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Parkinson's Disease: Mechanisms and Models

Review

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Parkinson's disease (PD) results primarily from the death of dopaminergic neurons in the substantia nigra. Current PD medications treat symptoms; none halt or retard dopaminergic neuron degeneration. The main obstacle to developing neuroprotective therapies is a limited understanding of the key molecular events that provoke neurodegeneration. The discovery of PD genes has led to the hypothesis that misfolding of proteins and dysfunction of the ubiquitin-proteasome pathway are pivotal to PD pathogenesis. Previously implicated culprits in PD neurodegeneration, mitochondrial dysfunction and oxidative stress, may also act in part by causing the accumulation of misfolded proteins, in addition to producing other deleterious events in dopaminergic neurons. Neurotoxin-based models (particularly MPTP) have been important in elucidating the molecular cascade of cell death in dopaminergic neurons. PD models based on the manipulation of PD genes should prove valuable in elucidating important aspects of the disease, such as selective vulnerability of substantia nigra dopaminergic neurons to the degenerative process.

Introduction

In his classic 1817 monograph "Essay on the Shaking Palsy," James Parkinson described the core clinical features of the second most common age-related neurodegenerative disease after Alzheimer's disease (AD). Although more than a century passed before the central pathological feature of Parkinson's disease (PD) was found to be the loss of neurons in the substantia nigra pars compacta (SNpc), the pace of discovery accelerated following Arvid Carlsson's 1958 discovery of dopamine (DA) in the mammalian brain. SNpc neurons were then found to form the nigrostriatal dopaminergic pathway, and this line of research culminated with two key discoveries. First, loss of SNpc neurons leads to striatal DA deficiency, which is responsible for the major symptoms of PD. Second, replenishment of striatal DA through the oral administration of the DA precursor levodopa (L-3,4-dihydroxyphenylalanine) alleviates most of these symptoms.

Although the discovery of levodopa revolutionized the treatment of PD, we soon learned that after several years of treatment most patients develop involuntary movements, termed "dyskinesias," which are difficult to control and significantly impair the quality of life. Current research is directed toward prevention of dopaminergic neuron degeneration. Nevertheless, despite advances toward this goal, all current treatments are symptomatic; none halt or retard dopaminergic neuron degeneration.

The main obstacle in the development of neuroprotective drugs is ignorance of the specific molecular events that provoke neurodegeneration in PD. Prior to the last 5 years, most of the current hypotheses about the etiology and pathogenesis of PD derived from postmortem tissue or neurotoxic animal models, most notably, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurodegeneration. Exposure of humans to MPTP causes a syndrome that mimics the core neurological symptoms and relatively selective dopaminergic neurodegeneration of PD, and MPTP toxicity in mice is the most commonly studied animal model of PD. These studies have focused on three types of cellular dysfunction that may be important in the pathogenesis of PD: oxidative stress, mitochondrial respiration defect, and abnormal protein aggregation. In addition, the MPTP monkey model has yielded valuable information regarding the functional alterations in basal ganglia circuits that occur subsequent to striatal DA depletion, and this model remains the gold standard for the preclinical evaluation of new therapies aimed at alleviating the symptoms of PD. While many findings from MPTP studies have been confirmed in human PD brains, there is intense debate about the relationship between MPTP and PD neurodegeneration.

This situation changed in 1997 with the discovery that mutations in the gene for α-synuclein cause an inherited form of PD. In just 5 years since this breakthrough, three additional PD-causing genes have been identified, and linkage has been reported for three more. As in AD, these rare PD genes appear to operate through a common molecular pathway, and their discovery may lead to the creation of novel animal models for the study of PD pathogenesis. It will also be important to determine whether these pathogenic proteins participate in the molecular events leading to neurodegeneration in existing animal models of PD, in order to evaluate how closely these models mimic the pathogenic events of the human disease.

Here, after discussing clinical and neuropathological characteristics of PD, we review current concepts of the etiology and pathogenesis of PD. We then focus on animal models of PD, evaluating how both well-established toxin-induced models and newer genetic models have contributed to the understanding of PD.

Clinical Characteristics of PD

PD is a progressive disease with a mean age at onset of 55, and the incidence increases markedly with age, from 20/100,000 overall to 120/100,000 at age 70. In about 95% of PD cases, there is no apparent genetic linkage (referred to as "sporadic" PD), but in the remaining cases, the disease is inherited. Over time, symptoms worsen, and prior to the introduction of levodopa, the mortality rate among PD patients was three times that of the normal age-matched subjects. While levo-

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Table 1. Parkinsonian Syndromes

Primary Parkinsonism
Parkinson disease (sporadic, familial)
Secondary Parkinsonism
Drug-induced: dopamine antagonists and depletors
Hemiatrophy-hemiparkinsonism
Hydrocephalus: normal pressure hydrocephalus
Hypoxia
Infectious: postencephalitic
Metabolic: parathyroid dysfunction
Toxin: Mn, CO, MPTP, cyanide
Trauma
Tumor
Vascular: multiinfarct state
Parkinson-plus Syndromes
Cortical-basal ganglionic degeneration
Dementia syndromes: Alzheimer disease, diffuse Lewy body disease, frontotemporal dementia
Lytico-Bodig (Guamanian Parkinsonism-dementia-ALS)
Multiple system atrophy syndromes: striatonigral degeneration, Shy-Drager syndrome, sporadic olivopontocerebellar degeneration (OPCA), motor neuron disease-parkinsonism
Progressive pallidal atrophy
Progressive supranuclear palsy
Familial Neurodegenerative Diseases
Hallervorden-Spatz disease
Huntington disease
Lubag (X-linked dystonia-parkinsonism)
Mitochondrial cytopathies with striatal necrosis
Neuroacanthocytosis
Wilson disease

MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; ALS, amytrophic lateral sclerosis.

dopa has dramatically improved the quality of life for PD patients, population-based surveys suggest that these patients continue to display decreased longevity compared to the general population (Hely et al., 1989; Morgante et al., 2000; Levy et al., 2002). Furthermore, most PD patients suffer considerable motor disability after 5–10 years of disease, even when expertly treated with available symptomatic medications.

Clinically, any disease that includes striatal DA deficiency or direct striatal damage may lead to "parkinsonism," a syndrome characterized by tremor at rest, rigidity, slowness or absence of voluntary movement, postural instability, and freezing (Table 1). PD is the most common cause of parkinsonism, accounting for ~80% of cases.

PD tremor occurs at rest but decreases with voluntary movement, so typically does not impair activities of daily living. Rigidity refers to the increased resistance (stiffness) to passive movement of a patient's limbs. Bradykinesia (slowness of movement), hypokinesia (reduction in movement amplitude), and akinesia (absence of normal unconscious movements, such as arm swing in walking) manifest as a variety of symptoms, including paucity of normal facial expression (hypomimia), decreased voice volume (hypophonia), drooling (failure to swallow without thinking about it), decreased size (micrographia) and speed of handwriting, and decreased stride length during walking. Bradykinesia may significantly impair the quality of life because it takes much longer to perform everyday tasks such as dressing or eating. PD patients also typically develop a stooped posture and may lose normal postural reflexes, leading to falls and, sometimes, confinement to a wheelchair. Freezing, the inability to begin a voluntary movement such as walking (i.e., patients remain "stuck" to the ground as they attempt to begin moving), is a common symptom of parkinsonism. Abnormalities of affect and cognition also occur frequently; patients may become passive or withdrawn, with lack of initiative; they may sit quietly unless encouraged to participate in activities. Responses to questions are delayed, and cognitive processes are slowed ("bradyphrenia"). Depression is common, and dementia is significantly more frequent in PD, especially in older patients.

Neurochemical and Neuropathological Features of PD

The pathological hallmarks of PD are the loss of the nigrostriatal dopaminergic neurons and the presence of intraneuronal proteinacious cytoplasmic inclusions, termed "Lewy Bodies" (LBs) (Figure 1). The cell bodies of nigrostriatal neurons are in the SNpc, and they project primarily to the putamen. The loss of these neurons, which normally contain conspicuous amounts of neuromelanin (Marsden, 1983), produces the classic gross neuropathological finding of SNpc depigmentation (Figure 1B). The pattern of SNpc cell loss appears to parallel the level of expression of the DA transporter (DAT) mRNA (Uhl et al., 1994) and is consistent with the finding that depletion of DA is most pronounced in the dorsolateral putamen (Bernheimer et al., 1973), the main site of projection for these neurons. At the onset of symptoms, putamenal DA is depleted ~80%, and ~60% of SNpc dopaminergic neurons have already been lost. The mesolimbic dopaminergic neurons, the cell bodies of which reside adjacent to the SNpc in the ventral tegmental area (VTA), are much less affected in PD (Uhl et al., 1985). Consequently, there is significantly less depletion of DA in the caudate (Price et al., 1978), the main site of projection for these neurons.

