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Value of Genetic Models in Understanding the Cause and Mechanisms of Parkinson's Disease

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized pathologically by the degeneration of nigrostriatal pathway dopaminergic neurons and other neuronal systems and the appearance of Lewy bodies that contain α -synuclein. PD is generally a sporadic disease, but a small proportion of cases have a clear genetic component. Mutations have been identified in six genes that clearly segregate with disease in rare families with PD. Transgenic, knockout, and virus-based models of disease have been developed in rodents to further understand how these genes contribute to the pathogenesis of PD. In general, these animal models recapitulate many key features of the disease, including derangements in dopaminergic synaptic transmission, selective neurodegeneration, neurochemical deficits, α -synuclein-positive neuropathology, and motor deficits. However, a genetic model with all or most of these pathogenic features has proved difficult to create. In this article, we discuss these mammalian genetic models of PD and what they have revealed about the cause and mechanisms of this disease.

Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the cardinal symptoms of muscular rigidity, bradykinesia, resting tremor, and later onset of postural instability [1, 2]. The motor symptoms of PD are primarily due to the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta with an accompanying reduction of striatal dopamine levels. PD is typically sporadic in origin with unclear etiology. However, over the past 10 years, a genetic basis for this disease has been firmly established through the identification of genetic mutations that segregate with disease in rare families with PD. Mutations in six genes are known to unambiguously cause familial PD. These genes are α -synuclein (PARK1 and 4), *parkin* (PARK2), *DJ-1* (PARK7), *PINK1* (PARK6), *LRRK2* (PARK8), and *ATP13A2* (PARK9) [3, 4]. Genetic mutations in α -synuclein and LRRK2 manifest disease through autosomal dominant inheritance, presumably through a toxic gain-of-function mechanism, whereas mutations in *parkin*, *DJ-1*, *PINK1*, and *ATP13A2* cause disease through autosomal recessive transmission and most likely through a typical loss-of-function mechanism. The identification of genes and their corresponding mutations that are associated with inherited forms of PD have provided

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tremendous insight into the molecular pathways and mechanisms underlying disease pathogenesis. Most importantly, they have provided critical clues for understanding the pathogenesis of the more common, sporadic form of disease. Following the initial identification of PD-associated genes, much of the focus has been on elucidating the normal biological function of their encoded proteins and the specific effects of disease-causing mutations. Many of the early studies have been conducted either in vitro or in cultured mammalian cells because these experimental systems provide useful insight into protein function and the nature of mutations, and also provide a sound basis for more physiologically relevant studies in vivo in whole organisms [4]. The development of model organisms based on genes associated with familial PD can provide mechanistic insight into the normal biology and pathobiology of their gene products and ultimately provide relevant models of PD (or certain features of disease) that may prove valuable as tools for identifying novel disease-modifying therapies or neuroprotective strategies. The most widely employed and perhaps most relevant genetic models for studying PD pathogenesis usually involve mammals, particularly mice [5], although non-mammalian models such as the fruit fly *Drosophila melanogaster* [6], the nematode *Caenorhabditis elegans* [7], and the budding yeast *Saccharomyces cerevisiae* [8] have also proved highly insightful. Here, however, we focus on mammalian genetic models and what they have revealed about the fundamental cause and mechanisms of PD.

Genetic Models of Parkinson's Disease: Mice and Rats

Genetic models of PD in rodents are the mainstay of recent research efforts because it is theoretically possible to model the essential features of PD, which are neuronal loss, neuropathology, neurochemical alterations, and motoric or other behavioral deficits. The mouse has been heavily used for such phenotypic studies because of the relative ease and availability of genetic manipulation in these animals. Mice have been developed that either exogenously express a human transgene (to genetically model dominant forms of disease) or with gene disruption (to genetically model recessive forms of disease). Most animal studies have focused on those genes for which there is a strong association with PD.

