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Animal Models of Parkinson's Disease Induced by Toxins and Genetic Manipulation

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1. Introduction

Parkinson's disease (PD) is one of the most common chronic neurodegenerative disorders. It is characterized by a variety of motor (bradykinesia, rigidity, tremor, and postural instability) and nonmotor (autonomic disturbances and psychosis) symptoms. Although it can be diagnosed accurately, no therapeutic strategies can cure or completely block the progression of PD. Pathologically, PD is characterized by the severe loss of dopaminergic (DAergic) neurons in the pars-compacta nigra and the presence of proteinaceous α-synuclein inclusions, called Lewy bodies (LBs), which are present in neurons of the central nervous system (specific cortical regions, brain stem, and spinal cord), peripheral autonomic nervous system, enteric nervous system (ENS), and cutaneous nerves (Braak et al., 2006; Ikemura et al., 2008; Lebouvier et al., 2009). Similar to other neurodegenerative diseases, such as Alzheimer's disease, age is the major risk factor for PD although 10% of the people with the disease are younger than 45.

Although PD is regarded as a sporadic disorder, remarkably few environmental causes or triggers have been identified (Dick et al., 2007; Tanner, 2003; Taylor et al., 2005). Pesticides and herbicides are the most likely candidates for environmental agents associated with the pathogenesis of PD. On the other hand, PD characteristics are seen in a number of familial motor disorders caused by different genetic factors. Animal models of neurodegenerative diseases, including PD, have in general been quite instructive in understanding their pathogenesis. Ideally, animal models of PD, whether induced by environmental risk factors (neurotoxins) or genetic manipulations, should faithfully reproduce the clinical manifestations (behavioral abnormalities), pathological features, and molecular dysfunctions characterizing the disease. Unfortunately, animal models rarely mimic the etiology, progression, and pathology of PD completely, and in most cases, only partial insight can be gained from these studies. Despite these difficulties, animal models are considered to be very helpful in the development of therapies to treat PD. In this paper, we discuss recently developed neurotoxin-induced and genetic model animals of PD.

Over the years, many chemical compounds and toxin have been identified causative agents of PD. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a representative strong neurotoxin that has been recognized from several young drug addicts developed severe parkinsonism. The addicts illegally achieved street preparations of drugs and products were contaminated with MPTP. In addition, epidemiologically, environmental neurotoxins such

as agricultural chemicals (pesticides, herbicides, and fungicides) are promising candidates for causative factors of PD. Rotenone and paraquat could promote and accelerate the development of PD. Oxidative stress and mitochondrial dysfunction induced by these toxins could contribute to the progression of PD. While most cases of PD are sporadic, specific mutations in genes that cause familial forms of PD have led to provide new insights into its pathogenesis. Analysis of these gene products may provide vital clues to our understanding of the molecular pathogenesis of dopaminergic neuronal death in PD.

Over 10 causative genes for autosomal-dominant (*α-synuclein*, *Ubiqitin carboxy-terminal hydrolase L1 (UCHL1)*, and *Leucine-rich repeat kinase 2 (LRRK2)*) or autosomal-recessive (*parkin*, *phosphatase and tensin homolog deleted on chromosome ten (PTEN)-induced putative kinase 1 (PINK1)*, and *DJ-1* inheritance PD have been identified and classified for PARK loci. Studying animal models are important tools in experimental medical science for understanding the pathogenesis and therapeutic intervention strategies of human diseases, including neurodegenerative diseases such as PD. However, it is quite difficult to completely reproduce symptomatic and pathological features of human disorders. Since many human diseases including PD do not arise spontaneously in animals, in particular, characteristic functional changes have to be mimicked by neurotoxic agents. Nevertheless, recent studies have indicated excellent neurotoxin-induced animal models of PD. In addition, many genetic animal models of familial PD have been generated and recognized valuable tools for investigating and understanding pathophysiology of familial and even sporadic PD. Apart from the obvious preference for vertebrate (rodents and primates) models to investigate PD, an increasing number of studies have also shown a number of advantages and the utility of invertebrate (flies and nematodes) models. The central nervous system of invertebrate animal has a rather small number of neuron and glia as compared to vertebrates, however, essential functional features such as neurotransmitter system of vertebrates and invertebrates are conserved. This chapter focuses on animal models of both toxin-induced and genetically determined PD that have provided significant insight for understanding this disease. We also discuss the validity, benefits, and limitations of representative models.

2. Neurotoxin-induced animal models of PD

PD is currently viewed as a multifactorial disease. Environmental exposures, particularly to pesticides, are thought to be involved in the pathogenesis of sporadic PD. Specifically, the herbicide Paraquat (PQ) and the fungicide Maneb (manganese ethylene-bis-dithiocarbamate) have been associated with the incidence of PD (Ascherio et al., 2006; Ferraz et al., 1988). However, a causal role for pesticides in the etiology of PD has yet to be definitively established. In animal models, PD-like disorders induced by neurotoxins or other chemical compounds have led to a better understanding of the pathophysiology of PD (Table 1).

2.1 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP)

In 1979 and 1983, MPTP was initially identified as a strong neurotoxin when heroin addicts accidentally self-administered MPTP and developed an acute form of parkinsonism that was indistinguishable from idiopathic PD (Davis et al., 1979; Langston et al., 1999). A detailed neuropathological study of MPTP-induced parkinsonism in humans showed severe neuronal degeneration in the substantia nigra and the absence of LBs (Langston, et al., 1999).

Neurotoxin	Behavioral and Pathological Features	Molecular Mechanisms
MPTP	1) Parkinsonism (akinesia, rigidity, and tremor) with acute onset 2) Relatively less potent in rodents 3) Good response to L-DOPA and DA-agonists 4) Loss of TH-neurons (-fibers) and DA-content in nigrostriatal region 5) Loss of TH-neurons (-fibers) in ENS 6) α-Synuclein-positive inclusions 7) No typical LBs	1) Easily crosses the BBB 2) Converted to MPP^+ in glial cells 3) Transferred into mitochondria by transporters 4) Inhibits electron transport chain complex I 5) Upregulation of iNOS, NADPH-oxidase, and ROS 6) Microglial activation
6-OHDA	1) Intracerebral administration 2) Quantifiable locomotor abnormalities (rotation, akinsesia) 3) Good response to L-DOPA and DA-agonists 4) Loss of TH-neurons (-fibers) and DA-content in nigrostriatal region 5) No typical LBs	1) Transferred into mitochondria by transporters 2) Inhibits electron transport chain complex I 3) Microglial activation
Rotenone	1) Parkinsonism (bradykinesia, fixed posture, and rigidity) 2) Good response to L-DOPA and DA-agonists 3) Loss of TH-neurons (-fibers) and DA-content in nigrostriatal region 4) α-Synuclein-positive inclusions, resemblance to true LBs 5) Loss of myenteric neurons	1) Easily crosses the BBB 2) Inhibits electron transport chain complex I 3) Upregulation of NADPH-oxidase 4) Microglial activation
Paraquat (+ Maneb)	1) Parkinsonism similar to that of induced by MPTP 2) Loss of DA-content in nigrostriatal region 3) α-Synuclein-positive inclusions with long exposure	1) Crosses the BBB by neutral amino acid transporter 2) Inhibits electron transport chain complex I 3) Reduction of nAchR-mediated DA release 4) Inhibits complex III (Maneb)