Neuropathological studies of PD-related neurodegeneration suggest possible clues to the pathogenesis of the disease. First, PD-associated loss of dopaminergic neurons has a characteristic topology, distinct from the pattern seen in normal aging. In PD, cell loss is concentrated in ventrolateral and caudal portions of the SNpc, whereas during normal aging the dorsomedial aspect of SNpc is affected (Fearnley and Lees, 1991). Thus, even though age is an important risk factor for PD, the processes that produce age-related dopaminergic neuronal death are probably different from those in PD. Second, the degree of terminal loss in the striatum appears to be more pronounced than the magnitude of SNpc dopaminergic neuron loss (Bernheimer et al., 1973), suggesting that striatal dopaminergic nerve terminals are the primary target of the degenerative process and that neuronal death in PD may result from a "dying back" process. Experimental support for the concept of dying back includes the observations that in MPTP-treated monkeys the destruction of striatal terminals precedes that of SNpc cell bodies (Herkenham et al., 1991), and in MPTP-treated mice, protection of striatal terminals prevents the loss of SNpc dopaminergic neurons (Wu et al., 2003). Third, the mechanism of

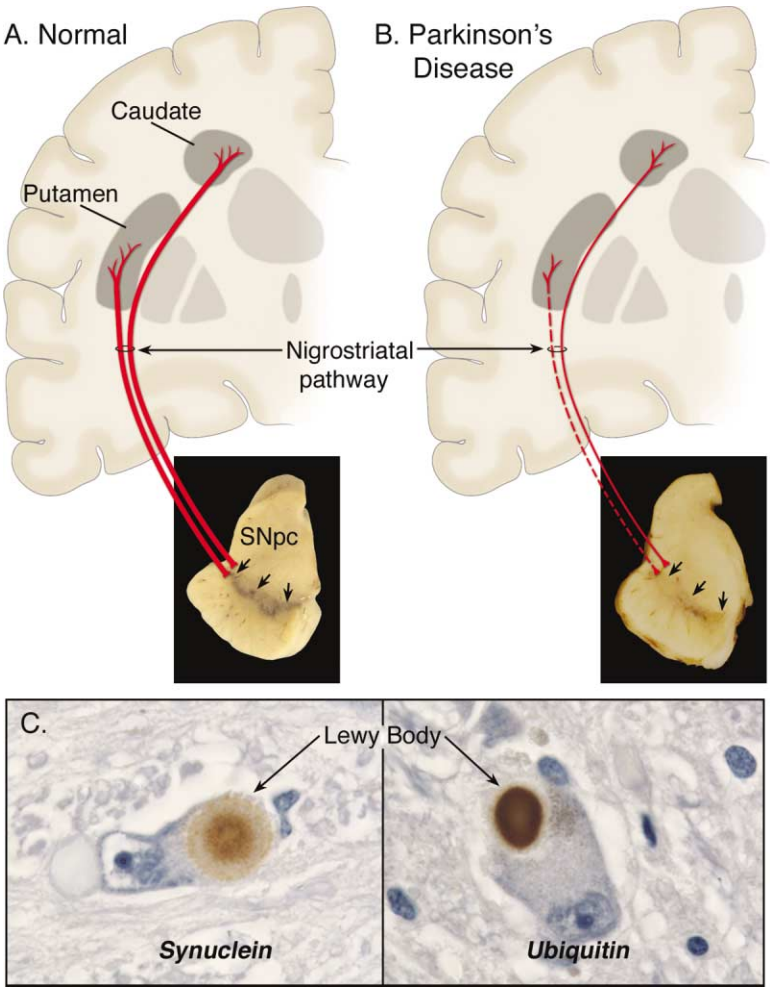

Figure 1. Neuropathology of Parkinson's Disease

(A) Schematic representation of the normal nigrostriatal pathway (in red). It is composed of dopaminergic neurons whose cell bodies are located in the substantia nigra pars compacta (SNpc; see arrows). These neurons project (thick solid red lines) to the basal ganglia and synapse in the striatum (i.e., putamen and caudate nucleus). The photograph demonstrates the normal pigmentation of the SNpc, produced by neuromelanin within the dopaminergic neurons.

(B) Schematic representation of the diseased nigrostriatal pathway (in red). In Parkinson's disease, the nigrostriatal pathway degenerates. There is a marked loss of dopaminergic neurons that project to the putamen (dashed line) and a much more modest loss of those that project to the caudate (thin red solid line). The photograph demonstrates depigmentation (i.e., loss of dark-brown pigment neuromelanin; arrows) of the SNpc due to the marked loss of dopaminergic neurons.

(C) Immunohistochemical labeling of intraneuronal inclusions, termed Lewy bodies, in a SNpc dopaminergic neuron. Immunostaining with an antibody against α-synuclein reveals a Lewy body (black arrow) with an intensely immunoreactive central zone surrounded by a faintly immunoreactive peripheral zone (left photograph). Conversely, immunostaining with an antibody against ubiquitin yeilds more diffuse immunoreactivity within the Lewy body (right photograph).

synaptic DA clearance in the striatum seems to be more dependent on DAT than in the prefrontal cortex, where other monoaminergic transporters and the synaptic enzyme catechol-*O*-methyltransferase play a greater role in terminating the actions of DA (Giros et al., 1996; Gogos et al., 1998; Mundorf et al., 2001). The prefrontal cortex is a primary site of projection for VTA dopaminergic neurons, so this difference may be of importance in understanding the relative resistance of VTA neurons to PD-related degeneration. Differences in neuronal milieu have also been identified surrounding SNpc dopaminergic cell bodies. The neuropil of the substantia nigra, composed of axon projections from the striatum and globus pallidus, stains strongly for calbindin D_{28K}, and most dopaminergic cell bodies reside within this calbindin-rich neuropil (Damier et al., 1999a). However, the susceptible neurons in PD tend to be in calbindin-poor areas of the substantia nigra (Damier et al., 1999b).

Although it is commonly thought that the neuropathology of PD is characterized solely by dopaminergic neuron loss, the neurodegeneration extends well beyond dopaminergic neurons (reviewed by Hornykiewicz and Kish, 1987). Neurodegeneration and LB formation are found in noradrenergic (locus coeruleus), serotonergic (raphe), and cholinergic (nucleus basalis of Meynert, dorsal motor nucleus of vagus) systems, as well as in the cerebral cortex (especially cingulate and entorhinal cortices), olfactory bulb, and autonomic nervous system. Degeneration of hippocampal structures and cholinergic cortical inputs contribute to the high rate of dementia that accompanies PD, particularly in older patients. However, the clinical correlates of lesions to the serotonergic and noradrenergic pathways are not as clearly characterized as are lesions in the dopaminergic systems. Thus, while involvement of these neurochemical systems is generally thought to occur in more severe or late-stage disease, the temporal relationship of damage to specific neurochemical systems is not well established. For example, some patients develop depression months or years prior to the onset of PD motor symptoms, which could be due to early involvement of nondopaminergic pathways.

In life, the diagnosis of PD is made on clinical grounds, but definite diagnosis requires the identification of both LB and SNpc dopaminergic neuron loss. LBs are not specific for PD, however, and are also found in AD, in a condition called "dementia with LB disease," and as an incidental pathologic finding in people of advanced age at a greater frequency than the prevalence of PD (Gibb and Lees, 1988). The role of LB in neuronal cell death is controversial, as are the reasons for their increased frequency in AD and the relationship of inciden-

tal LB to the occurrence of PD. LBs are spherical eosinophilic cytoplasmic protein aggregates composed of numerous proteins (Figure 1C), including α-synuclein, parkin, ubiquitin, and neurofilaments, and they are found in all affected brain regions (Forno, 1996; Spillantini et al., 1998). LBs are more than 15 μm in diameter and have an organized structure containing a dense hyaline core surrounded by a clear halo. Electron microscopy reveals a dense granulovesicular core surrounded by a ring of radiating 8–10 nm fibrils (Duffy and Tennyson, 1965; Pappolla, 1986).

Etiology of PD

The cause of sporadic PD is unknown, with uncertainty about the role of environmental toxins and genetic factors. The environmental toxin hypothesis was dominant for much of the 20th century, especially because of the example of postencephalitic PD (as described in the Oliver Sacks' book *Awakenings*) and the discovery of MPTP-induced parkinsonism. However, the discovery of PD genes (reviewed in "Gene-Based Models" section below) has renewed interest in hereditary susceptibility factors. Both probably play a role.

The environmental hypothesis posits that PD-related neurodegeneration results from exposure to a dopaminergic neurotoxin. Theoretically, the progressive neurodegeneration of PD could be produced by chronic neurotoxin exposure or by limited exposure initiating a self-perpetuating cascade of deleterious events. The finding that people intoxicated with MPTP develop a syndrome nearly identical to PD (Langston et al., 1983) is a prototypic example of how an exogenous toxin can mimic the clinical and pathological features of PD. Paraquat is structurally similar to 1-methyl-4-phenylpyridinium (MPP+), the active metabolite of MPTP, and has been used as herbicide. Like MPP+, rotenone is also a mitochondrial poison present in the environment, and it is used as an insecticide and to kill unwanted lake fish. Human epidemiological studies have implicated residence in a rural environment and related exposure to herbicides and pesticides with an elevated risk of PD (Tanner, 1992). Yet, there are no convincing data to implicate any specific toxin as a cause of sporadic PD, and chronic environmental exposure to MPP+ or rotenone is unlikely to cause PD. MPP+'s quaternary ammonium cation prevents its passage across the blood-brain barrier, and rotenone is so unstable in solution that it only lasts a few days in lakes (Hisata, 2002). Still, cigarette smoking and coffee drinking are inversely associated with the risk for development of PD (Hernan et al., 2002), reinforcing the concept that some environmental factors do modify PD susceptibility.

Another possibility, which does not fit neatly into a genetic or environmental category, is that an endogenous toxin may be responsible for PD neurodegeneration. Distortions of normal metabolism might create toxic substances because of environmental exposures or inherited differences in metabolic pathways. One source of endogenous toxins may be the normal metabolism of DA, which generates harmful reactive oxygen species (ROS) (Cohen, 1984). Consistent with the endogenous toxin hypothesis is the report that patients harboring specific polymorphisms in the gene encoding the xenobiotic detoxifying enzyme cytochrome P450 may be at greater risk of developing young-onset PD (Sandy et al., 1996). Further, isoquinoline derivatives toxic to dopaminergic neurons have been recovered from PD brains (Nagatsu, 1997).

Pathogenesis of PD

Whatever insult initially provokes neurodegeneration, studies of toxic PD models and the functions of genes implicated in inherited forms of PD suggest two major hypotheses regarding the pathogenesis of the disease. One hypothesis posits that misfolding and aggregation of proteins are instrumental in the death of SNpc dopaminergic neurons, while the other proposes that the culprit is mitochondrial dysfunction and the consequent oxidative stress, including toxic oxidized DA species.