Modeling Dominantly Inherited Disease: Transgenic Mice and Viral-Based Rat Models

Mutations in two genes, α -synuclein and *LRRK2*, cause dominantly inherited PD; therefore, disease can be modeled in vivo through the development of transgenic mice that express the human disease-associated variant in the brain. Point mutations (A30P, E46K, and A53T) or gene multiplications of α -synuclein cause rare familial forms of PD, and fibrillar α -synuclein forms the major component of Lewy bodies, the hallmark pathology of PD [3]. Since α -synuclein mutations were first identified over 10 years ago, a large number of transgenic models have been produced based largely on the A53T variant but also on the A30P variant, in addition to mice expressing wild-type (WT) human α -synuclein [5, 9]. To drive transgene expression selectively in neurons within the brain, α -synuclein transgenic mice have been produced using a variety of heterologous promoter elements, such as the broadly expressing prion protein PDGF- β and Thy-1 promoters or the catecholaminergic-specific tyrosine hydroxylase (TH) promoter. Each promoter element drives a unique pattern

of transgene expression in the brain that is further modified by the unique genomic integration site of each transgene and its copy number, inevitably producing a wide range of α -synuclein-related neurologic phenotypes in mice. A number of observations can be surmised from these studies: 1) expression of A53T α -synuclein can produce a lethal neurodegenerative motoric phenotype in mice, often with the formation of filamentous α -synuclein-positive inclusions that in some respects resemble Lewy bodies [10–12]; 2) the A53T variant generally tends to produce greater neurotoxicity or neurologic deficits in vivo compared with the A30P variant, whereas the WT protein is rarely toxic [11, 13]; 3) expression of A30P α -synuclein, in contrast to the A53T variant, does not consistently produce filamentous inclusions in mice [11, 14, 15]; and 4) WT or mutant α -synuclein transgenic models fail to exhibit a progressive loss of nigral dopaminergic neurons; only a few lines exhibit any loss of these neurons at all [10–16].

Are α -synuclein transgenic mice useful models of PD?

In general, α -synuclein transgenic mice have succeeded in reproducing many key features of PD, especially the A53T variant. These include progressive and often severe motoric deficits that in some cases lead to premature death [10, 11], the formation of pathologic α -synuclein-positive inclusions or aggregates that are composed of either fibrillar or non-fibrillar α -synuclein [10, 12, 13, 15], and frank neuronal loss (albeit in anatomic brain regions that are not necessarily primarily affected in PD) [10–12]. Therefore, no α -synuclein transgenic mouse model recapitulates all essential features of PD, but current models are useful for understanding the mechanism(s) of α -synuclein-induced neurodegeneration and pathologic inclusion body formation. As such, some α -synuclein transgenic mice represent useful yet broader models of α -synucleinopathy but not PD per se. What we have learned from these studies is that our current approach to faithfully model α -synuclein-linked disease in mice requires some refinement. For example, the prion protein promoter and to a lesser extent the Thy-1 promoter have been highly successful in driving robust levels of α -synuclein expression in the mouse brain, which have often produced the most pronounced motoric and neurodegenerative phenotypes [10–12, 14]. However, these motor-related phenotypes are most probably related to inappropriate and high-level neuronal expression of α -synuclein within the brainstem and spinal cord, particularly motor neurons [10, 12, 17•], and as such fail to model deficits in nigrostriatal dopaminergic pathway function that would make them refractory to dopamine-based or nigral-based therapies. Furthermore, despite some demonstrable transgene expression within the substantia nigra in a few of these models, the neurons within this structure are relatively spared from degeneration, even though α -synuclein aggregates are often present [10–14, 18•]. Attempts to address the issue of neuronal specificity have employed the catecholamine-specific TH promoter to directly drive α -synuclein expression within dopaminergic neurons of the nigrostriatal pathway. However, these models also fail to develop significant neuronal loss or pathology despite obvious transgene expression within the substantia nigra [16, 19]. The absence of dopaminergic neuronal loss in these mice may hint at inadequate promoter strength or duration throughout adulthood, an intrinsic property of dopaminergic neurons, and/or species differences between mouse and human dopaminergic neurons. In mice, it would appear that nigral dopaminergic neurons are refractory to α -synuclein-induced cell death, whereas other neuronal populations (ie, motor neurons) are not. What is apparent here is that

within the relatively short lifespan of the mouse, the absolute levels and sustainability of α -synuclein transgene expression are key determinants of neuronal toxicity (ie, α -synuclein-induced phenotypes are clearly dose-dependent in mice) [10, 11].