Abbreviations: MPTP, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA, 6-hydroxy-dopamine; Maneb, manganese ethylene-bis-dithiocarbamate; L-DOPA, L-3,4-dihydroxy-L-phenylalanine; TH, tyrosine hydroxylase; DA, dopamine; ENS, enteric nervous system; LB, Lewy body; BBB, blood-brain barrier; MPP^+, 1-methyl-4-phenylpyridinium; iNOS, inducible nitric oxide synthase; ROS, reactive oxygen species; NADPH, nicotinamide adenine dinucleotide phosphate; nAchR, nicotinic acetylcholine receptor

Table 1. Representative neurotoxin-induced mammalian models of PD

The lack of LBs may have reflected the age of the patient and the duration of exposure to MPTP. The tragic results of MPTP poisoning in the heroin addicts led to the development of MPTP-induced rodent and nonhuman primate animal models of PD, which have proved extremely valuable (Chiueh et al., 1984; Kopin & Markey, 1988; Langston et al., 1984; Langston & Irwin, 1986; Markey et al., 1984). The MPTP-exposed primates show good response to therapy with L-3,4-dihydroxy-L-phenylalanine (L-DOPA) and dopamine (DA) receptor agonists (Kopin & Markey, 1988; Langston & Irwin, 1986). However, rats are relatively insensitive to MPTP neurotoxicity compared with primates. Rats given MPTP at doses comparable to those used in mice do not show remarkable neurodegeneration

(Giovanni et al., 1994; Giovanni et al., 1994). Only high doses of MPTP cause DAergic neurodegeneration in rats, indicating that complete blockade of the DA receptors is required for them to display signs of parkinsonism. Mice, like rats, are also less sensitive to MPTP than primates (Przedborski et al., 2001; Schmidt & Ferger, 2001). This model also shows pathological changes in the ENS, as observed in PD. In PD, gastrointestinal (GI) dysfunction was hypothesized to depend on neuronal degeneration in the ENS that is similar to that seen in the CNS. Recent studies show that the administration of MPTP results in decreased tyrosine hydroxylase- (TH-) positive enteric neurons in mice, indicating that the MPTP model mice should be suitable for understanding the extranigral pathophysiology of PD (Anderson et al., 2007; Natale et al., 2010).

2.2 6-Hydroxy-Dopamine (6-OHDA)

Like MPTP, 6-OHDA is a neurotoxin that has been successfully used in induction animal models of PD. 6-OHDA's strong neurotoxic effects were described by Ungerstedt in 1971, in a study presenting the first example of using a chemical agent to produce an animal model of PD (Ungerstedt, 1971). Since 6-OHDA cannot cross the blood-brain barrier (BBB), systemic administration fails to induce parkinsonism. This induction model requires 6-OHDA to be injected into the substantia nigra, medial forebrain bundle, and striatum (Perez & Palmiter, 2005; Przedborski et al., 1995). The effects resemble those in the acute MPTP model, causing neuronal death over a brief time course (12 hours to 2-3 days). Interestingly, the intrastriatal injection of 6-OHDA causes progressive retrograde neuronal degeneration in the substantia nigra and ventral tegmental complex (ST-VTA) (Berger et al., 1991; Przedborski, et al., 1995; Sauer & Oertel, 1994). As in PD, DAergic neurons are killed, and the non-DAergic neurons are preserved. However LBs do not form. Typically, 6-OHDA is used as a hemiparkinson model, in which its unilateral injection into the substantia nigra causes asymmetric motor behavior (turning, rotation) when apomorphine, a DAergic receptor agonist, or amphetamine, a dopamine releasing agent, is given systemically. In this model, the quantifiable motor behavior is a major advantage for screening pharmacological screening agents for their effects on the DAergic system and for testing cell replacement therapies (Beal, 2001; Deumens et al., 2002; Hirsch et al., 2003).

2.3 Rotenone

Rotenone is a naturally occurring complex ketone pesticide derived from the roots of *Lonchocarpus* species. It can rapidly cross cellular membranes without the aid of transporters, including the BBB. Rotenone is a strong inhibitor of complex I, which is located at the innermitochondrial membrane and protrudes into the matrix. In 2000, Betarbet et al. demonstrated in rats that chronic systemic exposure to rotenone causes many features of PD, including nigrostriatal DAergic degeneration (Betarbet et al., 2000). Importantly, pathological features match those seen in typical PD. For example, many of the degenerating neurons have intracellular inclusions that are morphologically similar to LBs. These inclusions also show immunoreactivity for α–synuclein and ubiquitin, like true LBs (Betarbet, et al., 2000; Sherer et al., 2003). The rotenone-administered model animals also reproduce all the behavioral and pathological features seen in the typical form of human PD. However, rotenone-injected rats without nigrostriatal DAergic neuronal loss demonstrate the same abnormal motor behaviors as those with such pathological features (Lapointe et al., 2004; Sherer, et al., 2003). This finding suggested that the abnormal behaviors of PD could depend, at least partly, on the damage to

non-DAergic neurons in the nigrostriatal area. Furthermore, rotenone exposure also causes the loss of myenteric neurons in the rat (Drolet et al., 2009).

