et al., 2005). Incidentally, treatment with deprenyl, a drug that reportedly delays symptom progression in early PD, or a deprenyl derivative (CGP3466B) prevents S-nitrosylation of GAPDH and thus prevents the associated apoptotic cell death. However, CGP3466B has not yet proven to be neuroprotective in human clinical trials for PD (Hara et al., 2006). These studies nevertheless indicate that nitrosylation-based therapies should be aimed at specifically blocking the pathologic forms of S-nitrosylated proteins and augmenting the neuroprotective effects of NO without propagating its neurodestructive actions to improve their clinical utility.

XV. Repairing Defects in Synaptic Dysfunctions

Neuropathological studies of the PD brain and animal models support the idea that nigrostriatal synapses could be affected during the early stages of the neurodegenerative process. Many PD-associated genes are also implicated in synaptic function and trafficking at axon terminals. For instance, a regulatory function in synaptic vesicle release is ascribed to α-synuclein (Scott and Roy, 2012) that is predominantly localized to presynaptic terminals in the normal brain. Further molecular evidence linking α-synuclein to presynaptic function stems from studies showing its impact on vesicle fusion and soluble N-ethylmaleimidesensitive factor activating protein receptor complex assembly through interactions with different presynaptic proteins including synaptobrevin-2, synapsin III, and vesicular monoamine transporter 2 (VMAT2) (Burre et al., 2010), as well as DA and serotonin transporters (Lee et al., 2001; Wersinger and Sidhu, 2009). LRRK2 kinase activity is involved in the regulation of clathrin-mediated endocytosis of synaptic vesicles and neurotransmission (Shin et al., 2008; Matta et al., 2012; Arranz et al., 2015). LRRK2 also phosphorylates a subset of Rab GTPases (Steger et al., 2016; Jeong et al., 2018) that have crucial roles in intracellular vesicle trafficking (Stenmark, 2009). Pathogenic LRRK2 variants mapping to different functional domains in LRRK2 increase phosphorylation of these Rab GTPases and inhibit their activity, which could in turn perturb vesicle trafficking (Steger et al., 2016). VPS35 and retromers are involved in endosomal recycling of neurotransmitter receptors, and PD-linked mutations in VPS35 cause significant alterations in synaptic transmission (Munsie et al., 2015). A fully cosegregating mutation in the gene encoding transmembrane protein 230, a transmembrane protein of synaptic vesicles in neurons, has been linked to autosomal dominant parkinsonism, providing further genetic evidence that defects in synaptic vesicle trafficking could underlie PD pathogenesis (Deng et al., 2016). Localization of Parkin to synaptic vesicles at the presynaptic terminals (Kubo et al., 2001) and its enrichment at postsynaptic densities in the brain (Fallon et al., 2002) are indicative of a role for Parkin in synaptic transmission as well. Parkin is also observed to interact with and ubiquitinate a number of synaptic proteins and, as such, orchestrates a range of synaptic functions including neurotransmitter release, potentiation of excitatory ion channel currents, and regulation of synaptic vesicle docking/release [reviewed in Sassone et al. (2017)].