New and emerging α -synuclein transgenic models

Newer, more sophisticated models of α -synuclein-linked disease are attempting to readdress neuronal specificity and expression levels as well as identify pathogenic α -synuclein variants that potentially induce greater neurotoxicity. Models based on bacterial artificial chromosome (BAC) and P1-derived artificial chromosome (PAC) technology, which use the entire human α -synuclein gene with the idea of faithfully recapitulating endogenous expression patterns, are currently being developed. Our laboratories have developed conditional Cre-loxP-based transgenic mouse models that can direct α -synuclein neuronal expression selectively within the nigrostriatal dopaminergic pathway from the relatively robust and stable ROSA26 promoter (Ying, Dawson, Moore, and Dawson, unpublished data). These models are currently being evaluated. Other conditional models are using tetracycline-regulated systems that combine a tetO-promoter-regulated α -synuclein transgene in one line with a tetracycline-controlled transactivator (tTA) driven by a neuronal-specific promoter element in another line to provide robust α -synuclein expression. Such tetracycline-regulated systems are currently limited by the lack of tTA driver lines that direct prominent expression throughout the nigrostriatal dopaminergic pathway. However, as a promising recent example of this approach, tetracycline-regulated WT α -synuclein transgenic mice were developed based upon the forebrain-specific CamKII α -tTA driver [18•], with transgene expression localized to cortical and subcortical regions, the olfactory bulb, thalamus, and basal ganglia (including the striatum and substantia nigra). Mice developed nonfibrillar α -synuclein-positive aggregates, progressive motoric deficits, signs of neurodegeneration within the hippocampus and substantia nigra pars reticulata, and a mild yet nonsignificant reduction of TH-positive dopaminergic neurons within the pars compacta without an accompanying reduction in striatal dopamine levels [18•]. Future models based on pathogenic α -synuclein variants with dopamine pathway-specific tTA drivers may hold particular promise and offer the potential for investigating the reversibility or cessation of these phenotypes by turning off transgene expression.

Recent studies have investigated the pathologic significance of posttranslational modifications to α -synuclein, in particular truncation of the C-terminal region of this protein, a pathologic event that has been consistently detected in brain tissue from human PD patients and α -synuclein transgenic mice, leading to the appearance of a number of truncated variants including Syn119, Syn122, and Syn123 [10, 20•, 21]. In vitro data demonstrate that these truncated species (ie, Syn110 and Syn120) assemble more readily into 6- to 9-nm-wide filaments that resemble filaments in Lewy bodies [21–23], accumulate at greater levels from mutant α -synuclein than WT protein [20•, 21], and that truncated species (ie, Syn102, Syn110, Syn120, and Syn123) can enhance the aggregation of full-length WT α -synuclein at low substoichiometric ratios, suggesting an inherent seeding capacity [20•, 21, 23]. These properties of C-terminal truncation variants suggest a potential mechanism for the increased fibrillization and toxicity of α -synuclein pathogenic mutants relative to WT protein. To model these effects in vivo, transgenic mice have recently been