2.4 Paraquat and maneb

Because of its close structural similarity to 1-methyl-4-phenylpyridinium (MPP+, the active metabolite form of MPTP), an herbicide, 1,1′-dimethyl-4,4′-bipyridinium, named paraquat has been suggested as a risk factor for PD (Di Monte et al., 1986). The systemic administration of paraquat to adult mice results in a significant decrease in substantia nigra DAergic neurons, a decline in striatal dopamine nerve terminal density, and a neurobehavioral syndrome characterized by reduced ambulatory activity (Brooks et al., 1999). These data support the idea that paraquat crosses the BBB to cause destruction of the dopamine neurons in the substantia nigra, like MPP+ (Brooks, et al., 1999). The prolonged exposure to paraquat leads to a remarkable accumulation of α–synuclein-like aggregates in neurons of the substantia nigra pars compacta in mice (Manning-Bog et al., 2002). Chronic exposure to paraquat also reduces the expression of the nicotinic acetylcholine receptor (nAChR) subunit $\alpha 3/\alpha 6\beta 2*$ (the asterisk indicates the possible presence of additional subunits). Normally, the activation of both nAChR subtypes stimulates DA release in the striatum (Khwaja et al., 2007; McCallum et al., 2005; Wonnacott et al., 2000). The injection of paraquat selectively reduces the $\alpha 3/\alpha 6\beta 2*$-mediated DA release from the striatum in primates (O'Leary et al., 2008). Maneb is an organomanganese fungicide that is broadly used in agriculture and is a putative causative agent for PD. Surprisingly, Thiruchelvam et al. found that the neurotoxic effects of maneb or paraquat on the nigrostriatal DA system in mice are synergistically potentiated in combination (Thiruchelvam et al., 2000). Their report argued that this finding has important implications for the human risk of PD, because the marked geographical overlap in the estimated annual agricultural applications of paraquat and maneb means that people living in these areas may be exposed to the synergistic neurotoxicity of these two agents (Thiruchelvam, et al., 2000; Thiruchelvam et al., 2000).

3. Pathophysiological mechanisms of DAergic neurotoxins

All the representative neurotoxin-induced PD models described above show defective mitochondrial function, manifested by the inhibition of mitochondrial complex I or III. MPTP is a highly lipophilic agent. After its systemic administration, MPTP rapidly crosses the BBB. Once in the brain, MPTP is converted to 1-methyl-4-phenyl-2,3- dihydropyridium (MPDP+) in glial cells (astrocytes) and serotonin neurons by monoamine oxidase B (MAO-B) and then spontaneously oxidizes to MPP+ (Nicklas et al., 1985; Przedborski & Vila, 2003). Thereafter, MPP+ is released into the extracellular space. Unlike MPTP, MPP+ is a polar molecule that cannot freely enter DAergic neurons. Thus, a plasma membrane transport system is required. MPP+ has a high affinity for dopamine transporter (DAT) as well as for norepinephrine and serotonin transporters (Bezard et al., 1999; Mayer et al., 1986). Once inside DAergic neurons, MPP+ can accumulate in mitochondria and impair mitochondrial respiration by inhibiting complex I in the electron transport chain (Nicklas, et al., 1985; Ramsay & Singer, 1986), which induces the generation of reactive oxygen species (ROS). MPP+ can also bind to vesicular monoamine transporters (VMATs), which help move selected materials into synaptic vesicles containing DA (Del Zompo et al., 1993). MPP+ can also remain in the cytoplasm and interact with cytosolic enzymes (Klaidman et al., 1993).

Inducible nitric oxide synthase (iNOS) is also involved in the pathogenesis of MPP+-induced parkinsonism in animal models. Increased iNOS has also been found in the substantia nigra of autopsied PD patients, indicating that nitric oxide (NO) overproduction is a feature of the human disease (Huerta et al., 2007; Hunot et al., 1996). Excess NO could contribute to the formation of free radicals, which could damage DAergic neurons, leading to the development of PD symptoms. Mice null for iNOS show a resistance to neuronal damage by MPTP, and iNOS inhibitors protect against the degeneration of DAergic neurons in MPTP-treated mice (Dehmer et al., 2000; Liberatore et al., 1999). Furthermore, microglial cells can be activated by the formation of free radicals and iNOS-mediated damage, and thereby exacerbate the toxicity of MPTP (Barcia et al., 2004; Breidert et al., 2002; Wu et al., 2002). Finally, MPTP can also upregulate nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase in the substantia nigra of mice (Wu, et al., 2002), which is significant because NADPH-oxidase appears to be ubiquitously expressed in all brain regions and metabolizes molecular oxygen, generating superoxide as a product. In fact, MPTP toxicity is diminished in mice lacking functional NADPH-oxidase, indicating a pivotal role for superoxide ions in the neurotoxicity induced by MPTP (Wu, et al., 2002).

The toxicity of 6-OHDA also involves mechanisms of oxidative stress. 6-OHDA can be taken up by DAergic neurons through DAT (Bove et al., 2005; Schober, 2004). Once transported into neurons, 6-OHDA is oxidized like DA. The oxidized molecule generates free radicals inhibits mitochondrial complex I and produces superoxide and hydroxyl radicals (Bove, et al., 2005; Schober, 2004). It is not only toxic to the DAergic neurons but can also induce microglial activation (Bove, et al., 2005).

Like MPTP, the pesticide rotenone is very lipophilic, crosses the BBB, and is distributed evenly throughout the brain (Bove, et al., 2005; Uversky, 2004). It can enter mitochondria, where it inhibits complex I of the electron transport chain with high affinity (Bove, et al., 2005). Interestingly, the inhibition of microglial activation by an antibiotic,minocycline, can attenuate the neurotoxicity of rotenone (Casarejos et al., 2006). Gao et al. also showed that the neurotoxicity of rotenone is reduced in neuron-glia cocultures from NADPH oxidase-null mice (Gao et al., 2003). The DA uptake of the neuron-enriched cultures was not affected by the addition of microglia from NADPH oxidase-null mice, the addition of microglia from wild-type (WT) mice significantly increased the sensitivity of DAergic neurons either from WT or knockout (KO) mice to rotenone neurotoxicity. These data indicate that microglial NADPH oxidase, but not neuronal NADPH oxidase, is responsible for the NADPH oxidase-mediated neurotoxicity of rotenone (Gao, et al., 2003). Paraquat mainly crosses the BBB through the neutral amino acid transporter (McCormack & Di Monte, 2003; Shimizu et al., 2003; Yang & Sun, 1998). Once in the brain, it is selectively taken up by the terminals of DA-containing neurons in the substantia nigra by the DAT, and it inhibits mitochondrial complex I (Shimizu, et al., 2003). Maneb contains a major active fungicidal component, manganese ethylene-bis-dithiocarbamate (Mn–EBDC). In a rat model in which Mn–EBDC is directly delivered to the lateral ventricles, Mn–EBDC causes selective DAergic neurodegeneration (Zhang et al., 2003). Mn–EBDC preferentially inhibits mitochondrial complex III (Zhang, et al., 2003).