Within the dopaminergic nigrostriatal pathway, synaptic vesicles are involved in packaging transmitters for neurotransmission and sequestering compounds, such as cytosolic DA (Dunn et al., 2017). Confinement of DA to synaptic vesicles protects it from metabolic breakdown that would otherwise give rise to cytotoxic free radicals. In the dopaminergic terminals, the synaptic protein VMAT2 packages DA into synaptic vesicles and through continuous DA translocating activity, VMAT2 serves to keep cytosolic DA concentrations to a minimum (Lohr and Miller, 2014). Genetic reduction of VMAT2 in mice leads to age-related nigrostriatal DA dysfunction, which ultimately results in loss of nigral DA neurons (Caudle et al., 2007). VMAT2 mutation in humans leads to dramatic reduction in vesicular functions and is linked to an infantile parkinsonism-like condition typified by deficits in DA among other monoamines (Rilstone et al., 2013). Impaired vesicular uptake of DA is observed in the striatum of patients with PD (Piñeiro et al., 2014), and significantly lower VMAT2 densities are also observed in the putamen, caudate, and SN of patients with PD (Bohn et al., 2006; Okamura et al., 2010). Nonhuman primate models of MPTP intoxication exhibit selective loss of VMAT2 in the striatal dopaminergic synapses, even during the asymptomatic phase, suggesting that reduction in VMAT2 and DA storage capacity could represent early pathogenic events (Chen et al., 2008). Thus, defective DA handling appears to be a feature of PD, and if this abnormality is established during the preclinical stages of the disease, it could in turn lower the threshold for other pathways of dopaminergic neurodegeneration in patients with PD. A direct inference
from these studies is that DA neurotransmission could be enhanced by increasing vesicular packaging. Indeed, VMAT2 overexpression in mice leads to increased vesicle capacity and synaptic DA release and also reduces vulnerability to MPTP-induced toxic insults (Lohr et al., 2014) by sequestering the active metabolite MPP+ into vesicular lumens and away from its site of action at complex I of the mitochondria (Edwards, 1993). Of note, increased VMAT2 expression resulting from polymorphisms within the VMAT2 promoter region are associated with a reduced risk for PD (Glatt et al., 2006; Brighina et al., 2013). Besides the VMAT2 locus, variations within the synaptic vesicle glycoprotein 2C (SV2C) gene encoding for yet another synaptic protein appear to influence PD patient response to L-dihydroxyphenylalanine (Altmann et al., 2016). The normal distribution of human SV2C throughout the basal ganglia in dopaminergic regions of the SNpc and striatum is altered in patients with PD, and genetic deletion of SV2C in mice leads to significant reduction in striatal DA content, underscoring a presynaptic role for SV2C in regulating DA content and maintaining DA tone (Altmann et al., 2016). Together, these studies reinforce the notion that manipulating the vesicular capacity by therapeutically targeting synaptic proteins such as VMAT2 and SV2C could help sustain DA homeostasis and thereby preclude early nigrostriatal injury.

Several PD-linked genes have also been observed to disrupt presynaptic DA handling. In the absence of overt dopaminergic neuronal loss, nigral overexpression of α-synuclein in rats leads to impaired striatal DA release that progresses analogously with degenerative changes in the nigrostriatal axons and terminals (Gaugler et al., 2012; Lundblad et al., 2012). Such early changes in synaptic DA release suggest that gene mutations or factors that cause aberrant handling of DA may trigger a progressive degenerative process to which the axons and terminals succumb first. The ensuing synaptic dysfunction could then set forth further neuronal damage and cell loss, typical of the symptomatic and more advanced stages of PD (Lundblad et al., 2012). Cultured glutamatergic hippocampal pyramidal neurons and mesencephalic dopaminergic neurons moderately overexpressing α-synuclein also exhibit impairments in neurotransmitter release as a consequence of defective reclustering of synaptic vesicles near synaptic release sites after