developed that express C-terminal truncated forms of human α -synuclein. Mice expressing WT Syn120 were developed with transgene expression driven by the rat TH promoter serendipitously on a mouse α -synuclein-null genetic background [24]. Syn120 mice display prominent transgene expression within catecholaminergic areas (ie, the olfactory bulb and substantia nigra) and exhibit both filamentous and nonfilamentous α -synuclein-positive pathologic aggregates in these brain regions with concomitant microglial activation, progressive deficits in locomotion, and reduced striatal dopamine levels, but without loss of nigral dopaminergic neurons [24]. In a second study using the TH promoter, full-length α -synuclein and truncated Syn130 mice were developed that both harbored the A53T variant [25]. These mice display similar levels of α -synuclein transgene expression throughout the striatum, substantia nigra pars compacta, and adjacent ventral tegmental area (VTA), although Syn130 mice exhibited a nonprogressive loss of nigral TH-positive dopaminergic neurons, whereas VTA neurons were entirely spared. Full-length A53T α -synuclein mice did not exhibit nigral neuronal loss. Nigral neuronal loss in Syn130 mice was associated with markedly reduced striatal TH-positive nerve terminals, reduced striatal dopamine levels, and deficits in locomotion that could be reversed by treatment with L-DOPA [25]. These α -synuclein truncation models fulfill a number of key criteria for faithfully modeling PD (despite the developmental loss of dopaminergic neurons in one model) and highlight the utility of combining dopamine-specific promoter elements with more neurotoxic variants of human α -synuclein. These models show the important role of C-terminal truncation in regulating α -synuclein aggregation in vivo and the pathologic consequences of this event.

Mechanistic insight from α -synuclein transgenic models

From these studies, it is clear that expression of human α -synuclein variants in the mouse brain is sufficient to precipitate selective neurodegeneration, α -synuclein-positive intraneuronal inclusions, and motoric dysfunction. More recently, transgenic mice have provided insight into the mechanisms underlying α -synuclein-induced neuronal dysfunction. In prion promoter-driven A53T α -synuclein mice that develop a severe motor phenotype, neurodegeneration was identified in the brainstem, spinal cord, and neocortex that was typified by a severe loss of motor neurons, marked mitochondrial DNA damage and degeneration, axonal degeneration, and apoptosis-like neuronal cell death [17•]. Furthermore, there were signs of peripheral nerve degeneration and skeletal muscle damage [17•]. A30P α -synuclein mice with similar transgene expression developed a less pronounced yet similar neurodegenerative phenotype, whereas WT α -synuclein mice did not, thus further highlighting the greater neurotoxicity elicited by the A53T variant. A similar neurodegenerative phenotype was identified in Thy-1 promoter-driven A53T α -synuclein mice, including spinal cord motor neuron damage and neuromuscular degeneration [12]. Recent crossbreeding studies to generate double-mutant mice have further revealed the potential mechanism of α -synuclein-induced phenotypes. For example, neurodegeneration in one line of A53T α -synuclein mice is independent of parkin function [26], an E3 ubiquitin ligase, whereas in a similar A53T line, phenotypes including neuropathology, locomotor deterioration, and premature death are enhanced by deletion of septin4 (CDCrel-2) [27••], a polymerizing guanosine triphosphate-binding protein scaffold that co-localizes with α -synuclein in Lewy bodies in sporadic PD. Thus, septin4 normally provides a protective role against α -synuclein-induced neurotoxicity in vivo, possibly

through binding to and preventing α -synuclein self-aggregation and phosphorylation at serine-129 [27••]. Other mechanistic studies have shown that mutant α -synuclein-induced neurodegeneration in mice involves alterations in the ubiquitin-proteasome system (UPS), a marked increase in apolipoprotein E (apoE) levels, and accumulation of insoluble mouse A β peptide [28••]. The increase in apoE proved detrimental and was downstream of α -synuclein-induced neurodegeneration because deletion of apoE delayed disease onset and increased survival but did not abolish neurodegeneration. Deletion of apoE most likely delays α -synuclein-induced neurodegeneration through correction of UPS alterations, increasing the solubility of human α -synuclein and reducing A β levels and aggregation [28••]. Thus, apoE directly contributes to the pathogenesis of α -synuclein-induced neurodegeneration in transgenic mice. β -synuclein has also been shown to modulate β -synuclein-induced neurotoxicity in mice through a distinct mechanism [29]. Transgenic overexpression of α -synuclein in α -synuclein transgenic mice ameliorates motor deficits, neurodegenerative alterations, and intraneuronal α -synuclein accumulation, most likely through potentially inhibiting the aggregation of α -synuclein [29] and/or through reducing α -synuclein protein levels [30]. Hsp70 has similarly been shown to reduce pathologic α -synuclein aggregation in vivo [31]. These studies implicate some intriguing new players in modulating α -synuclein-induced neurodegeneration in mice and potentially offer new pathways and strategies for the development of disease-modifying therapies.