4. Genetic animal models of PD

Although the etiopathogenesis (including environmental factors) of PD is not fully understood, the extensive examination of human postmortem material, the genetic analysis

of patients, and the study of experimental animal models have shed significant light on the molecular mechanisms involved in its progression. However, since the number of patients with familial PD is extremely low compared to the number with sporadic PD, genetic studies in affected human families are very difficult. Therefore, the development of animal genetic models for PD is especially important, and such models provide an opportunity not only to investigate the genetic etiology of PD but also to identify new factors that could be invaluable in terms of diagnosis, drug design, and/or therapy (Gasser, 2009; Lees et al., 2009). Even invertebrate animals, for example, *Drosophila melanogaster*, are useful models for surveys of human PD. While their numbers of neurons and glia are obviously much smaller than in rodents and primates, *Drosophila* have the same types of neuron-glia systems, and a great number of genes and molecular transduction pathways are conserved between *Drosophila* and humans.

In recent years, several genetic animal models of PD have been reported, including models for autosomal-dominant (AD) inheritance patterns. The genes manipulated in these models include *α-synuclein*, *LRRK2*, *UCHL1*, and *high temperature requirement A2* (*HTRA2/Omi*) (Table 2). There are also models of autosomal-recessive (AR) inherited PD, which involve KO or knockdown genes for *parkin*, *DJ-1*, and *PINK1* (Table 3). In addition, we will review a PD mouse model deficient in *nuclear receptor-related 1* (*Nurr1*), also named *nuclear receptor subfamily 4, group A, member 2* (*NR4A2*), which is a susceptibility gene for familial PD (Table 3).

Gene	Animal	Manipulation	DA neuron loss	LB-like inclusions[1]	DA-responsive motor deficits[2]
α-synuclein (*PARK1*)	Nematode	Transgenic	Yes§	No	Yes
	Fly	Transgenic	Yes	Yes	Yes
	Mouse	Transgenic	No	Yes§ (PrP promoter)	Yes§ (PDGFβ promoter)
	Rat	Transgenic	Yes	No	Yes
	Monkey	Transgenic	Yes	No	ND
UCHL1 (*PARK5*)	Mouse	Transgenic	Yes	No	Yes
LRRK2 (*PARK8*)	Nematode	Transgenic	Yes	ND	ND
	Fly	Transgenic	Yes	No	Yes
	Mouse	Transgenic	No	No	Yes

Abbreviations: UCHL1, ubiqitin carboxy-terminal hydrolase L1; LRRK2, leucine-rich repeat kinase 2; DA, dopamine; LB, Lewy body; ND, not determined; PrP, prion; PDGFβ, platelet-derived growth factor β

1; LB-like inclusions by definition contain filamentous α-synuclein

2; ND could include some degree of behavioral impairment in spontaneous and locomotor activity and in response to sensory stimulation

§; Controversial. The opposite result has also been shown.

Table 2. Autosomal-dominant PD models

Gene	Animal	Manipulation	DA neuron loss	LB-like inclusion[1]	DA-responsive motor deficits[2]
parkin (*PARK2*)	Nematode	Knockout	No	No	No
	Fly	Knockout	Yes	No	Yes
		Transgenic	Yes	No	Yes
	Mouse	Knockout	No	No	ND
		Transgenic	Yes	Yes	ND
PINK1 (*PARK6*)	Fly	Knockout	Yes	No	Yes
	Mouse	Knockout	No	No	ND
DJ-1 (*PARK7*)	Fly	Knockout	Yes	No	Yes
	Mouse	Knockout	No	No	ND
HtrA2/Omi (*PARK13*)	Fly	Knockout	No	No	No
	Mouse	Knockout	No	No	ND
Nurr1 (NR4A2)	Mouse	Knockout	Yes	No	ND

Abbreviations: PINK1, phosphatase and tensin homolog deleted on chromosome ten (PTEN)-induced putative kinase 1; HtrA2, high temperature requirement A2; Nurr1, nuclear receptor-related 1; NR4A2, nuclear receptor subfamily 4, group A, member 2; DA, dopamine; LB, Lewy body; ND, not determined
1; LB-like inclusions by definition contain filamentous α-synuclein
2; ND could include some degree of behavioral impairment in spontaneous and locomotor activity and in response to sensory stimulation

Table 3. Autosomal-recessive PD models and other causative genes of PD

4.1 α-synuclein

α-synuclein was the first gene linked to an AD-type familial PD, called Park1. The identification of an *α-synuclein* mutation in this family revolutionized PD research, since *α*-synuclein is the main component of LBs, which are observed in the sporadic PD brain. This striking result strongly indicates that genetic and sporadic PD may share similar etiologies and that investigating *α*-synuclein-mediated pathogenesis in familial PD could uncover important information about sporadic PD. Three missense mutations of *α-synuclein*, encoding the substitutions A30P, A53T, and E46K, have been identified in familial PD (Gasser, 2009; Kruger et al., 1998; Lees, et al., 2009; Polymeropoulos et al., 1997). Furthermore, the duplication or triplication of *α-synuclein* is sufficient to cause PD, suggesting that the level of *α*-synuclein expression is a critical determinant of PD progression (Singleton, 2005; Singleton et al., 2003). Even though no direct relationship between sporadic PD and *α*-synuclein expression has yet been shown, the existence of several polymorphisms in the promoter or 3′-UTR of the *α-synuclein* gene suggests that its expression level might be a risk factor (Holzmann et al., 2003; Pals et al., 2004; Winkler et al., 2007).

Human *α*-synuclein is an abundant 140-amino acid presynaptic phosphoprotein involved in vesicle handling and neurotransmitter release. Mutations in *α*-synuclein that increase the propensity for misfolding are probably deleterious, because the misfolded forms are toxic, and they induce cell death *in vitro* (Cookson, 2005; Lee & Trojanowski, 2006). Among the

variety of abnormal forms that mutant α-synuclein can adopt, protofibrils and fibrils seem to be the most toxic (Lee & Trojanowski, 2006). These demonstrations of α-synuclein toxicity *in vitro* led to the creation and extensive analysis of many *α-synuclein*-based animal models of PD.

Although flies (*Drosophila*) and nematodes (*C. elegans*) do not have complex nervous systems compared to vertebrates and do not express endogenous α-synuclein, they are useful for identifying genetic and pharmacological modifiers of *α-synuclein* and its product. In *Drosophila*, the overexpression of WT and mutated (A30P, A53T) human α-synuclein causes the age-dependent loss of dorsomedial DAergic neurons, an accumulation of LB-like filamentous inclusions with α-synuclein immunoreactivity, and compromised locomotor activity (climbing ability) (Feany & Bender, 2000). In *C. elegans*, α-synuclein overexpression leads to accelerated DAergic neuronal loss and motor impairment (Kuwahara et al., 2006; Lakso et al., 2003). However, the neurons of these nematodes do not contain notable synuclein-containing inclusions.