The pathogenic factors cited above are not mutually exclusive, and one of the key aims of current PD research is to elucidate the sequence in which they act and whether points of interaction between these pathways are key to the demise of SNpc dopaminergic neurons. Potential points of interaction are diagrammed in Figure 2. The finding that oxidative damage to α-synuclein can enhance its ability to misfold and aggregate is one example of such an interaction (Giasson et al., 2000). Another uncertain issue is whether the multiple cell death-related molecular pathways activated during PD neurodegeneration ultimately engage common downstream machinery, such as apoptosis, or remain highly divergent. Clearly, this issue is of great consequence in deciding about possible therapeutic strategies for PD.

Misfolding and Aggregation of Proteins

The abnormal deposition of protein in brain tissue is a feature of several age-related neurodegenerative diseases, including PD. Although the composition and location (i.e., intra- or extracellular) of protein aggregates differ from disease to disease, this common feature suggests that protein deposition per se, or some related event, is toxic to neurons.

Aggregated or soluble misfolded proteins could be neurotoxic through a variety of mechanisms. Protein aggregates could directly cause damage, perhaps by deforming the cell or interfering with intracellular trafficking in neurons. Protein inclusions might also sequester proteins that are important for cell survival. If so, there should be a direct correlation between inclusion formation and neurodegeneration. However, a growing body of evidence, particularly from studies of Huntington disease (HD) and other polyglutamine diseases (Saudou et al., 1998; Cummings et al., 1999), suggests that there is no correlation between inclusion formation and cell death. Cytoplasmic protein inclusions may not result simply from precipitated misfolded protein but rather from an active process meant to sequester soluble misfolded proteins from the cellular milieu (reviewed by Kopito, 2000). Accordingly, inclusion formation, while possibly indicative of a cell under attack, may be a defensive measure aimed at removing toxic soluble misfolded proteins (Cummings et al., 1999; Warrick et al., 1999; Cummings et al., 2001; Auluck et al., 2002). The ability of chaperones such as Hsp-70 to protect against neurodegeneration provoked by disease-related proteins (including α-synuclein-mediated dopaminergic

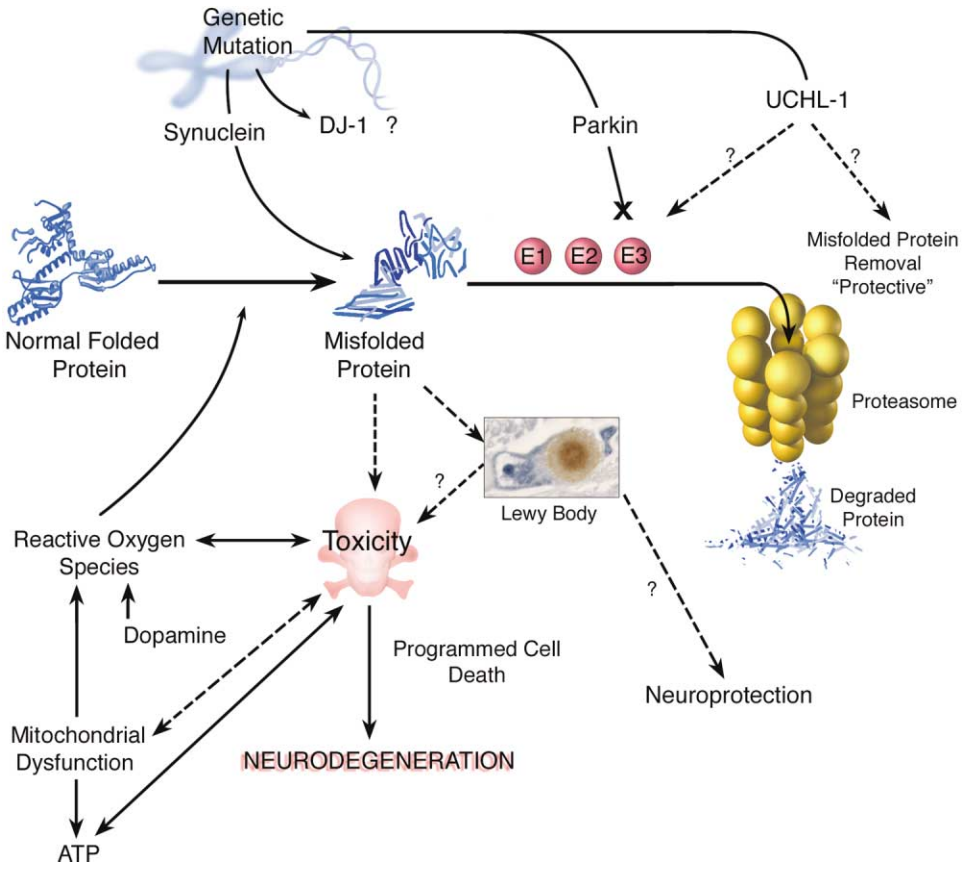

Figure 2. Mechanisms of Neurodegeneration

A growing body of evidence, detailed in this review, suggests that the accumulation of misfolded proteins is likely to be a key event in PD neurodegeneration. Pathogenic mutations may directly induce abnormal protein conformations (as believed to be the case with α-synuclein) or damage the ability of the cellular machinery to detect and degrade misfolded proteins (Parkin, UCH-L1); the role of DJ-1 remains to be identified. Oxidative damage, linked to mitochondrial dysfunction and abnormal dopamine metabolism, may also promote misfolded protein conformations. It remains unclear whether misfolded proteins directly cause toxicity or damage cells via the formation of protein aggregates (Lewy body). Controversy exists regarding whether Lewy bodies promote toxicity or protect a cell from harmful effects of misfolded proteins by sequestering them in an insoluble compartment away from cellular elements. Oxidative stress, energy crisis (i.e., ATP depletion) and the activation of the programmed cell death machinery are also believed to be factors that trigger the death of dopaminergic neurons in Parkinson's disease.

neuron loss) is consistent with the view that soluble misfolded proteins are neurotoxic (reviewed by Muchowski, 2002; Auluck et al., 2002).

In patients with *inherited* PD, pathogenic mutations are thought to cause disease directly by inducing abnormal and possibly toxic protein conformations (e.g., Bussell and Eliezer, 2001) or indirectly by interfering with the processes that normally recognize or process misfolded proteins (the function of genes identified in inherited PD is reviewed in the "Gene-Based Models" section below). In *sporadic* PD, there is a similar focus both on direct protein-damaging modifications and on dysfunction of chaperones or the proteasome that may indirectly contribute to the accumulation of misfolded proteins. The triggers for dysfunctional protein metabolism in sporadic PD are only just beginning to be elucidated. One trigger may be oxidative stress, long thought to play a key role in the pathogenesis of PD through damage caused by ROS (reviewed by Przedborski and Jackson-Lewis, 2000). The tissue content of abnormally oxidized proteins (which may misfold) increases with age (reviewed by Beckman and Ames, 1998), and neurons may be particularly susceptible because they are postmitotic. In PD, LBs contain oxidatively modified α-synuclein, which in vitro exhibits a greater propensity to aggregate than unmodified α-synuclein (Giasson et al., 2000). Several herbicides and pesticides induce misfolding or aggregation of α-synuclein (Uversky et al., 2001; Manning-Bog et al., 2002; Lee et al., 2002a). There also appears to be an age-related decline in the ability of cells to handle misfolded proteins (reviewed by Sherman and Goldberg, 2001). Cells respond to misfolded proteins by inducing chaperones, but if not properly refolded they are targeted for proteasomal degradation by polyubiquitination. With aging, the ability of cells to induce a variety of chaperones is impaired as is the activity of the proteasome. Proteasomal dysfunction and the consequent accumulation of misfolded proteins may provoke a vicious cycle, with excess misfolded proteins further inhibiting an already compromised proteasome. Thus, factors that have been previously implicated in the pathogenesis of PD, including aging and oxidative stress, may converge

to generate a proteotoxic insult to cells. The discoveries regarding the genetics of inherited PD are consistent with this scenario (see below).

Mitochondrial Dysfunction and Oxidative Stress

The possibility that an oxidative phosphorylation defect plays a role in the pathogenesis of PD was fueled by the discovery that MPTP blocks the mitochondrial electron transport chain by inhibiting complex I (Nicklas et al., 1987). Subsequent studies identified abnormalities in complex I activity in PD (reviewed by Greenamyre et al., 2001). In vitro studies indicate that such a complex I defect may subject cells to oxidative stress and energy failure. The abnormality of oxidative phosphorylation identified in PD is not confined to the brain (Schapira et al., 1990), as reduced complex I activity has been found in platelets from PD patients (Parker et al., 1989) and in cybrid cells (cells lines engineered to contain mitochondria derived from platelets of PD patients [Swerdlow et al., 1996]). This latter finding suggests either that the observed complex I deficit is inherited from the mitochondrial genome or that some systemic toxicity leads to mutations in mitochondrial DNA. However, mitochondrial DNA mutations have not yet been identified in PD patients.

Nearly 100% of molecular oxygen is consumed by the mitochondrial respiration, and powerful oxidants are normally produced as byproducts, including hydrogen peroxide and superoxide radicals. Inhibition of complex I increases the production of the ROS superoxide, which may form toxic hydroxyl radicals or react with nitric oxide to form peroxynitrite. These molecules may cause cellular damage by reacting with nucleic acids, proteins, and lipids. One target of these reactive species may be the electron transport chain itself (Cohen, 2000), leading to mitochondrial damage and further production of ROS. Several biological markers of oxidative damage are elevated in the SNpc of PD brains (reviewed by Przedborski and Jackson-Lewis, 2000). Also, the content of the antioxidant glutathione is reduced in the SNpc of PD brains (Sian et al., 1994), consistent with increased ROS, although this could also indicate a primary reduction of protective mechanisms against ROS.