Viral-based rat models of PD

Despite the lack of success in modeling the progressive loss of nigral dopaminergic neurons in α -synuclein transgenic mice, studies in rats using viral-mediated gene transfer to express human α -synuclein variants in the nigra have proved more successful. Expression of human α -synuclein variants by injection of lentiviral-based expression vectors directly into the substantia nigra leads to a progressive and selective loss of dopaminergic neurons and their striatal nerve terminals [32]. Neurodegeneration was associated with the formation of α -synuclein-positive inclusions resembling Lewy bodies and extensive neuritic pathology. Intriguingly, expression of rat α -synuclein did not produce nigral neuronal loss but did form inclusions, thus revealing intrinsic differences between the rat and human proteins and bringing into doubt the pathologic significance of α -synuclein inclusions [32]. In contrast to crossbreeding studies in mice, coexpression of human parkin in this lentiviral rat model could afford significant protection against A30P α -synuclein-induced dopaminergic neurodegeneration, suggesting a unique interaction between these two PD-associated proteins in this model [33]. A second rat model displaying progressive α -synuclein-induced neurodegeneration has been developed based upon expression mediated by adeno-associated viral vectors that exhibit dopaminergic pathway degeneration, reduced striatal dopamine levels, α -synuclein-positive inclusions, and motor impairment [34]. Thus, viral-based models hold particular promise for modeling key features of PD and for testing neuroprotective strategies in a more rapid manner than afforded by transgenic mouse models. Perhaps the success of these models relates to differential sensitivity to α -synuclein-induced neurotoxicity between rat and mouse dopaminergic neurons because it has been difficult to replicate this model in mice. Other factors might include the lack of developmental compensation in viral models that often confounds transgenic studies, or a higher level and stability of nigral transgene expression. This viral technology can be easily

translated to other mammals, as has recently been demonstrated in nonhuman primates [35]. Viral-mediated expression is largely independent of transgene integration site, in contrast to transgenic studies, making it possible to directly compare variants of the same protein. It provides strong tropism for neurons and is not confounded by pronounced neurotoxic effects in other brain regions less relevant to PD (ie, spinal cord motor neurons). Disadvantages include the acute nature of the viral models, with most neuronal degeneration usually complete within 6 to 8 weeks after viral injection [32, 34], its reproducibility and labor intensiveness, and the inability to concurrently model extranigral degeneration and pathology without the simultaneous need for multiple injection sites. Clearly, viral-based rat models hold considerable promise for rapidly advancing our understanding of the mechanisms of α -synuclein-induced neurotoxicity.

Collectively, rodent models of α -synuclein-linked disease are currently our most promising and relevant avenue for developing a faithful and robust animal model of PD, and for elucidating the molecular pathways and mechanisms underlying disease pathogenesis. Following the recent discovery of *LRRK2* mutations, transgenic models expressing human *LRRK2* variants have been developed but not reported as yet. Because mutations in *LRRK2* give rise to late-onset disease that is indistinguishable from idiopathic PD and because mutations represent the most common cause of familial and sporadic PD known to date, the results from these transgenic models are eagerly awaited and may hold great promise for accurately modeling disease.