Many different mouse lines that overexpress α-synuclein under various promoters have been generated in the last ten years, and most have been described in recent reviews (Chesselet, 2008; Fernagut & Chesselet, 2004; Fleming & Chesselet, 2006). Mice expressing α-synuclein containing two mutations (A30P + A53T) under the TH promoter show progressive declines in locomotor activity and the loss of substantia nigra neurons and striatal DA content (Richfield et al., 2002; Thiruchelvam et al., 2004). Similarly, mice overexpressing WT human (-synuclein under the neuron-specific platelet-derived growth factor β (PDGFβ) promoter show reduced TH immunoreactivity and DA content in the striatum and impaired motor performance (Masliah et al., 2000). Mice overexpressing WT human α-synuclein under another neuron-specific promotor, Thy1, show strong widespread expression in cortical and subcortical neurons, including the substantia nigra pars compacta, but no glial, spinal, or neuromuscular pathology (Kahle et al., 2001; Rockenstein et al., 2002; Song et al., 2004). These mice have an increased sensitivity to mitochondrial damage from low doses of MPTP (Song, et al., 2004). Mice in which the mouse prion promoter (mPrP) is used to drive the expression of α-synuclein A53T show α-synuclein aggregation, fibrils and truncation, α-synuclein phosphorylation, ubiquitination, and progressive age-dependent neurodegeneration, just as in humans (Giasson et al., 2002; Lee et al., 2002).

Several viral vectors, primarily lentiviruses and adenoassociated viruses (AAVs), have been used to drive exogenous α-synuclein. Because viral vector delivery requires stereotactic injections within or near the site of the neuronal cell bodies in the substantia nigra pars compacta, rats are generally used for these studies although the model has been reproduced in other rodents (Kirik et al., 2002; Klein et al., 2002; Lauwers et al., 2003; Lo Bianco et al., 2002). The overexpression of human WT or A53T mutant α-synuclein by AAVs in the SNc neurons of rats causes the progressive age-dependent loss of DA neurons, motor impairment, and α-synuclein-positive cytoplasmic inclusions (Kirik, et al., 2002). Kirik et al. also overexpressed WT or A53T mutant α-synuclein in marmosets (Kirik et al., 2003), in which the α-synuclein protein was expressed in 90%–95% of all substantia nigra DA neurons. The transduced neurons showed evidence of severe pathology, including α-synuclein-positive cytoplasmic inclusions, granular deposits, and loss of the TH-positivity.

It is particularly notable that the phenotypic outcome of α-synuclein overexpression in mice heavily depends on the promoter used to drive transgene expression. Unfortunately, most

of these models fail to accurately mimic PD in that there is no progressive loss of DA neurons. The loss of TH-positive cell bodies in the substantia nigra does not necessarily indicate cell death. Despite the lack of overt degenerative pathology in the DA-positive neurons, obvious locomoter abnormalities due to degeneration of the nigrostriatal system and a lack of DA responsiveness are observed in the various mouse α-synuclein models. Thus, most of these lines are excellent models of α-synuclein-induced neurodegenerative disorders, such as PD.

Although mutated α-synuclein causes human familial PD, α-synuclein's physiological roles in PD are not fully understood. In KO mice of α-synuclein, neuronal development and the formation of presynaptic terminals are normal (Abeliovich et al., 2000). Moreover, double KO mice that lack α- and β-synuclein exhibit normal basic brain functions and survive to adulthood (Chandra et al., 2004). Thus, the loss of α-synuclein function is unlikely to play a role in the pathogenesis of α-synuclein-induced neurodegeneration. Meanwhile, α-synuclein KO mice show reduced rearing activity in the open field, decreased DA content in the striatum, and a decrease in the reserve pool of vesicles in the hippocampus (Abeliovich, et al., 2000; Cabin et al., 2002). These results indicate that α-synuclein may play a regulatory role *in vivo*, possibly in the fine tuning of synaptic plasticity and/or vesicle maintenance. Interestingly, several lines of α-synuclein-null mice have a complete or partial resistance to the MPTP (Dauer et al., 2002; Schluter et al., 2003). Dauer et al. showed that this resistance is not due to abnormalities of the DA transporter, which appears to function normally in α-synuclein null mice (Dauer, et al., 2002). These reports indicate that α-synuclein is not obligatorily coupled to MPTP sensitivity, but can influence MPTP toxicity on some genetic background.

4.2 *UCHL1*

A rare AD-inherited form of PD, PARK5, is caused by a missense mutation in the *UCHL1* gene. UCHL1 constitutes 1%-2% of the brain proteins and functions in the ubiquitin-proteasome system. The ubiquitin hydrolase activity of UCHL1 is important for freeing reusable ubiquitin monomers. The missense mutation in PARK5 causes an Ile93Met substitution in the UCHL1 protein (UCHL1Ile93Met), and this mutant was initially shown to have decreased ubiquitin hydrolase activity (Leroy et al., 1998). Interestingly, UCHL1 is detected in LBs in sporadic PD cases (Lowe et al., 1990). These findings initiated a debate on whether the Ile93Met mutation causes a gain of function (toxicity) or loss of function (deficiency).

The gracile axonal dystrophy (*gad*) mouse is an AR-mutant that shows sensory ataxia at an early stage, followed by motor ataxia. Saigoh et al. showed that these mice exhibit spontaneous intragenic deletion of the *UCHL1* gene and do not express the UCHL1 protein (Saigoh et al., 1999). These mice do not show obvious pathological changes in the nigrostriatal DA pathway; in particular, there is no loss of DA cell bodies in the substantia nigra. Setsuie et al. generated transgenic mice that overexpressed UCHL1Ile93Met and reported a reduction in the DAergic neurons of the substantia nigra and of the DA content in the striatum (Setsuie et al., 2007). These mice show behavioral and pathological phenotypes of parkinsonism at 20 weeks of age. Moreover, recently, Yasuda et al. performed a viral vector-mediated *α-synuclein* injection into the substantia nigra of the UCHL1Ile93Met transgenic mice (Yasuda et al., 2009). These mice show a significantly enhanced loss of DA-positive cell bodies in the substantia nigra and of DA content in the striatum. The

neurotoxicity is enhanced by PARK5-associated UCHL1Ile93Met mutant, but not influenced by the loss of UCH-L1 WT protein *in vivo*, indicating that the UCHL1Ile93Met toxicity results from a gain of function.