The presence of ROS would increase the amount of misfolded proteins, increasing the demand on the ubiquitin-proteasome system to remove them. Dopaminergic neurons may be a particularly fertile environment for the generation of ROS, as the metabolism of DA produces hydrogen peroxide and superoxide radicals, and autooxidation of DA produces DA-quinone (Graham, 1978), a molecule that damages proteins by reacting with cysteine residues. Mitochondria-related energy failure may disrupt vesicular storage of DA, causing the free cytosolic concentration of DA to rise and allowing harmful DA-mediated reactions to damage cellular macromolecules. Thus, DA may be pivotal in rendering SNpc dopaminergic neurons particularly susceptible to oxidative attack. Nevertheless, despite the literature documenting mitochondrial dysfunction and indices of oxidative damage in tissue from PD patients, all of these observations are correlative in nature, and the supportive data from postmortem studies of PD patients suffers from the fact that such specimens primarily consist of glial cells and nondopaminergic neurons, as most dopaminergic neurons die long before these specimens become available. There are no data that convincingly link a *primary* abnormality of oxidative phosphorylation or ROS generation with PD. Furthermore, parkinsonism is rare in many diseases known to result from mutations directly affecting oxidative phosphorylation ("mitochondrial cytopathies"). When parkinsonism is encountered in these diseases, it is generally accompanied by other symptoms not typical of PD. Therefore, many of the oxidative phosphorylation and ROS abnormalities documented in PD tissues could be nonspecific features of dying cells.

Mode of Cell Death

How do cells ultimately die in PD? Does a common downstream pathway mediate all PD-related cell loss, or is there significant heterogeneity in the pathways activated in different sick neurons in a single patient, or among different patients with PD? The answers to these questions are important for the rational development of therapeutic strategies for PD. In programmed cell death (PCD), intracellular signaling pathways are activated to cause cell demise. Although physiological PCD is crucial during normal development and as a homeostatic mechanism in some systems (e.g., immune system), dysregulation of this pathway in the brain may contribute to neurodegeneration. Until recently, investigators have explored the possibility that PCD occurs in PD autopsy specimens by searching for neurons that display features of apoptosis, a morphological correlate of PCD. These morphological studies have yielded conflicting results (reviewed by Vila and Przedborski, 2003). Complicating matters, if apoptosis does occur in PD, it may be difficult to detect by morphological criteria because the rate of neuronal loss may be low (McGeer et al., 1988) and apoptotic cells seem to disappear rapidly (Raff et al., 1993). In addition, there may be nonapoptotic forms of PCD (Clarke, 1999; Sperandio et al., 2000). For these reasons, some studies of PCD in PD have measured molecular components of PCD instead of relying on morphological criteria. For example, investigations of the PCD molecule Bax demonstrate an increased number of Bax-positive SNpc dopaminergic neurons in PD (Hartmann et al., 2001a), and compared to controls, there is increased neuronal expression of Bax in PD, suggesting that these cells are undergoing PCD (Tatton, 2000). SNpc dopaminergic neurons with increased expression and subcellular redistribution of the anti-PCD protein Bcl-xL and with activated PCD effector protease caspase-3 have also been found in greater proportion in PD (Hartmann et al., 2000, 2002). Other molecular markers of PCD are altered in PD, including the activation of caspase-8 (Hartmann et al., 2001b) and caspase-9 (Viswanath et al., 2001). Taken together, these studies suggest that the PCD machinery is activated in postmortem PD tissue. Nevertheless, because these studies are single time point-descriptive assessments of patient tissue they cannot address whether the findings reflect a primary abnormality of PCD regulation or an appropriate "suicide" decision by injured cells damaged by one of the processes reviewed above.

Modeling PD in Animals

While recent genetic discoveries have led to significant insight into molecular pathways of likely importance in

PD pathogenesis, these discoveries have not contributed to an understanding of other important aspects of the disease. Why is there a relatively selective loss of dopaminergic neurons in PD? Is the toxicity provoked by these disease alleles a cell-autonomous effect in dopaminergic neurons? What is the role of aging in both sporadic and inherited PD, or posed differently, why does it take many decades even for inherited PD to develop? Does pharmacological or genetic manipulation of the ubiquitin-proteasome pathway prevent (or provoke) dopaminergic neurodegeneration? Do the different genetic forms of PD display unique responses to cell-based (e.g., stem cell) or pharmacological therapies? What is the relationship between the neurodegeneration provoked by disease allele-related pathways and that occurring in sporadic PD? Although aspects of these questions can be assessed in PD patients, postmortem tissue, and in vitro systems, it is clear that these and related questions will be addressed most powerfully in animal models.

The crucial requirement for a disease gene-based model of PD (also referred to as an "etiologic model") is the adult onset of relatively specific and progressive dopaminergic neuron degeneration. A behavioral correlate of the nigrostriatal dopaminergic pathway degeneration is also desirable but, in rodents, will not likely parallel the motor deficits of PD because rodents do not develop typical parkinsonism. Alternatively, behaviors that involve striatal function, such as habituation to a novel environment or the ability to learn a stimulus-response paradigm, may be useful in assessing the striatal dopaminergic function. Because motor system organization differs in rodents and humans, the value of a particular behavioral phenotype depends upon its relationship to striatal dopaminergic function rather than apparent similarity to a symptom of PD. Specifically, behaviors claimed to result from striatal DA deficiency should improve with DA replacement. The formation of LBs is also a desirable but not essential feature. While LBs are characteristic of PD, they are not specific, are not found in a minority of clinically defined PD cases, and are not seen in parkin-related PD.

Other valuable approaches to modeling PD in animals do not depend on disease-related genes. These "pathologic models" use toxins or non-PD-related genetic mutations (Kostic et al., 1997) to mimic the selective degeneration of dopaminergic neurons or exploit the loss of dopaminergic neurons that normally occurs in rodents during early postnatal development (Macaya et al., 1994; Jackson-Lewis et al., 2000). These strategies are based on the premise that dopaminergic neurons have a stereotyped death cascade that can be activated by a range of insults or developmental signals. Clearly defining this cascade of events may lead to the identification of new molecules of potential relevance to PD pathogenesis or treatment. Most notable is the MPTP model, partially because of the striking similarity between PD and individuals intoxicated with MPTP. Finally, "symptomatic" or "pathophysiologic" models recapitulate the motor symptoms of PD and are used to develop symptomatic therapies or to study circuit-related questions. Only nonhuman primates accurately mimic the motor symptoms of PD and are therefore the only suitable animal for such studies.

The remainder of this review will focus on pathologic and genetic animal models of PD. We will first review toxin-induced models, with an emphasis on the MPTP model, to date the best characterized of this class. We will then focus on PD genes and review early attempts to exploit them to better model the disease.

Toxin-Based Models

Among the neurotoxins used to induce dopaminergic neurodegeneration, 6-hydroxydopamine (6-OHDA), MPTP, and more recently paraquate and rotenone have received the most attention. Presumably, all of these toxins provoke the formation of ROS. Rotenone and MPTP are similar in their ability to potently inhibit complex I, though they display significant differences, including, importantly, their ease of use in animals. Only MPTP is clearly linked to a form of human parkinsonism, and it is thus the most widely studied model.

6-Hydroxydopamine

6-hydroxydopamine, the first animal model of PD associated with SNpc dopaminergic neuronal death, was introduced more than 30 years ago (Ungerstedt, 1968). Although 6-OHDA-induced pathology differs from PD, it is still extensively used. 6-OHDA-induced toxicity is relatively selective for monoaminergic neurons, resulting from preferential uptake by DA and noradrenergic transporters (Luthman et al., 1989). Reminiscent of PD, there is a range of sensitivity to 6-OHDA between the ventral midbrain dopaminergic neuronal groups; greatest loss is observed in the SNpc, while tuberoinfundibular neurons are almost completely resistant (reviewed by Jonsson, 1980). Inside neurons, 6-OHDA accumulates in the cytosol, generating ROS and inactivating biological macromolecules by generating quinones that attack nucleophilic groups (reviewed by Cohen and Werner, 1994).

Because 6-OHDA cannot cross the blood-brain barrier, it must be administered by local stereotaxic injection into the substantia nigra, median forebrain bundle (MFB; which carries ascending dopaminergic and serotonergic projections to the forebrain), or striatum to target the nigrostriatal dopaminergic pathway (Javoy et al., 1976; Jonsson, 1983). After 6-OHDA injections into substantia nigra or the MFB, dopaminergic neurons start degenerating within 24 hr and die without apoptotic morphology (Jeon et al., 1995). When injected into the striatum, however, 6-OHDA produces a more protracted retrograde degeneration of nigrostriatal neurons, which lasts for 1–3 weeks (Sauer and Oertel, 1994; Przedborski et al., 1995). So far, however, none of the modes of 6-OHDA intoxication have led to the formation of LB-like inclusions. For striatal stereotaxic lesions, 6-OHDA is injected unilaterally, with the contralateral side serving as control (Ungerstedt, 1971). These injections produce an asymmetric circling behavior in the animals, the magnitude of which depends on the degree of the nigrostriatal lesion (Ungerstedt and Arbuthnott, 1970; Hefti et al., 1980; Przedborski et al., 1995). The unilateral lesion can be quantitatively assayed; thus, a notable advantage of this model is the ability to assess the anti-PD properties of new drugs (Jiang et al., 1993) and the benefit of transplantation or gene therapy to repair the damaged pathways (Bjorklund et al., 2002). However, it is not clear whether the mechanism by which 6-OHDA kills dopa-

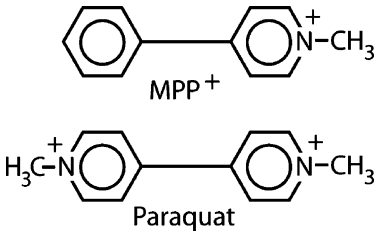

Figure 3. Structural Similarity between Paraquat and MPP+

The only difference between these two compounds is the second *N*-methyl-pyridinium group that paraquat has instead of the phenyl group as seen in MPP+.

minergic neurons shares key molecular features with PD.