endocytosis (Nemani et al., 2010). This suggests that in vivo, α-synuclein overexpression could indiscriminately affect synaptic transmission in most, if not all, brain circuits, which could also account for the widespread neural network dysfunctions prevalent in PD. In α-synuclein–overexpressing mice, elevated extracellular DA concentrations in the striatum are observed to precede DA loss in the region and prior to DA neuron degeneration (Lam et al., 2011). Synaptic DA levels are regulated by DA transporter (DAT), which functions to remove DA from the synaptic cleft, thereby terminating DA signaling (Cavelli et al., 2008). Overexpression of wild-type α-synuclein in immortalized DA-like neurons decreases the magnitude and rate of substrate uptake by DAT, suggesting that interactions between α-synuclein and DAT could negatively impact DA transmission (Swant et al., 2011). Nigral administration of human wild-type α-synuclein in rats also leads to significant reductions in DA reuptake prior to the onset of any overt signs of axonal damage, consistent with early dysfunctions of DAT (Lundblad et al., 2012). High concentrations of exogenous DA in the striatum are observed to be selectively toxic to dopaminergic terminals through production of ROS and reactive quinone intermediates that inactivate detoxifying enzymes by binding their cysteinyl residues (Hastings et al., 1996; Rabinovic et al., 2000), indicating that modulation of DAT activity and DA transmission by α-synuclein could result in extracellular neurotoxic effects. Moreover, both wild-type and A53T mutant α-synuclein are observed to decrease VMAT2 expression and vesicular activity in cultured dopaminergic cell lines and contribute to intracellular superoxide production (Lotharius et al., 2002; Fountaine et al., 2008; Guo et al., 2008). Besides α-synuclein, loss of PINK1 or Parkin function is also observed to impinge on DA utilization. In midbrain DA neurons derived from patients with PD, Parkin mutations increase spontaneous release of DA while decreasing DA uptake via DAT (Jiang et al., 2012). Germline-deleted Parkin mouse models also exhibit increased spontaneous DA release from nigral neurons in the absence of dopaminergic degeneration (Goldberg et al., 2003). On the contrary, PINK1 inactivation in mouse models impairs DA release in the striatum, which leads to deficits in corticostriatal long-term potentiation and long-term depression (LTD) (Kitada et al., 2007). Defects in corticostriatal LTD resulting from increased DA uptake also persist in DJ-1–deficient mice (Goldberg et al., 2005). Such alterations to the spatial and temporal precision of DA transmission could augment DA-induced oxidative stress in the nigrostriatal region. Collectively, these studies indicate that disruption of synaptic DA homeostasis could initiate protracted, low-grade DA toxicity in nigral DA neurons and serve as precursors to dopaminergic denervation and eventual cell loss. Thus, disease-modifying therapies aimed at restoring optimal synaptic DA transmission could have therapeutic benefit. In this regard, Parkin is observed to control oxidative stress through ubiquitination and degradation of estrogen-related receptors (ERRs) that promote transcription of monoamine oxidases, the mitochondrial enzymes involved in DA oxidation (Ren et al., 2011). Monoamine oxidase-B inhibitors have neuroprotective effects and have demonstrated a modest but significant effect toward slowing down the progression of PD (Olanow et al., 2009). Similar neuroprotective effects could be achieved in the absence of Parkin through ERR-specific antagonists such as XCT790 (Willy et al., 2004) or selective small-molecule modulators
of ERR. Parkin also enhances DAT expression on the cell surface expression by ubiquitinating and degrading misfolded DAT (Jiang et al., 2004). As such, pharmacological agents with the potential to enhance native folding of DAT and/or eliminate misfolded conformers of DAT and thereby promote DA uptake could potentially substitute for Parkin. Similarly, agents that stimulate DA release or DA receptor agonists could help rectify impairments in bidirectional corticostral synaptic plasticity.