Modeling Recessively Inherited Disease: Knockout Mice

Homozygous mutations in the *parkin*, *DJ-1*, *PINK1*, and *ATP13A2* genes cause autosomal recessive forms of familial PD, most likely through a loss-of-function mechanism [3]. Accordingly, disease can be modeled in vivo through the development of knockout (KO) mice that usually harbor exonic deletions that ultimately lead to a loss of protein expression. Because *parkin* was the first recessive PD gene identified [3], parkin KO mice have been most extensively characterized to date.

Parkin knockout mice

Parkin KO mice are generally unremarkable and most importantly do not exhibit frank neuronal loss or other PD-related phenotypes; thus they do not provide an adequate model of disease. However, one KO model exhibits a developmental loss of TH-positive noradrenergic neurons in the locus coeruleus accompanied by a reduction in norepinephrine levels in the spinal cord and olfactory bulb and a reduction in the norepinephrine-dependent acoustic startle response [36]. Parkin-null mice fail to develop obvious protein inclusion pathology, largely consistent with postmortem neuropathologic observations in *parkin*-linked human autosomal recessive–juvenile parkinsonism (AR-JP) patients [4]. One KO model exhibits subtle deficits in nigrostriatal dopaminergic function, including increased extracellular striatal dopamine levels, reduced synaptic excitability of striatal neurons, and deficits in behavioral motor tests sensitive to nigrostriatal pathway impairment [37]. In another parkin-deficient model, there are subtle deficits in motor and cognitive function, including locomotion and working memory, together with abnormalities in dopaminergic and glutamatergic neurotransmission [38]. These subtle alterations in nigrostriatal

dopaminergic function in the absence of neuronal loss suggest that parkin KO mice may represent a presymptomatic model of PD akin to the functional consequences of dopaminergic denervation. Because similar phenotypes are also observed in DJ-1-null and PINK1-null mice, this has raised doubt about our ability to model key features of recessive PD within the short lifespan of the mouse.

Where parkin KO mice have proved most useful is in understanding the normal biology of parkin. Proteomic analysis of ventral midbrain tissue from parkin KO mice relative to litter-mate controls has revealed decrements in a number of key proteins involved in mitochondrial function and protection from oxidative stress [39]. These were accompanied by deficits in mitochondrial respiratory capacity together with reduced antioxidant capacity and increased oxidative damage [39]. Similar observations have been made in parkin-null *Drosophila* models, including mitochondrial pathology and increased oxidative stress as the earliest manifestations of reduced lifespan, locomotor defects, and apoptotic muscle degeneration [40]. Thus, parkin may play a normal role in maintaining mitochondrial integrity. Despite such a role, deletion of parkin does not confer sensitivity to the mitochondrial complex I inhibitor and PD toxin MPTP [41]. The mitochondrial abnormalities caused by parkin deletion and MPTP toxicity in mice may therefore operate through distinct mechanisms. Parkin is an E3 ubiquitin protein ligase that mediates the ubiquitination of specific protein substrates that may target some of these proteins for degradation by the 26S proteasomal complex [4]. As such, accumulation of these substrates in the absence of parkin through improper ubiquitination and turnover may mediate neurotoxicity. Although a number of putative parkin substrates have been identified [4], only p38/AIMP2 and FUSE-binding protein 1 have been shown to accumulate in brain tissue from parkin KO mice and *parkin*-linked AR-JP patients as well as sporadic PD brains and MPTP-treated mice [42, 43]. Accordingly, viral-mediated expression of p38/AIMP2 in the mouse substantia nigra causes a loss of dopaminergic neurons [43]. Other parkin substrates that apparently accumulate in brains of *parkin*-linked AR-JP patients but not parkin KO mice, including CDCrel-1 [44] and Pael-R [45], can elicit dopaminergic neuronal loss through a dopamine-dependent mechanism when expressed in rodent brain, although their pathologic significance remains uncertain. Parkin-deficient mice will continue to prove instrumental for the identification of parkin substrates that are critical to the pathogenesis of *parkin*-linked PD.