Paraquat

The herbicide paraquat (N,N′-dimethyl-4-4′-bipiridinium) also induces a toxic model of PD. As noted above, paraquat shows structural similarity to MPP+ (Figure 3) and is present in the environment. Exposure to paraquat may confer an increased risk for PD (Liou et al., 1997). However, paraquat does not easily penetrate the blood brain barrier (Shimizu et al., 2001), and its CNS distribution does not parallel any known enzymatic or neuroanatomic distribution (Widdowson et al., 1996a, 1996b). The toxicity of paraquat appears to be mediated by the formation of superoxide radicals (Day et al., 1999). Systemic administration of paraquat to mice leads to SNpc dopaminergic neuron degeneration accompanied by α-synuclein containing inclusions, as well as increases in α-synuclein immunostaining in frontal cortex (Manning-Bog et al., 2002; McCormack et al., 2002). This study was the first to include stereologic cell counts to assess neurodegeneration, which may explain why the investigators found clear evidence of cell loss, compared to earlier inconsistent reports (Brooks et al., 1999; Thiruchelvam et al., 2000a, 2000b). It remains to be seen whether the dopaminergic toxicity is selective or whether other cell types are similarly affected. Regardless of the outcome of those investigations, the ability to induce dopaminergic neuronal loss and α-synuclein-positive inclusions in a reliable fashion may prove valuable for studies of the role of α-synuclein in neurodegeneration.

Rotenone

Rotenone is the most potent member of the rotenoids, a family of natural cytotoxic compounds extracted from tropical plants; it is widely used as an insecticide and fish poison. Rotenone is highly lipophilic and readily gains access to all organs (Talpade et al., 2000). Rotenone binds (at the same site as MPP+) to and inhibits mitochondrial complex I.

As discussed in the section on the etiology of PD, epidemiological studies suggest that exposure to pesticides may be a risk factor. Greenamyre and colleagues reported that the administration of low-dose intravenous rotenone to rats produces selective degeneration of nigrostriatal dopaminergic neurons accompanied by α-synuclein-positive LB-like inclusions (Betarbet et al., 2000). Because rotenone may freely enter all cells, this study suggested that dopaminergic neurons are preferentially sensitive to complex I inhibition. Rotenone-intoxicated animals developed abnormal postures and slowness of movement, but it is unknown whether these features improved with levodopa administration. Nevertheless, this model was the first to link an environmental toxin of possible relevance to PD to the pathologic hallmark of α-synuclein aggregation, an association also seen in cell culture studies (Uversky et al., 2001; Sherer et al., 2002; Lee et al., 2002a).

In contrast to the findings of Betarabet and colleagues, acute intoxication with rotenone seems to spare dopaminergic neurons (Ferrante et al., 1997). Furthermore, a subsequent study of rats chronically infused with rotenone demonstrated significant reductions in striatal DARPP-32-positive, cholinergic, and NADPH diaphorase-positive neurons (Hoglinger et al., 2003). These results suggest that rotenone exerts a more widespread neurotoxicity than originally proposed, challenging the concept that dopaminergic neurons display preferential sensitivity to complex I inhibition (Betarbet et al., 2000). In addition, the use of rotenone in rodents is technically challenging (Betarbet et al., 2000). Nevertheless, the characteristic of LB-associated dopaminergic neurodegeneration in this model should enable investigators to perform a novel series of experiments exploring the relationship between aggregate formation and neuronal death.

MPTP: False Narcotic, Real Parkinsonian Toxin

In 1982, young drug users developed a rapidly progressive parkinsonian syndrome traced to intravenous use of a street preparation of 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP), an analog of the narcotic meperidine (Demerol) (Langston et al., 1983). MPTP was the responsible neurotoxic contaminant, inadvertently produced during the illicit synthesis of MPPP in a basement laboratory. In humans and monkeys, MPTP produces an irreversible and severe parkinsonian syndrome characterized by all of the features of PD, including tremor, rigidity, slowness of movement, postural instability, and freezing. In MPTP-intoxicated humans and nonhuman primates, the beneficial response to levodopa and development of long-term motor complications to medical therapy are virtually identical to that seen in PD patients. Also similar to PD, the susceptibility to MPTP increases with age in both monkeys and mice (Rose et al., 1993; Irwin et al., 1993; Ovadia et al., 1995).

The data regarding the comparison between PD- and MPTP-related neuropathology derive largely from MPTP studies in monkeys (Forno et al., 1993), because only four human MPTP cases have come to autopsy (Davis et al., 1979; Langston et al., 1999). These studies show that, as in PD, monkeys treated with low-dose MPTP exhibit preferential degeneration of putamenal versus caudate dopaminergic nerve terminals (Moratalla et al., 1992). Similarly, MPTP damages the dopaminergic pathways in a pattern similar to that seen in PD, including relatively greater cell loss in the SNpc than the VTA and a preferential loss of neurons in the ventral and lateral segments of the SNpc (Sirinathsinghji et al., 1992; Varastet et al., 1994); this regional pattern is also found in MPTP-treated mice (Seniuk et al., 1990; Muthane et al., 1994). Also reminiscent of PD (Hirsch et al., 1988), dopaminergic neurons that contain neuromelanin are more susceptible to MPTP-induced degeneration (Herrero et al., 1993). Neuromelanin may contribute neurodegenera-

tion in PD and MPTP-treated monkeys by catalyzing ROS formation through an interaction with iron selectively in pigmented neurons (Zecca et al., 2001). A variety of organic molecules interact with neuromelanin, including pesticides, MPTP, and MPP+ (D'Amato et al., 1986), so it may contribute to toxicity of pigmented neurons by acting as a depot for toxic compounds.

The monkey MPTP model does not include two characteristic features of PD. First, neurons are not consistently lost from other monaminergic nuclei, such as the locus coeruleus, a typical feature of PD (Forno et al., 1986, 1993). Second, although intraneuronal inclusions resembling LBs have been described (Forno et al., 1986), classical LBs have not been demonstrated convincingly in the brains of MPTP-intoxicated patients or monkeys (Forno et al., 1993). These cases were exposed to acute regimens of MPTP, so the lack of LB-like formation in MPTP-intoxicated humans and monkeys may reflect the fact that in these cases dopaminergic neurons were rapidly injured. Chronic infusion of rotenone does produce intraneuronal α-synuclein-containing proteinacious aggregates (Betarbet et al., 2000), consistent with the possibility that the speed of intoxication may influence the subsequent neuropathologic features.

Despite these neuropathologic shortcomings, the monkey MPTP model is the gold standard for the assessment of novel strategies and agents for the treatment of PD symptoms. For example, electrophysiologic studies of MPTP monkeys revealed that hyperactivity of the subthalamic nucleus is a key factor in the genesis of PD motor dysfunction (Bergman et al., 1990). This seminal discovery led to the targeting of this structure using chronic high-frequency stimulation procedures (also called deep brain stimulation) to effectively ameliorate the motor function of PD patients whose symptoms cannot be further improved with medical therapy (Limousin et al., 1998). In addition, MPTP-treated monkeys (Gash et al., 1996; Kordower et al., 2000) were used to demonstrate that the delivery of glial-derived neurotrophic factor (GDNF) both significantly limits MPTP-induced nigrostriatal dopaminergic neurodegeneration and can lead to behavioral recovery when given to previously lesioned animals (Kordower et al., 2000). These studies form the basis for current attempts to use GDNF in PD patients (Gill et al., 2003). Because of practical considerations, MPTP monkeys have not generally been used to explore the molecular mechanisms of dopaminergic neurodegeneration; the MPTP mouse model is typically used for such studies.

MPTP Metabolism and PD Neurodegeneration Selectivity. Since the initial discovery of MPTP-induced parkinsonism, much has been learned about the molecular pathway used by this toxin, as illustrated in Figure 4. Importantly, this knowledge enables investigators to use MPTP as a biological probe to explore the functions of PD genes and dissect the molecular events that occur during neurodegeneration of dopaminergic neurons. For example, mice mutant for PD genes (or other genes of possible relevance to dopaminergic neuronal death) can be injected with MPTP, and if these mice display markedly enhanced or suppressed dopaminergic neuronal death, one can then investigate which of the known molecular targets of MPTP are altered.