Within the basal ganglia circuitry, other neurotransmitter systems—namely, GABAergic, cholinergic, and glutamatergic—function together with the dopaminergic system. In PD, glutamatergic transmission is also considerably affected and contributes to neurotransmission alterations prevalent in the disorder (Martin, 2007). SN DA neurons exhibit heightened responsiveness to excessive levels of extracellular glutamate that can hyperactivate the ionotropic glutamatergic receptors located on these neurons (Sassone et al., 2017). This hyperactive pattern triggers a cascade of excitotoxic events that contribute to the neurodegenerative process. Neuronal delayed calcium accumulation and mitochondrial membrane depolarization are key mediators of such glutamate-induced neuronal loss (Ambrosi et al., 2014; Dawson and Dawson, 2017). Excessive stimulation of glutamate receptors can also increase NO generation with detrimental consequences (Penttice et al., 2015; Dawson and Dawson, 2018). Moreover, nigrostriatal dopaminergic depletion can cause overactivation of glutamatergic projections to the basal ganglia and striatal release of glutamate, resulting in excitotoxicity (Blandini et al., 2000; Centonze et al., 2005). In fact, DA at lower concentrations is observed to inhibit glutamate toxicity and neuronal death by preventing the onset of calcium deregulation (Vaaermann et al., 2013). The striatal depletion of DA in PD could thus result in uninhibited glutamate-mediated calcium signaling in the nigrostriatal pathway and sustain the progression of the degenerative process. Neuroprotective effects against glutamatergic insults are also exerted by Parkin, which regulates the strength and abundance of postsynaptic excitatory synapses to curb glutamatergic excitatory synaptic inputs, demonstrable in cultured rat hippocampal neurons (Helton et al., 2008). Lack of such regulation of synaptic strength and connectivity in the absence of Parkin activity could increase neuronal vulnerability to synaptic excitotoxicity. Similarly, disease-linked mutations in Parkin could enhance excitatory synapses that alter the neural circuitry and thereby contribute to neuronal loss in PD (Helton et al., 2008). Both monomeric and filamentous forms of z-synuclein are observed to preferentially increase calcium/depolarization-dependent glutamate release from mouse forebrain synaptoneurosomes enriched in synaptic endings (Sarafian et al., 2017). Large z-synuclein oligomers also engage glutamate receptors to enhance both pre- and postsynaptic neurotransmission, thereby perturbing intracellular calcium homeostasis in neuronal cultures and contributing to excitotoxic cell death (Huls et al., 2011). Similarly, cortical cultures expressing the G2019S LRRK2 mutant exhibit elevated glutamate release in the absence of any discernible effect on synaptic density (Beccano-Kelly et al., 2014). Such imbalances between excitatory and inhibitory neurotransmitters perpetuated by PD-linked genes/factors are likely to impact the threshold for the induction of glutamate and calcium-dependent synaptic plasticity in the striatum. For instance, electrophysiological studies in 6-OHDA rat models of PD show that partial dopaminergic denervation, reminiscent of early stages of PD, dramatically alters the maintenance of long-term potentiation, whereas complete dopaminergic denervation, representative of advanced stages of PD, abolishes corticostriatal LTD (Paillé et al., 2010). This indicates that the strength of glutamatergic signaling is dynamically regulated during the disease progression and that the integrity of nigrostriatal dopaminergic pathway is critical for the maintenance of long-lasting synapses in the striatum. Together, these studies raise the possibility that strategies targeting the glutamate-induced excitotoxicity pathway could have therapeutic benefit. Indeed, blockage of the ionotropic NMDA and z-amino-3-hydroxy-5-methyl-4-isoxazole-propionate receptors that are mainly responsible for glutamate-induced excitotoxicity is observed to have antiparkinsonian action in mouse and primate models of PD (St-Pierre and Bédard, 1995; Nash et al., 2000; Steece-Collier et al., 2000). Selective noncompetitive antagonists of the metabotropic glutamate receptor subtype 5 also ameliorate parkinsonian symptoms in rat models of PD (Ossowska et al., 2001). Furthermore, alterations of the subthalamic nucleus that sends glutamatergic projections to the SN either by lesion or deep brain stimulation offer neuroprotection to the dopaminergic neurons in MPTP-treated primates by reducing subthalamic nucleus firing and blunting release of glutamate onto the SN (Wallace et al., 2007). Glutamate receptor stimulation is also involved in promoting motor complications resulting from chronic administration of levodopa in patients with PD. As such, NMDA or z-amino-3-hydroxy-5-methyl-4-isoxazole-propionate antagonists as well as nonselective inhibitors of glutamatergic transmission block the development of levodopa-induced motor complications in rodent and nonhuman primate models of PD (Calon et al., 2003). Studies targeting glutamate excitotoxicity as a disease-modifying or symptomatic therapy in PD have not been successful. This is likely due to intolerable side effects of glutamate receptor antagonism (Zhang et al., 2019). Amantadine, a noncompetitive antagonist of NMDA receptor is currently the only antiglutamatergic pharmacotherapy used in human PD treatment and, as an adjuvant to levodopa, reduces dyskinesia severity...
Similar antidyskinetic effects are observed with the NMDA subunit glutamate ionotropic receptor NMDA type subunit 2A-selective antagonist CP-101,606 (Nutt et al., 2008). Memantine, a partial NMDA antagonist approved for moderate to severe Alzheimer disease treatment, also improves parkinsonian symptoms independently of other dopaminergic drugs (Merello et al., 1999; Aarsland et al., 2009a; Emre et al., 2010). Although these studies underscore the utility of targeting glutamatergic neurotransmission in the development of disease-modifying therapies in PD, the challenge ahead lies in the identification of compounds that are highly selective in their mode of action toward specific types of glutamate receptors and exhibit high tolerability while sustaining the normal physiologic functions of such receptors to avert possible adverse side effects.