4.3 *LRRK2*

The *LRRK2* mutation is another type of ADPD, called *PARK8*. LRRK2 is a large protein containing a serine/threonine kinase and a GTPase domain that is localized to membranous structures (Biskup et al., 2006). The frequency of the common LRRK2 Gly2019Ser mutation was 1% in patients with sporadic PD and, interestingly, 4% of patients with hereditary PD (Healy et al., 2008). The risk of PD when the LRRK2 Gly2019Ser mutation was present was 28% at age 59 years, 51% at 69 years, and 74% at 79 years. The motor symptoms and non-motor symptoms of LRRK2-associated PD are more benign than those of idiopathic PD. In autopsied tissue, the LB pathology was present in a representative LRRK2 G2019S case, indicating that LRRK2 and α-synuclein share some pathogenic mechanisms (Ross et al., 2006). Yet, LRRK2 may play a role in neuronal outgrowth and guidance, and its precise physiological function remains to be clarified (MacLeod et al., 2006).

dLRRK is a *Drosophila* orthologue of LRRK2, and it shows elevated expression in DA neurons of the head (Imai et al., 2008; Lee et al., 2007). Liu et al. overexpressed constructs with mutations similar to those found in patients (G2019S), in *Drosophila* (Liu et al., 2008). The neuronal expression of LRRK2 or LRRK2-G2019S produces an adult-onset selective loss of DAergic neurons, locomotor dysfunction, and early mortality. However, the phenotype caused by the G2019S-LRRK2 mutant is more severe than that cause by the expression of equivalent levels of WT LRRK2. Treatment with L-DOPA improves the mutant LRRK2-induced locomotor impairment but does not prevent the loss of TH-positive neurons. Some fly models that overexpress other LRRK2 mutations, such as I1122V, Y1699C, and I2020T, show similar results, in terms of an age-dependent impairment of locomotor activity that improves with DA stimulation, and the loss of DA neurons (Liu, et al., 2008; Ng et al., 2009; Venderova et al., 2009). Moreover, in transgenic *C. elegans*, DA marker loss is greater in those expressing G2019S LRRK2 than WT LRRK2 (Saha et al., 2009).

Transgenic mice made using bacterial artificial chromosome (BAC) technology and expressing WT LRRK2, or the R1441G or G2091S mutation exhibit mild axonal pathology in the nigrostriatal DA projection (Li et al., 2010; Li et al., 2009). However, the conditional overexpression of neither WT LRRK2 nor its G2019S mutation causes degeneration of the DA-containing neurons (Lin et al., 2009). Interestingly, although the LRRK2 conditional transgenic mice show minimal nigrostriatal pathologies, they exhibit a progressive age-dependent motor impairment that is improved by DA stimulation. LRRK2 involvement in the pathogenesis of PD may be limited, and other genetic and/or environmental factors are probably required to trigger DA neuronal degeneration.

LRRK2 KO mice are viable, have no major abnormalities, and live to adulthood, and there is no significant difference in the susceptibility of LRRK2-deficient and WT mice to MPTP (Andres-Mateos et al., 2009). In *LRRK2*-KO *Drosophila* models, differing results on the pathology of the DA neurons have been obtained (Imai, et al., 2008; Wang et al., 2008). Lee et al. showed that *LRRK* loss-of-function mutants exhibited severely impaired locomotive activity (Lee, et al., 2007). Moreover, DAergic neurons in *LRRK* mutants showed a severe reduction in tyrosine hydroxylase immunostaining and shrunken morphology. Conversely, Wang et al. demonstrated that mutants lacking *dLRRK* kinase activity are viable with

normal development and life span as well as unchanged number and pattern of DAergic neurons (Wang, et al., 2008). Nematode deletion mutants indicate that LRRK2 is dispensable for the development and maintenance of DA neurons (Sakaguchi-Nakashima et al., 2007).

4.4 *Parkin*

Parkin covers approximately 1.3 Mb of genomic DNA and is the causative gene for representative AR juvenile PD (*PARK2*). Mutations in *parkin* are not only a cause of familial PD but are also seen in 20% of young-onset sporadic PD cases (Lucking et al., 2000). Parkin is an E3 ubiquitin ligase that functions in the ubiquitin-proteasome system. The loss of parkin function is believed to result in abnormal accumulations of parkin's substrates. Springer et al. demonstrated that *pdr-1* (the nematode parkin homolog) mutants are viable and display no obvious morphological defects or alterations in motility, egg-laying behavior, brood size, or life span under standard growth conditions (Springer et al., 2005). Moreover, the authors did not detect any effect of the mutations on the survival of the DA neurons in the worms. However, overexpression of the α-synuclein A53T mutation in *pdr-1* mutants leads to developmental arrest and lethality, indicating this *C. elegans* model recapitulates parkin insolubility and aggregation similar to several AR juvenile PD-linked parkin mutations (Springer, et al., 2005).

Drosophila parkin-null mutants exhibit a reduced lifespan, locomotor defects (flight and climbing abilities), and male sterility (Greene et al., 2003; Whitworth et al., 2005). The locomotor defects derive from the apoptotic cell death of muscle subsets whereas the male sterile phenotype derives from a spermatid individualization defect at a late stage of spermatogenesis. Mitochondrial pathology is the earliest manifestation of muscle degeneration and a prominent characteristic of individualizing spermatids in parkin mutants. These mutants also display a decrement in the TH level and degeneration of a subset of DA neurons in the brain (Whitworth, et al., 2005). Several parkin-null mice have been generated and display motor and cognitive deficits including reduced locomotor activity and decreased spontaneous alteration in the T-maze; however, they show no substantial DAergic behavioral abnormalities (Goldberg et al., 2003; Itier et al., 2003; Perez & Palmiter, 2005; Von Coelln et al., 2004). Pathologically, KO mice exhibit slightly abnormal DA nigrostriatal and locus coeruleus noradrenergic regions (Goldberg, et al., 2003; Von Coelln, et al., 2004).

The overexpression of human mutant *parkin* in *Drosophila* causes an age-dependent, selective degeneration of DA neurons accompanied by progressive motor impairment (Sang et al., 2007; Wang et al., 2007). *Parkin-Q311X* mice also exhibit multiple late-onset and progressive hypokinetic motor deficits (Lu et al., 2009). Stereological analyses revealed that the mutant mice develop age-dependent DA neuron degeneration in the substantia nigra and a significant reduction of the striatal DA level, accompanied by a significant loss of DA neuron terminals in the striatum. These results indicate that *parkin* mutants may play a pivotal role in the dominant-negative etiological mechanisms of PD.