After systemic administration, MPTP, which is highly lipophilic, crosses the blood-brain barrier within minutes (Markey et al., 1984). Once in the brain, the pro-toxin MPTP is oxidized to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP+) by monoamine oxidase B (MAO-B) in glia and serotonergic neurons, the only cells that contain this enzyme. It is then converted to MPP+ (probably by spontaneous oxidation), the active toxic molecule, and released by an unknown mechanism into the extracellular space. Since MPP+ is a polar molecule, it depends on the plasma membrane carriers to enter cells. MPP+ is a high-affinity substrate for the DAT, as well as for norepinephrine and serotonin transporters (Javitch et al., 1985; Mayer et al., 1986). Pharmacological inhibition or genetic deletion of DAT prevents MPTP-induced dopaminergic damage (Javitch et al., 1985; Bezard et al., 1999), demonstrating the obligatory character of this step in MPTP neurotoxicity. However, uptake by DAT does not entirely explain the selectivity of the nigrostriatal dopaminergic lesion caused by MPTP. While there are quantitative differences in DAT expression between more susceptible SNpc neurons and less susceptible VTA neurons in monkeys (Haber et al., 1995), differences in DA uptake activity of comparable magnitudes between rats and mice and among mouse strains do not correlate with differences in MPTP sensitivity (Giovanni et al., 1991, 1994). Furthermore, while MPP+ is concen-

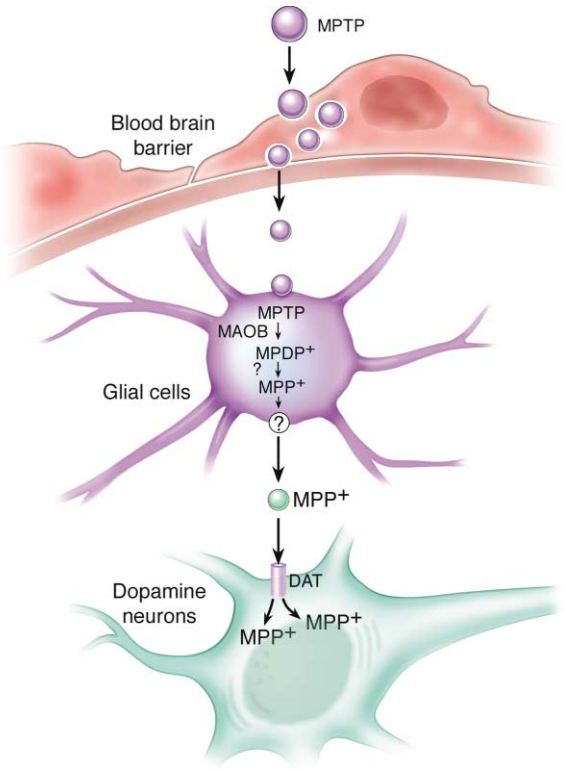

Figure 4. Schematic Representation of MPTP Metabolism

After systemic administration, MPTP crosses the blood-brain barrier. Once in the brain, MPTP is converted to MPDP+ by MAO-B within nondopaminergic cells, such as glial cells and serotonergic neurons (not shown), and then to MPP+ by an unknown mechanism (?). Thereafter, MPP+ is released, again by an unknown mechanism (?), into the extracellular space. MPP+ is concentrated into dopaminergic neurons via the dopamine transporter (DAT).

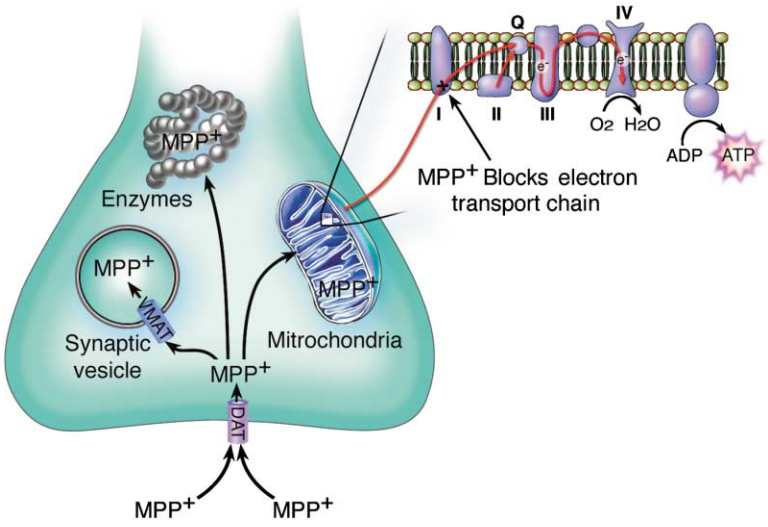

Figure 5. Schematic Representation of MPP+ Intracellular Pathways

Inside dopaminergic neurons, MPP+ can follow one of three routes: (1) concentration into mitochondria through an active process (toxic); (2) interaction with cytosolic enzymes (toxic); (3) sequestration into synaptic vesicles via the vesicular monoamine transporters (VMAT; protective). Within the mitochondria, MPP+ blocks complex I (X), which interrupts the transfer of electrons from complex I to ubiquinone (Q). This perturbation enhances the production of reactive oxygen species (not shown) and decreases the synthesis of ATP.

trated in (Speciale et al., 1998) and produces biochemical alterations in all monoaminergic neurons (Burns et al., 1983; Hallman et al., 1984; Wallace et al., 1984; Rose et al., 1993; Ovadia et al., 1995), degeneration is most prominent in dopaminergic neurons. In this regard, it is particularly striking that the highest levels of MPP+ are found in the adrenal medulla without causing the loss of chromaffin cells (Reinhard et al., 1987).

Inside neurons (Figure 5), MPP+ can follow at least three routes: (1) it can bind to the vesicular monoamine transporter-2 (VMAT2), which translocates MPP+ into synaptosomal vesicles (Liu et al., 1992); (2) it can be concentrated within the mitochondria by a mechanism that relies on the mitochondrial transmembrane potential (Ramsay and Singer, 1986); and (3) it can remain in the cytosol to interact with cytosolic enzymes, especially those carrying negative charges (Klaidman et al., 1993). Vesicular sequestration of MPP+ appears to protect cells from MPTP-induced neurodegeneration by sequestering the toxin and preventing it from accessing mitochondria, its likely site of action (see below). The importance of vesicular sequestration has been established by a number of experiments, including those showing that cells transfected to express greater density of VMAT2 are converted from MPP+-sensitive to MPP+-resistant cells (Liu et al., 1992) and that heterozygous VMAT2 null mice display enhanced sensitivity to MPTP-induced neurodegeneration (Takahashi et al., 1997). It appears that the ratio of DAT to VMAT2 expression predicts the likelihood of neuronal degeneration both in PD and the MPTP model. For instance, the putamenal dopaminergic terminals, which are most severely affected by both MPTP and PD, have a higher DAT/VMAT2 ratio than those in the caudate, which are less affected (Miller et al., 1999).

Mechanisms of Nigrostriatal Neurodegeneration: Hints from MPTP. Once inside the mitochondria, MPP+ impairs oxidative phosphorylation by inhibiting the multienzyme complex I of the mitochondrial electron transport chain (Nicklas et al., 1985). This blockade rapidly leads to decreases in tissue ATP content, particularly in the striatum and ventral midbrain (Chan et al., 1991; Fabre et al., 1999), the brain regions the most sensitive to MPTP. In vitro experiments in mitochondria isolated from *whole brain* demonstrate that complex I activity must be inhibited by ~70% to significantly impair ATP production (Davey and Clark, 1996), but data from PD postmortem tissues demonstrate only a ~40% inhibition of complex I activity (Schapira et al., 1990). Interestingly, in vitro experiments with *synaptic-derived* mitochondria demonstrate that significant ATP depletion results from as little as ~25% inhibition of complex I (Davey et al., 1998), indicating a much tighter functional relationship between complex I activity and ATP production in synaptic than in somatic mitochondria. Thus, mitochondria from phenotypically distinct neuronal populations may be differentially affected in PD, and the current approach of assessing mitochondrial function in specimens from whole tissue may not depict accurately abnormalities present in only a minority of cells. Furthermore, even the small alterations in complex I activity observed in PD may be particularly harmful to dopaminergic nerve terminals, which are rich in synaptic mitochondria.

Another early effector of complex I inhibition due to MPP+ may be oxidative stress. Indeed, by hampering the flow of electrons through complex I, MPP+ can stimulate the production of ROS, especially superoxide (Hasegawa et al., 1990, 1997). MPP+ effects on mitochondria can also indirectly stimulate the production of ROS by triggering DA leakage from synaptic vesicles to the cytosol, likely due to the inability of VMAT2 to maintain concentration gradients in the face of the ATP depletion (reviewed by Johnson, 1988). Findings from in vivo studies provide support for the importance of ROS in MPTP-induced neurodegeneration. Mice transgenic for superoxide dismutase-1 (SOD1), a key ROS scavenging enzyme, are resistant to MPTP-induced dopaminergic neuron degeneration (Przedborski et al., 1992), and other studies in mice imply a key role for reactive species, including NO, as critical effectors in MPTP toxicity (reviewed by Przedborski and Vila, 2003; Przedborski et al., 2003).

Alterations in energy metabolism and generation of ROS peak within hours of MPTP administration, days

before overt neuronal death has occurred (Jackson-Lewis et al., 1995). Therefore, these initial events are not likely to directly kill most cells but rather set into play downstream cellular events that ultimately kill most dopaminergic neurons (Mandir et al., 1999; Saporito et al., 2000; Vila et al., 2001).

Prolonged administration of low to moderate doses of MPTP to mice leads to morphologically defined apoptosis of SNpc dopaminergic neurons (Tatton and Kish, 1997). Under this regimen of MPTP intoxication, Bax, a potent PCD agonist and member of the Bcl-2 family, is upregulated in SNpc dopaminergic neurons (Vila et al., 2001). Bax upregulation coincides with its translocation to mitochondria, mitochondrial release of cytochrome c (an electron carrier and a mediator of PCD), and activation of caspases 9 and 3 (Viswanath et al., 2001). At the same time, PCD antagonists such as Bcl-2 are downregulated in the SNpc (Vila et al., 2001). Consistent with these observations, Bax null and Bcl-2 transgenic mice are both resistant to MPTP neurotoxicity (Yang et al., 1998; Offen et al., 1998; Vila et al., 2001).

How MPTP provokes these changes in Bcl-2 family members remains to be elucidated. MPTP causes oxidative damage to DNA (Mandir et al., 1999; Mandavilli et al., 2000), which may be important in inducing Bax via p53 activation. The tumor suppressor protein p53 is one of the few molecules known to regulate Bax expression and is activated by DNA damage. Furthermore, pharmacological inhibition of p53 attenuates MPTP-induced Bax upregulation and the subsequent SNpc dopaminergic neuron death (Duan et al., 2002), and p53 null mice are resistant to MPTP-induced neurodegeneration (Trimmer et al., 1996).