Glutamate concentrations in the synaptic cleft and postsynaptic ionotropic glutamate receptor activity is tightly regulated by glutamate release and its clearance, the latter being mediated primarily by astrocytes. Excitatory amino acid transporters (EAATs) play a major role in the clearance of excessive synaptic glutamate. Of the five mammalian EAATs, glutamate transporter 1 (GLT-1, also known as EAAT2) is responsible for ~90% of glutamate uptake by astrocytes (Lin et al., 2012). Decreased glutamate transporter expression and glutamate uptake are observed in 6-OHDA and MPTP rodent models of PD (Aoyama et al., 2008; Chung et al., 2008). Besides clearing extracellular glutamate, EAATs also provide substrate for the synthesis of the major cellular antioxidant, glutathione (Hayes et al., 2005). EAAT dysfunction could thus sustain oxidative stress in addition to excitotoxicity. Indeed, nigral administration of a substrate inhibitor of EAATs in rats decreases the availability of glutathione precursors and leads to preferential loss of DA neurons, likely by lowering the resistance threshold for glutamate excitotoxicity (Nafia et al., 2008; Assous et al., 2014). Collectively, these studies indicate that increasing the activity of glutamate transporters could prevent excitotoxic cell death as well as curtail oxidative stress in PD.

In this regard, many β-lactam antibiotics appear to be potent stimulators of GLT-1 expression and increase transcription of the GLT-1 gene. In particular, the β-lactam ceftriaxone increases brain expression of GLT-1 and its function in wild-type rodent models (Rothstein et al., 2005), and its beneficial therapeutic effects have also been observed in rodent models of amyotrophic lateral sclerosis, Huntington disease, and spinal muscular atrophy (Hsu et al., 2015). Ceftriaxone also increases glutamate uptake by upregulating GLT-1 in astrocytes and alleviates MPP⁺-induced neurotoxicity through suppression of NF-κB/c-Jun N-terminal kinase/c-Jun signaling (Zhang et al., 2015). Improvements in behavioral motor functions are also observed after ceftriaxone treatment in MPTP-infused rats through upregulation of GLT-1 expression (Kaur and Prakash, 2017). The neuroprotective effects observed with ceftriaxone suggest that it might be possible to design and develop more potent derivatives of ceftriaxone with enhanced pharmacological properties that can cross the BBB more readily to counter the detrimental excitotoxic effects. Recent screenings of small-molecule activators of EAAT2 expression identified compounds such as LDN/OSU-0212320 and harmine with pharmacokinetic properties that conferred protection against glutamate-induced excitotoxicity in cultured neurons as well as neuroprotection in vivo in animal models of amyotrophic lateral sclerosis (Li et al., 2011; Kong et al., 2014). Although it remains to be seen whether such compounds are effective in alleviating PD symptoms, studies such as these highlight the therapeutic possibilities in small molecules that can potentially enhance glutamate transporter functions for disease-modifying treatments in PD. Trafficking of glutamate transporters between the membrane and cytosol is also regulated by ubiquitination, as is observed in the case of the E3 ligase Nedd4-2, which decreases the membrane expression of GLT-1 and its function. Notably, knockdown of Nedd4-2 increases GLT-1 expression in MPP⁺-treated astrocytes, promotes glutamate uptake, and also ameliorates movement disorders and DA neuron loss in an MPTP mouse model of PD, raising the possibility that selective modulators of Nedd4-2 could have therapeutic success in PD treatment (Zhang et al., 2017). Collectively, these studies indicate that screening paradigms aimed at identifying modulators of glutamate transporters could serve as early entry points for developing new classes of neuroprotective drugs that function by potentially regulating synaptic levels of glutamate (Kim et al., 2011).

### XVI. Neurotrophic Factors May Repair Dopaminergic Neurons

Although we have been discussing the numerous pathways through which dopaminergic neurons degrade and methods of stopping that degradation, treatment of PD may also involve supporting the remaining DA neurons through neurotrophic factors. Neurotrophic factors work to induce, support, and protect neurons and lead to their specification, survival, and maturation. It therefore follows that providing further support for dopaminergic neurons with neurotrophic factors would decrease dopaminergic cell death and slow the disease course. Although there are several neurotrophic families, the glial cell line–derived neurotrophic factor (GDNF) family of ligands, which includes GDNF and neurturin, is the farthest along for use in the treatment of PD. In fetal human and rat cortical cultures, GDNF has been shown to support dopaminergic neurons through numerous lines of evidence, including induction of tyrosine

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(Verhagen Metman et al., 1998; Del Dotto et al., 2001).

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hydroxylation and promotion of the survival and differentiation of dopaminergic neurons (Sullivan and Toulouse, 2011).