4.5 *PINK1*

PINK1 is another causative gene for the AR inherited PD called *PARK6*. PARK6 is the second most frequent early-onset AR PD. PINK1 is located in mitochondria and is a putative mitochondrial kinase, because it contains a conserved serine/threonine kinase domain with an N-terminal mitochondrial-targeting motif (Silvestri et al., 2005). Thus, the PD-causative

mutations of *PINK1* may cause loss of function. Park et al. and Clark et al. generated and characterized loss-of-function *Drosophila* PINK1 mutants (Clark et al., 2006; Park et al., 2006). These flies exhibit male sterility, apoptotic muscle degeneration, defects in mitochondrial morphology, and increased sensitivity to multiple stresses, including oxidative stress.
Park et al. showed an age-dependent decrease in DA levels and a mild loss of DA neurons in these *Drosophila* mutants (Park, et al., 2006). Notably, the PINK1mutants share marked phenotypic similarities with parkin mutants. Parkin overexpression is able to rescue the mitochondrial defects found in PINK1, although the double mutants do not show an enhanced phenotype. PINK1 overexpression does not rescue parkin phenotypes. Together, the data indicate that parkin and PINK1 function, at least partly, in a common pathway, and PINK1 acts upstream of parkin. Whereas PINK1-deficient mice show age-dependent mitochondrial dysfunction, increased sensitivity to oxidative stress, decreased evoked DA release, and DA receptor agonist-responsive impairment of striatal plasticity, the number of DA neurons, the level of striatal DA, and the level of DA receptors are the same as in WT animals (Gautier et al., 2008; Gispert et al., 2009; Kitada et al., 2007). These phenotypes are similar to those of *parkin*-KO mice.

4.6 *DJ-1*

Deletion or point mutations in *DJ-1* have been identified in early onset AR PD (*PARK7*). DJ-1 plays a role as an antioxidant and chaperone, and it is expressed ubiquitously in the cytosol, mitochondrial matrix, and intermembranous space (Zhang et al., 2005). In vitro, downregulation or KO of the endogenous DJ-1 increases cells' vulnerability to oxidative stress and proteasome inhibition, implicating it in the cellular response to oxidative stress (Martinat et al., 2004; Mitsumoto et al., 2001; Yokota et al., 2003). *Drosophila* possesses two different orthologs of the human DJ-1 gene, named *DJ-1*α and *DJ-1*β. While loss-of-function DJ-1β mutants have normal numbers of DA neurons, classical genetic analyses and RNAi experiments have yielded contradictory results regarding the function of DJ-1α in DA neuron maintenance (Lavara-Culebras & Paricio, 2007; Menzies et al., 2005; Meulener et al., 2005; Park et al., 2005; Yang et al., 2005). However, DA neuron loss cannot be detected inDJ-1α/DJ-1β double-deletion mutants, which are also viable, fertile, and have a normal life span. Some studies have reported a loss of DA neurons upon acute RNA silencing of DJ-1α (Lavara-Culebras & Paricio, 2007; Yang, et al., 2005).
Similar to α-synuclein and parkin KO mice, DJ-1 KO mice do not show major DA-agonist-responsive behavioral abnormalities or the loss of nigrostriatal DA neurons (Andres-Mateos et al., 2007; Goldberg et al., 2005; Kim et al., 2005). In particular, although the levels of striatal DA and DA receptors are unchanged, the evoked dopamine release from striatal slices is clearly reduced, most likely as a consequence of increased reuptake. DJ-1 mutant mice also show an increased sensitivity to MPTP (Kim, et al., 2005). This is rescued by restoring the DJ-1 expression in mutant mice, further indicating a role for DJ-1 in the oxidative stress response.

4.7 HtrA2/Omi

HtrA2/Omi has been identified as the causative gene for a rare inherited PD, *PARK13*. HtrA2/Omi has a PDZ (PDZ is based on three proteins that led to its discovery, postsynaptic density protein (PSD-95), *Drosophila* disc large tumor suppressor (DLG1), and zonula occludens-1 protein (ZO-1)) domain in addition to a serine protease domain and is

localized to the mitochondrial intermembrane space by its mitochondria-targeting sequence. Whitworth et al. have demonstrated a genetic interaction between *HtrA2/Omi* and *PINK1*, described below, by investigating the eye phenotype of double mutant flies (Whitworth et al., 2008). Their study revealed that *HtrA2/Omi* acts downstream of *PINK1* and is independent of the *parkin* gene. Yet, Yun et al. indicated that *HtrA2/Omi* null fly mutants show neither mitochondrial morphological defects nor DAergic neuronal loss (Yun et al., 2008). They also generated a *Drosophila HtrA2/Omi* mutant analogue to the human mutation G399S, which was identified in *PARK13* patients. HtrA2/Omi G399S retains a significant, if not complete, function of HtrA2/Omi, compared with protease-compromised versions of the protein, indicating that *HtrA2/Omi* is unlikely to play a pivotal role in PD pathogenesis or as an etiological factor. The targeted deletion of *HtrA2/Omi* in mice increases their sensitivity to stress-induced cell death (Jones et al., 2003; Martins et al., 2004). Animals lacking HtrA2/Omi display a progressive movement disorder similar to progressive akinesia, a rigidity syndrome, showing lack of coordination, decreased mobility, bent posture, tremor, and a decreased number of TH-positive striatal neurons (Martins, et al., 2004).

4.8 Nurr1 (NR4A2)

Nurr1 is a member of the nuclear receptor superfamily and is involved in the differentiation and development of nigrostriatal DA neurons. Le et al. identified two mutations in *Nurr1* associated with PD (–291Tdel and –245T→G), which map to the first exon of *NR4A2* and affected one allele in 10 of 107 individuals with familial PD (Le et al., 2003). Mutations in *Nurr1* alter the transcription of *TH* and the DA transporter, suggesting that alterations in Nurr1 may cause chronic DA alterations that could increase susceptibility to PD (Sacchetti et al., 2001). Nurr1 is essential for the development of the ventral mesencephalic DA neurons, because homozygous Nurr1-KO mice do not develop DA neurons in the substantia nigra and die soon after birth (Zetterstrom et al., 1997). Heterozygous Nurr1-KO mice exhibit a significant decrease in rotarod performance and locomotor activities (Jiang et al., 2005). These phenotypes are associated with decreased DA levels in the striatum, decreased numbers of DAergic neurons, and a reduced expression of Nurr1 and DAT in the substantia nigra. Moreover, Le et al. reported that heterozygous Nurr1-KO mice show a significant decrease in the total number of TH-positive neurons in the substantia nigra and reduced DA in the striatum after MPTP administration (Le et al., 1999). Thus, these mice show a progressive DA phenotype that bears some resemblance to that found in α-synuclein-overexpressing and mutant mice. Therefore, *Nurr1*-knockdown mice may provide a good model for investigating the later stages of PD characterized by severe DA neuron loss.