Activation of the JNK pathway following DNA damage is required in vitro for Bax mitochondrial translocation and the ensuing recruitment of the mitochondrial apoptotic pathway (Ghahremani et al., 2002; Lei et al., 2002). Activation of the JNK pathway follows MPTP administration (Saporito et al., 2000; Xia et al., 2001), and pharmacological blockade of JNK (Saporito et al., 1999) or adenoviral-directed expression of the JNK binding domain of JNK-interacting protein-1 (Xia et al., 2001) results in marked attenuation of MPTP-induced SNpc dopaminergic cell death.

Approaches aimed at inhibiting PCD at a more downstream level, such as by interfering with activation of caspases, have yielded inconsistent results. Adenoviral gene transfer of X chromosome-linked inhibitor of apoptosis (XIAP), a protein caspase inhibitor, prevents MPTP-induced SNpc dopaminergic neuron death, although it does not prevent the loss of striatal dopaminergic terminals (Eberhardt et al., 2000). In contrast, transgenic neuronal expression of the general caspase inhibitor protein baculoviral p35 specifically attenuates both MPTP-induced neuronal death and DA depletion (Viswanath et al., 2001). As with XIAP, some in vitro studies suggest that resistance to PCD can be induced selectively in the cell body. The broad-spectrum caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone and peptide inhibitors of caspases 2, 3, and 9 prevent the loss of dopaminergic cell bodies of cultured ventral midbrain neurons exposed to MPP+, but the neurites are not spared (Bilsland et al., 2002); the molecular pathways governing neuronal death may differ from those governing axonal destruction (Raff et al., 2002).

MPTP administration also leads to the accumulation and nitration of α-synuclein in the cytosol of SNpc dopaminergic neurons (Vila et al., 2000; Przedborski et al., 2001), and ablation of α-synuclein in mutant mice prevents MPTP-induced dopaminergic neurodegeneration (Dauer et al., 2002). While it is not clear whether α-synuclein plays any direct role in regulating PCD, the expression of mutant α-synuclein in cell cultures may promote apoptosis (Xu et al., 2002), and cytochrome c has been reported to stimulate in vitro aggregation of α-synuclein (Hashimoto et al., 1999). Collectively, these data demonstrate that the activation of PCD is instrumental in MPTP toxicity. They also suggest that PCD alterations in PD postmortem samples are of pathological significance and that targeting specific PCD molecules may be a valuable neuroprotective strategy for the treatment of PD (Vila and Przedborski, 2003).

Gene-Based Models

As discussed above, uncertainty remains regarding which of the molecular events provoked by toxins relate to human PD. The discovery of PD genes is particularly exciting because theoretically it will allow the generation of novel models of definite significance to specific forms of the human disease, and evidence is emerging to link these genetic forms to idiopathic PD. Here, we will briefly review the current state of knowledge of PD genes and then discuss early attempts to exploit these discoveries to generate novel PD models.

The rationale for studying rare genetic forms of a common sporadic illness is the expectation that the phenotypic similarity between the genetic and sporadic forms of the disease indicates that they share important pathogenic mechanisms and, consequently, that genetic information will help focus research on a key biochemical pathway (Figure 2). Indeed, all of the PD genes that have been identified and studied in some detail—α-synuclein, parkin, and ubiquitin C-terminal hydrolase L1 (UCHL-1)—appear to participate in the ubiquitin-proteasome pathway, a particularly compelling finding considering the LB protein aggregates that characterize PD neuropathology. Although PD-causing mutations in the gene DJ-1 have only recently been identified, this protein also appears to have a potential link to the ubiquitin-proteasome pathway (Takahashi et al., 2001). Much of the current research in PD is focused on the normal role and functional interaction between these PD proteins and how these functions are disrupted by pathogenic mutations. Polymorphisms at the parkin and synuclein loci may also contribute to the risk of idiopathic PD (Farrer et al., 2001), and parkin mutations are found in patients without a family history of PD, especially with symptom onset before the age of 30 (Lucking et al., 2000). A number of epidemiological studies suggest that single-nucleotide polymorphisms at different loci may be associated with PD susceptibility (Martin et al., 2001; Li et al., 2002; Zareparsi et al., 2002), but the lack of concordance for PD in monozygotic twins argues against a *strong* genetic contribution in sporadic PD (Tanner et al., 1999).

Synuclein

Two missense mutations [Ala^{53} → Thr (A53T) and Ala^{30} → Pro (A30P)] in α-synuclein cause dominantly inherited PD (Polymeropoulos et al., 1997; Kruger et al., 1998). Clinical and pathological features typical of PD have been found in brains from patients with either mutation, although some atypical features have also been noted (Kruger et al., 1998; Spira et al., 2001). Mutations in α-synuclein have not been found in sporadic PD (Lynch et al., 1997; Munoz et al., 1997; Chan et al., 1998), so the concept that α-synuclein-mutant and sporadic PD share common pathogenic mechanisms rests predominantly on the observation that α-synuclein is a major component of LBs in sporadic PD (Spillantini et al., 1998).

The normal physiological role of α-synuclein is just beginning to be elucidated, and this prevalent presynaptic protein may modulate synaptic vesicle function (reviewed by Kahle et al., 2002). α-Synuclein is widely expressed in the nervous system, where it is found in presynaptic nerve terminals in close association with synaptic vesicles (Maroteaux et al., 1988; George et al., 1995). It binds reversibly to brain vesicles and components of the vesicular trafficking machinery (Jensen et al., 1998, 1999, 2000). In striatal dopaminergic terminals, α-synuclein participates in the modulation of synaptic function, possibly by regulating the rate of cycling of the readily releasable pool (Abeliovich et al., 2000). Downregulation of this protein by antisense oligonucleotide in hippocampal cell culture is reported to decrease the distal pool of synaptic vesicles and alters the expression of vesicular-associated proteins in cultured hippocampal glutamatergic neurons (Murphy et al., 2000). However, no abnormalities were identified in an extensive quantitative analysis of synaptic-related proteins from either whole-brain homogenates (Schluter et al., 2003) or hippocampal cultures (Cabin et al., 2002) from synuclein null mice. While the ultrastructure of striatal synapses appears normal in brain sections from mice that lack synuclein (Abeliovich et al., 2000), there may be fewer "non-docked" distal synaptic vesicles in hippocampal brain sections from synuclein null mice (Cabin et al., 2002). Nevertheless, since quantitative EM studies are challenging to perform, this finding awaits confirmation. Unfortunately, none of the studies of synuclein null mice specifically assessed dopaminergic nerve terminal synaptic protein expression and morphology; this remains a significant gap in the characterization of these animals.

Biochemical and biophysical evidence is also consistent with a role for α-synuclein in cellular membrane dynamics. As seen with synaptic vesicles, α-synuclein binds to lipid membranes, and this binding changes the conformation of the previously unfolded N terminus of the protein to a stable α-helical secondary structure (Davidson et al., 1998; Eliezer et al., 2001), suggesting that membrane binding elicits a functionally important alteration in the protein. Additional observations support the view that the cellular membrane is a key site of α-synuclein action (Pronin et al., 2000; Ahn et al., 2002). One membrane-related function of α-synuclein may be trafficking proteins to the plasma membrane, as suggested by the demonstration that α-synuclein could be involved in the membrane localization of DAT (Lee et al., 2001).

The fact that α-synuclein is abundant in LBs suggests that its propensity to misfold and form amyloid fibrils may be responsible for its neurotoxicity in pathological situation such as PD and that pathogenic mutations endow it with a toxic gain of function. A growing literature supports this notion and links the pathogenesis of PD to other neurodegenerative diseases that involve protein aggregation (reviewed by Goedert, 2001). Misfolding of α-synuclein may interfere with its normal functions, but it is unlikely that loss of function plays a major role in α-synuclein-related neurodegeneration (Abeliovich et al., 2000; Dauer et al., 2002).

Both wild-type and mutant α-synuclein form amyloid fibrils resembling those seen in LBs (Conway et al., 1998; Giasson et al., 1999) as well as nonfibrillary oligomers (Conway et al., 1998), termed "protofibrils." Since the two known pathogenic α-synuclein mutations promote the formation of protofibrils (Conway et al., 2000), they may be the toxic species of α-synuclein. Consistent with this view and the association of α-synuclein with synaptic vesicles, protofibrils may cause toxicity by permeabilizing synaptic vesicles (Volles et al., 2001; Lashuel et al., 2002), allowing DA to leak into the cytoplasm and participate in reactions that generate oxidative stress (reviewed above). Furthermore, the selective vulnerability of dopaminergic neurons in PD may derive from the ability of DA itself to stabilize these noxious α-synuclein protofibrils (Conway et al., 2001). Nevertheless, protofibrils have only been observed and studied in vitro, so further work will need to explore whether they form in neurons and if their formation correlates with neurotoxicity.

Parkin

Loss-of-function mutations in the gene encoding parkin cause recessively inherited parkinsonism (Kitada et al., 1998). Although this form of parkinsonism was originally termed autosomal recessive juvenile parkinsonism, the clinical phenotype is now known to include older-onset patients (Lincoln et al., 2003). In general, however, parkin mutations are found in PD patients with onset before age 30, particularly those with a family history consistent with recessive inheritance (Mizuno et al., 2001). Clinically, parkin mutant patients display the classical signs of parkinsonism but with marked improvement of symptoms with sleep, abnormal dystonic movements, and a striking response to levodopa. Heterozygote mutations in parkin may also lead to dopaminergic dysfunction and later onset of PD (Hilker et al., 2001; Hedrich et al., 2002). Pathologically, parkin-related PD is characterized by loss of SNpc dopaminergic neurons, but it is not typically associated with LBs (Mizuno et al., 2001).