DJ-1-null mice

DJ-1 KO mice have been developed but these mice lack neuronal loss or inclusion pathology related to PD. They also have normal numbers of nigral dopaminergic neurons and locus coeruleus noradrenergic neurons [46•, 47, 48••]. Neuropathology in *DJ-1*-linked PD patients is not yet known and so it is unclear whether protein inclusion pathology is part of the disease spectrum. DJ-1-null mice do however exhibit alterations in nigrostriatal dopaminergic function and motor deficits. KO mice exhibit reduced evoked dopamine overflow in the striatum, mainly as a result of increased dopamine reuptake, and reduced sensitivity to the inhibitory effects of D2 autoreceptor stimulation [48••]. Mice also displayed an absence of corticostriatal long-term depression (LTD) that was associated with reduced D2 dopamine receptor-dependent activity because it could be reversed by treatment

with D2 receptor agonists [48••]. KO mice also reveal decreased spontaneous locomotor activity in the open field [48••], a behavioral paradigm sensitive to alterations in dopaminergic neurotransmission. Thus, DJ-1 is required for normal nigrostriatal dopaminergic neurotransmission and is important for mediating downstream signaling in nigral neurons following D2 receptor activation. Other DJ-1–null models have revealed increased dopamine transporter (DAT) levels, binding, and activity in striatal synaptosomes consistent with increased dopamine reuptake in KO mice [49], whereas another KO model displayed increased dopamine reuptake and enhanced dopamine tissue content [47]. Thus, DAT function is enhanced in DJ-1–null mice. Consistent with altered dopaminergic neurotransmission, DJ-1 KO mice are hypersensitive to MPTP-induced dopaminergic neuronal toxicity that in one model has been ascribed to increased striatal uptake of the active metabolite MPP⁺ by DAT [49, 50]. KO mice have also provided clues to the normal function of DJ-1 in vivo. For example, a proportion of DJ-1 is normally localized to mitochondria in the mouse brain [51], and mitochondria isolated from brains of young KO mice exhibit excess hydrogen peroxide production together with the loss of mitochondrial aconitase activity in the absence of overt mitochondrial damage or dysfunction [46•]. In older KO mice, these alterations are not apparent, most likely because of a compensatory increase in the levels and activity of mitochondrial glutathione peroxidase [46•]. These observations are consistent with a role for DJ-1 in vivo as an atypical peroxidase involved in the elimination of mitochondrial hydrogen peroxide [46•]. DJ-1–deficient *Drosophila* models have similarly revealed a role for DJ-1 in protection against oxidative stress [52]. The effects of DJ-1 may also relate to its putative function as a mitochondrial redox-sensitive molecular chaperone, although such a role has not yet been examined in vivo [4].

PINK1-deficient mice

PINK1-deficient mice have recently been developed. Similar to the subtle dopaminergic phenotypes observed in parkin-null and DJ-1–null mice, PINK1-null mice reveal decreased evoked dopamine overflow in the striatum due to a reduction in dopamine release [53••]. Furthermore, adrenal chromaffin cells derived from PINK1-deficient mice exhibit reductions in the quantal size and release frequency of catecholamines. Directly related to reduced synaptic dopamine release is the impairment of corticostriatal long-term potentiation (LTP) in striatal medium-sized spiny neurons of PINK1 KO mice [53••], a measure of striatal synaptic plasticity that is modulated by nigral dopaminergic inputs. Deficits in LTP could be corrected by D1 receptor agonists and were specific to corticostriatal glutamatergic synapses because synaptic plasticity in a hippocampal glutamatergic synapse was normal in PINK1-null mice. Corticostriatal LTD induction was also absent in PINK1-null mice, but this could be restored by D1 and D2 receptor agonists, consistent with reduced D1 and D2 receptor signaling caused by reduced synaptic dopamine release [53••]. As such, amphetamine, which increases dopamine release, and the dopamine precursor L-DOPA, which increases dopamine availability, both rescued striatal LTD induction in PINK1-null mice [53••]. Thus, reduced dopamine release in the striatum of PINK1 KO mice underlies the deficits in striatal synaptic plasticity. Because the numbers of nigral dopaminergic neurons, striatal dopamine and dopamine receptor levels, and dopamine synthesis are normal in PINK1-null mice [53••], impaired dopaminergic synaptic transmission due to reduced presynaptic dopamine release may model the early functional