5. Genetic risk factors of PD

The identification and characterization of susceptibility genes for common human disease, including PD, is a difficult challenge. The usual approach of focusing a study on just one or a few candidate genes limits our ability to identify novel genetic effects associated with disease. In addition, many susceptibility genes may exhibit effects that are partially or solely dependent on interactions with other genes and/or the environment. Recently, Genome-wide association studies (GWAS) have been proposed as a solution to these problems. GWAS analyses must embrace abundant clinical and environmental data available to complement the rich genotypic data with the ultimate goal of revealing the genetic and environmental factors important for disease risk.

In 2009, two reports of GWAS demonstrated that several genes and loci could be genetic risk factors for PD in the different population (Satake et al., 2009; Simon-Sanchez et al., 2009). These studies indicated that *α-synuclein, LRRK2*, and locus on 1q32 (designated as *PARK16*) showed strong association to PD. Interestingly, *BST1* on 4q15 is only identified a new risk locus in the Japanese study (Satake, et al., 2009) and *microtubule-associated protein tau (MAPT)* is only found association in the European study (Simon-Sanchez, et al., 2009), indicating that population-specific genetic heterogeneity involves in the pathogenesis of PD.

MAPT is primarily expressed in neurons and plays a key role in the organization and integrity of the cytoskeleton and filamentous neuronal tau inclusions define a set of neurodegenerative diseases referred to as the "tauopathies," which include Alzheimer's disease, corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17). While tau pathology is sometimes found in PD, it is not pathognomic. Thus, the relationship between the MAPT and the pathophysiology of PD still remains to be elucidated, however, brain pathology in Alzheimer's disease cases with amyloid precursor protein mutation exhibits not only β-amyloid deposition, tangle, but also sometimes LB pathology (Hardy, 1994). This finding indicates that there are genetic pathologic connections between α-synuclein and tau.

Other GWAS demonstrated that the strongest evidence of association was obtained on chromosome 4p16 in the gene *cyclin G associated kinase (GAK)*, designated as PARK17 (Hamza et al., 2010; Pankratz et al., 2009). This gene might be a promising candidate since the expression of cell cycle regulators altered in the substantia nigra pars compacta with PD (Grunblatt et al., 2004).

More recent GWAS showed a new genetic association with PD in the *human leukocyte antigen (HLA)-DRA* region (6p21), designated as PARK18 (Hamza, et al., 2010). Interestingly, this result suggests an involvement of immune system in the pathogenesis of PD. Furthermore, genetic variability of *HLA* region potentially has impact on damage repair and cleaning up risk for disease. The adaptive or innate immune systems had previously been implicated in disease pathology in the late-onset neurodegenerative diseases such as PD and Alzheimer's disease (McGeer et al., 2005).

Gaucher disease (GD) is an autosomal recessive glycolipid storage disorder with multisystemic manifestations caused by loss of function mutations in the *glucocerebrosidase (GBA)* gene, which encodes the enzyme glucocerebrosidase. A small subset of patients with GD develops parkinsonism with brain stem or diffuses LB-related pathology (Wong et al., 2004). An increased incidence of parkinsonism has also been reported in relatives of patients with PD (Halperin et al., 2006). In 2009, multicenter analysis demonstrated that there is a strong association between GBA mutations (L444P and N370S) and PD (Sidransky et al., 2009). While both gain-of- and loss-of- function hypotheses have been proposed, he mechanism by which GBA mutations increase risk for PD is not fully known (Velayati et al., 2010).

Additional susceptibility loci will likely be uncovered in the near future, as the wealth of recent data from GWASs is further analyzed. Such efforts will include meta-analysis, consideration of gene-gene and gene -environment interaction, and analysis of copy number variation. Although important progress has been made, the mechanisms by which variation in PD-linked genes leads to neurodegeneration remains poorly understood. However, data accumulated thus far has implicated mitochondrial dysfunction, oxidative damage, aberrant protein aggregation, deficits in ubiquitin-mediated protein degradation, and malfunction of immune system as playing key roles in the etiopathogenesis of PD. Actually, animal models

of these risk factor gene mutations have been described very few, but once they are available (if pathological features including LB and clinical manifestation are replicated by candidate genes manipulation), they will undoubtedly shed new light on the mechanisms of PD.

6. Concluding remarks

The symptoms of PD become apparent after more than 80% of the DA neurons have died. The rate of substantia nigral cell loss is assumed to be about 2,500 per year in normal people. The loss of DA function can be accelerated by exposure to neurotoxins and by molecular (genetic) abnormalities, leading to a fast and significant decrease in the number of DA neurons. Consequently, these pharmacological and/or genetic insults can cause early onset of PD. This scenario indicates that critical pathological changes could be initiated one or two decades prior to the onset of PD. As described above, whether the causative factor is a toxic compound or a mutated gene, we have no perfect animal models of PD. So far, the neurotoxin-induced vertebrate models of PD are suitable for investigating disease-modifying therapies, since they have already proved predictive. Several genetic animal models of PD are useful for understanding the early processes of degeneration in the nigrostriatal DA system. In particular, transgenic α-synuclein animals are valuable for researching general toxicity effects and the mechanisms of α-synuclein pathology, as well as for confirming potential therapeutic strategies.

Neurotoxic and genetic models of PD have opened new perspectives for modeling and understanding the progression of PD but the advantages and disadvantages of each approach must be carefully considered. As described above, some models of PD induced by toxins and mutations exhibit insoluble α-synuclein inclusions in the pathological feature, however, they fail to exhibit true LBs. It is important to distinguish models that reproduce the progressive degeneration of nigrostriatal DA neurons from those that model disease progression in the whole organism. Genetic modeling of nigrostriatal degeneration complements toxin-induced neuronal loss by reproducing insults that are mechanistically linked to PD in humans. These models can provide useful information on stages of neurodegeneration, in particular on the interplay between protective and detrimental mechanisms, which are likely to contribute to the late onset of the disease and the effect of aging, a main risk factor for PD.

7. References

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Mechanisms in Parkinson's Disease - Models and Treatments
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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 mechanisms 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 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.

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