It is uncertain how loss of parkin function leads to dopaminergic neuron degeneration, but clues are emerging from the identification of its normal function. Parkin, a 465 amino acid protein, contains two RING finger domains separated by an in-between RING (IBR) finger domain at the C terminus and an ubiquitin-like homology domain at the N terminus. The presence of an IBR led to the finding that parkin is an E3 ubiquitin ligase (Zhang et al., 2000; Shimura et al., 2000), a component of the ubiquitin-proteasome system that identifies and targets misfolded proteins to the proteasome for degradation (reviewed by Sherman and Goldberg, 2001). The upstream ubiquitin ligases (E1 and E2) cooperate nonspecifically to tag misfolded proteins with a single

ubiquitin, while E3 ligases confer target specificity by binding to specific molecules or classes of molecules facilitating the polyubiquitination necessary for targeting to the proteasome. Many parkin mutations abolish this E3 ligase activity, suggesting that the accumulation of misfolded parkin substrates could be responsible for the demise of SNpc dopaminergic neurons in PD.

A number of parkin substrates have been identified (Zhang et al., 2000; Shimura et al., 2001; Chung et al., 2001; Imai et al., 2001; Staropoli et al., 2003). Some of these substrates appear to link parkin and synuclein function, and one—cyclin E—links parkin function to a molecule previously implicated in neuronal apoptosis. Three reports suggest a relationship between parkin and synuclein function (Shimura et al., 2001; Petrucelli et al., 2002) or aggregation (Chung et al., 2001). Notably, the E3 ligase activity of parkin modulates the sensitivity of cells to both proteasome inhibitor- and mutant synuclein-dependent cell death (Petrucelli et al., 2002). A number of observations suggest that the functional interaction between synuclein and parkin may involve the proteasome: synuclein interacts with and may be degraded by the proteasome (Ghee et al., 2000; Snyder et al., 2003), overexpression of synuclein inhibits the proteasome (Stefanis et al., 2001), and mutant synuclein increases the sensitivity of cells to proteasome inhibition (Tanaka et al., 2001; Petrucelli et al., 2002). Parkin has also been found to function in a multiprotein ubiquitin ligase complex that ubiquitinates cyclin E (Staropoli et al., 2003). Importantly, these investigators also demonstrated that there is an accumulation of cyclin E in midbrain extracts from parkin mutant as well as idiopathic PD and that in excitotoxin-treated cultured postmitotic neurons parkin overexpression attenuates cyclin E accumulation and promotes survival. Thus, a number of findings are beginning to strengthen the functional links between parkin, synuclein, and proteasome function as well as to highlight parkin substrates that might play a key role in cell death. However, none of the identified parkin substrates normally display a pattern of selective or enriched expression in dopaminergic neurons. Thus, these data have yet to suggest a molecular explanation for the relative specificity of dopaminergic neuron degeneration in PD.

Ubiquitin C-Terminal Hydrolase-L1

A dominant mutation (I93M) in UCH-L1 was identified in one family with inherited PD (Leroy et al., 1998), but no pathological data were included in this report. This enzyme catalyzes the hydrolysis of C-terminal ubiquityl esters and is thought to play a role in recycling ubiquitin ligated to misfolded proteins after their degradation by the proteasome (reviewed by Wilkinson, 2000). Although the I93M mutation decreases the activity of this deubiquitinating enzyme, mice null for UCH-L1 do not display dopaminergic neurodegeneration (Saigoh et al., 1999). Rather, they develop an axonopathy affecting primary sensory axons in the gracile nucleus of the medulla, whose cell bodies reside in the dorsal root ganglia (Saigoh et al., 1999). Additionally, a polymorphism (S18Y) of UCH-L1 appears to be protective for the development of PD (Maraganore et al., 1999; Levecque et al., 2001; Satoh and Kuroda, 2001). Aside from its deubiquitinating function, UCH-L1 exerts a previously unrecognized ubiquitin ligase activity upon dimerization (Liu et al., 2002). Both the I93M mutation and the S18Y polymorphism alter UCH-L1 ligase activity in a manner consistent with the hypothesis that impaired activity of the ubiquitin proteasome system is critical in PD pathogenesis: UCH-L1 ligase activity is decreased by the pathogenic I93M mutation and increased by the protective S18Y polymorphism (Liu et al., 2002).

DJ-1

DJ-1 mutations were identified in two consanguineous pedigrees with autosomal recessive PD (Bonifati et al., 2002). One family carried a deletion predicted to abolish protein function, while the other harbored a missense mutation that results in the insertion of a proline into an α-helical region. Expression of this proline mutant form of DJ-1 appears to lead to its accumulation in mitochondria (Bonifati et al., 2002), and DJ-1 has been implicated as a cellular monitor of oxidative stress (Mitsumoto and Nakagawa, 2001; Mitsumoto et al., 2001).

Synuclein-Based Models

All published genetic models of PD have been based on α-synuclein, primarily the transgenic overexpression of mutant or wild-type forms in mice or flies (Masliah et al., 2000; van der Putten et al., 2000; Feany and Bender, 2000; Matsuoka et al., 2001; Giasson et al., 2002; Lee et al., 2002b). In general, these studies demonstrate that transgenic overexpression of α-synuclein causes neurotoxicity but that α-synuclein ablation is not associated with neuropathological changes, supporting the notion that PD-causing mutations operate via a toxic gain-of-function mechanism. However, a striking disappointment of the α-synuclein transgenic mice has been a complete failure to model dopaminergic neurodegeneration (i.e., actual cell death). Instead, these mice display a variety of neuropathologic changes, including neuronal atrophy, dystrophic neurites, and astrocytosis accompanied by α-synuclein-positive LB-like inclusions. Indeed, compared to other neuronal populations, murine dopaminergic neurons appear inexplicably resistant to α-synuclein-induced neurotoxicity, even in the face of marked accumulations of the protein (Matsuoka et al., 2001; Giasson et al., 2002; Lee et al., 2002b), significantly limiting the utility of these models.

In contrast to the transgenic mouse studies, two groups have demonstrated that the injection of human α-synuclein expressing viral vectors into the substantia nigra of adult rats causes the selective death of dopaminergic neurons accompanied by synuclein-containing inclusions and other pathologic changes reminiscent of those observed in PD (Kirik et al., 2002; Lo Bianco et al., 2002). The reasons for the discrepancy between the rat and mouse studies are not clear. Significantly higher levels of α-synuclein expression may be achieved with the viral vectors, or it may be important that, in contrast to transgenic mice, in these models α-synuclein is suddenly overexpressed during adulthood. It is also possible that a species-dependent difference in susceptibility to α-synuclein toxicity exists between mice and rats. While the viral vector approach will be useful for certain studies, it has significant limitations. Most importantly, because the investigator must generate each individual animal, it is technically challenging to produce large cohorts of rats that express similar amounts of protein in

a consistent anatomic pattern. Thus, unlike the situation with heritable transgenes, each rat is in effect an independent experiment. Furthermore, this approach does not allow investigators to take advantage of the large number of mouse mutants or genetic strategies available in mice that would greatly facilitate the further assessment of the molecular mechanisms of this synuclein-dependent dopaminergic neurodegeneration.

Overexpression of either wild-type or mutant α-synuclein in *Drosophila* leads to LB-like synuclein-containing inclusions and loss of dopaminergic neurons, as well as a behavioral abnormality that appears to be corrected by levodopa or DA agonists (Feany and Bender, 2000; Pendleton et al., 2002). This model should be particularly useful for genetic screens to identify novel genes involved in α-synuclein-mediated neurodegeneration.

While these transgenic studies suggest that a component of cellular toxicity may derive from α-synuclein aggregates, the relationship between aggregate formation and neurodegeneration is not straightforward. Although all reports of α-synuclein-related toxicity feature α-synuclein-containing aggregates, there exist clear examples of dissociation between aggregate formation and neurodegeneration. For example, in *Drosophila*, transgenic coexpression of the chaperone Hsp-70 prevented the dopaminergic neuronal loss caused by α-synuclein but did not affect the number of α-synuclein-containing aggregates (Auluck et al., 2002), arguing against a direct role for inclusions. Similarly, lentiviral-mediated expression of wild-type rat α-synuclein in rats led to aggregates but no cell loss (Lo Bianco et al., 2002). Thus, it is possible that a soluble misfolded species of α-synuclein or an increase in normal α-synuclein function rather than or in addition to inclusion formation contributes to the cellular toxicity observed in some of these studies.

Conclusions and Future Directions

In the past 20 years, two discoveries have profoundly influenced our understanding of PD pathogenesis, provided a conceptual framework for novel therapies, and spawned an accelerating research effort. First, the discovery of MPTP-induced PD and subsequent research exploring the molecular basis of MPTP-induced neurodegeneration established relationships between mitochondrial function, oxidative stress, and neurodegeneration. Second, the discovery of genetic causes of PD and the demonstration that dysfunction of these genes probably plays a role in sporadic PD has highlighted the importance of protein misfolding-related toxicity as a fundamental insult in neurodegeneration. The identification of PD-causing genes has also demonstrated how dysfunction of the ubiquitin-proteasome system can provoke neurodegeneration, presumably by leading to an excess of misfolded proteins.

Looking forward, a number of goals clearly emerge from the discovery of multiple PD-related genes. Future work must search for links between the molecular pathways modified by these disease-associated genes. A related goal will be to understand the relationship between previously identified factors in PD neurodegeneration (e.g., mitochondrial dysfunction, ROS) and the molecular events provoked by disease alleles. A specific aspect of this work should be to clarify primary initiating events from those that may be a nonspecific consequence of neuronal demise. While the identification of PD genes has also allowed the generation of etiologic-specific PD animal models, none of these models manifests the crucial feature of the disease: relatively selective degeneration of dopaminergic neurons. This is a vital future goal, as it would enable investigators to explore the unique features of dopaminergic neurons that make them preferentially susceptible to neurodegeneration in PD as well as to test novel therapies.

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