consequences of dopaminergic striatal denervation in PD, possibly as a precursor to nigrostriatal pathway dysfunction and degeneration. PINK1 may function as a mitochondrial serine/threonine kinase [4], raising the possibility of a novel functional relationship between mitochondria and the regulation of presynaptic dopamine release.

Utility of knockout mice for modeling PD

In general, genetic mammalian models of recessive PD, such as parkin-null, DJ-1-null, and PINK1-null mice, fail to recapitulate the hallmark features of PD, especially the progressive loss of nigral dopaminergic neurons. These models do however display subtle impairments in dopaminergic neurotransmission, often with associated deficits in motoric function, perhaps antecedent to nigrostriatal pathway dysfunction. Because parkin, DJ-1, and PINK1 appear to have prominent yet distinct roles in maintaining or protecting normal mitochondrial physiology [4], an observation also evident in *Drosophila* null genetic models [6, 40, 52, 54, 55], mitochondrial defects may represent the earliest manifestation of disease that would eventually lead to dopaminergic dysfunction. These mice may therefore represent useful presymptomatic models of PD indicative of the earliest derangements in dopaminergic neuronal function. The development of ATP13A2-null mice may help to strengthen this hypothesis. In contrast to transgenic models of dominant PD, in which high, nonphysiologic levels of transgene expression can often help to accelerate the development of neurologic phenotypes within the short lifespan of a mouse, gene disruption as a means of modeling recessive PD is clearly not sufficient to precipitate disease. The absence of overt PD-related phenotypes in these KO models could further reflect differences in the functional roles or redundancy of these proteins between humans and mice in preserving the integrity of the nigrostriatal dopaminergic pathway. Perhaps disease could be exacerbated in these null mice by combining different genetic models to introduce a second or even third pathogenic insult. However, initial results have not been encouraging, with parkin-null mice and α -synuclein transgenic mice failing to reveal an interaction [26], although many other interesting genetic combinations are sure to follow soon. However, these types of genetic interaction studies may be better suited to viral-based rodent models or *Drosophila* for developing new models of PD [6, 33, 54, 55].

Conclusions

No mammalian genetic model of PD has so far been able to recapitulate all key features of disease [5]. Although parkin, DJ-1, and PINK1 KO mice that model recessive PD do not develop many essential aspects of disease, they have been instructive in potentially revealing preclinical dopaminergic phenotypes. α -Synuclein transgenic mice that model dominant disease have succeeded in recreating neurodegeneration, neuropathology, and a movement disorder with similarities to PD. Furthermore, whereas genetically engineered mice have generally failed in reproducing progressive dopaminergic neuronal loss, viral-based models in rats have succeeded. What is ultimately needed, however, is a highly reproducible, predictive rodent model that exhibits progressive and selective neurodegeneration of key neuronal populations relevant to PD with associated neurochemical, neuropathologic, and behavioral alterations. Once we have succeeded in recreating a movement disorder in mice based at least on dopaminergic neuronal loss that closely resembles PD, then perhaps we can

focus on modeling the plethora of nonmotor symptoms of PD that are often considered more debilitating to those affected. This inherent bias in our view of modeling PD solely as a disorder of movement likely stems from our traditional understanding of this disease and our lack of understanding of the cause and mechanisms of extranigral pathology. The development of new, refined, transgenic or viral models of α -synuclein-linked disease and the emergence of *LRK2*-linked disease models may hold particular promise and will allow us to further explore the causes and mechanisms of PD and may help to identify novel disease-modifying therapies.

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