In early studies on nonhuman primate models, lentiviral delivery of GDNF showed functional improvement in animals when GDNF was delivered directly to the striatum and SN (Kordower et al., 2000). A subsequent open-label, phase I trial in humans showed clinical improvement with direct GDNF delivery into the putamen of five patients with PD (Gill et al., 2003). Given these promising results, a small randomized controlled trial was undertaken to evaluate the safety and efficacy of recombinant human GDNF infusion into the putamen of the patients. Among the 34 participants in this study, there was not a significant difference in off time between those receiving the GDNF and placebo, although there was a difference in F-dopa influx constant (Lang et al., 2006). Neurturin also has potential as a neurotrophic factor treatment of PD. In two separate randomized trials, neurturin was injected into both the bilateral putamen and bilateral SN pars compacta of individuals with moderate to advanced stage. Delivery was with a stereotactic injection of a viral vector, adeno-associated virus (AAV) serotype 2. The AAV2-neurturin combination was generally found to be safe and tolerable to patients. However, no significant differences in efficacy endpoints (change in UPDRS part III) were observed between those who received AAV2-neurturin injections into the putamen and SN versus sham surgery (Warren Olanow et al., 2015). Subsequent long-term autopsy follow-up (8 and 10 years after injection) of two of the participants in this investigation showed persistent transgene expression in the SN, although there was no difference in Lewy pathology between treated and untreated patients (Chu et al., 2020). The disappointing clinical findings may be related to the disease stage at which the injection occurred such that DA cell loss and dysfunction was great enough that the neurons could not respond to neurotrophic therapy (Chu et al., 2020).

A new approach based on reprogramming astrocytes to DA neurons has been shown to be promising, as in the early studies of growth factors in animal models of PD (Qian et al., 2020). Although exploration into neurotrophic factors and genetic reprogramming of astrocytes into DA neurons is now ongoing, these approaches rest on the clinical assumption that growth of the DA cells will successfully treat the symptoms of PD. Although the motor symptoms of PD are likely related to DA cell degeneration, the nonmotor symptoms, including cognitive changes, are more likely due to α-synuclein accumulation in the cortex, as discussed previously. Therefore, DA cell regeneration would be an important step toward disease modification but would not impact some of the more disabling aspects of the disease.

XVII. Conclusion

Although described by James Parkinson as a single disease, PD is a clinically and pathophysiologically variable disease. Bridging the connection between the underlying pathophysiology and the clinical heterogeneity is a necessary next step toward the development of disease-modifying therapies and ultimately a cure for the disease. Biomarkers will help us toward this goal by informing clinical trials, but identification of better biomarkers will also be informed by our growing understanding of the different disease mechanisms. Aberrant α-synuclein is emerging as possibly a common endpoint to a number of different disease pathophysiologies and the inciting factor toward others, including inflammatory pathways and increased stress on the neuron. The subsequent and related mitochondrial dysfunction and oxidative and nitrosylated stress, as well as the other pathways that we discussed, ultimately lead to cell death. The unique susceptibilities of the dopaminergic neurons also seem to make them inherently more vulnerable than other neuronal populations. Finally, genetic and environmental influences overlay and contribute to all of the pathophysiologic changes in ways that we are still investigating.

The profound pathophysiologic heterogeneity is reflected in the phenotypic heterogeneity of the disease. Our patients have variable symptoms ranging from bothersome tremors to severe rigidity to significant changes in balance. Determining the degree to which these different phenotypes are reflective of specific underlying pathophysiologies is unknown and would be incredibly instructive toward disease-modifying therapies. It is likely that PD symptomatology can be clustered into specific phenotypes that we are still working to determine. One ongoing division is between tremor dominant and akinetic rigid, but patients are actually quite fluid in that they move between these designations. The large patient data sets that are being created through platforms including the National Parkinson’s Foundation’s Parkinson’s Outcomes Project, as well as the investigations of PPMI, PDPB, and others, will facilitate the clustering of patients into specific groups with higher-level statistical techniques. Importantly, the current assessment methods may not allow for this clustering; rather, we may need to incorporate the growing interest into wearables and other new technologies to truly understand our patients and their different phenotypic clusters.

Authorship Contributions

Participated in research design: Pirrooznia, Rosenthal, V. L. Dawson, T. M. Dawson.
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