Molecular Pathophysiology of Parkinson's Disease

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Key Words

Abstract

Parkinson's disease (PD) is a progressive neurodegenerative move-
ment disorder that results primarily from the death of dopaminergic
neurons in the substantia nigra. Although the etiology of PD is in-
completely understood, the recent discovery of genes associated with
rare monogenic forms of the disease, together with earlier studies
and new experimental animal models, has provided important and
novel insight into the molecular pathways involved in disease patho-
genesis. Increasing evidence indicates that deficits in mitochondrial
function, oxidative and nitrosative stress, the accumulation of aber-
rant or misfolded proteins, and ubiquitin-proteasome system dys-
function may represent the principal molecular pathways or events
that commonly underlie the pathogenesis of sporadic and familial
forms of PD.

Contents

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative movement disorder that is estimated to affect approximately 1% of the population older than 65 years of age (Lang & Lozano 1998a,b). Clinically, most patients present with the cardinal symptoms of bradykinesia, resting tremor, rigidity, and postural instability. A number of patients also suffer from autonomic, cognitive, and psychiatric disturbances. The major symptoms of PD result from the profound and selective loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc), but there is widespread neuropathology with the SNc becoming involved later toward the middle stages of the disease (Braak et al. 2003). The pathological hallmarks of PD are round

eosinophilic intracytoplasmic proteinaceous inclusions termed Lewy bodies (LBs) and dystrophic neurites (Lewy neurites) present in surviving neurons (Forno 1996). PD is primarily a sporadic disorder and its specific etiology is incompletely understood, but important new insights have recently been provided through studying the genetics, epidemiology, and neuropathology of PD, in addition to the development of new experimental models. Until recently, PD had been considered the prototypical nongenetic disorder. In the past seven years, the identification of distinct genetic loci responsible for rare Mendelian forms of PD has challenged this view and has provided us with vital clues to understanding the molecular pathogenesis of the more common sporadic forms of this disease. These genetic advances have revolutionized the way we think about PD and have opened up new and exciting areas of research. Despite such advances, much recent research has continued to focus on the contribution of nongenetic or environmental factors to the development of sporadic forms of PD.

THE HERITABILITY OF PD

For most of the twentieth century, genetic predisposition to Charcot's "la maladie de Parkinson" was thought to play a negligible role in development of the syndrome. The notion that environmental factors, not heredity, caused PD was further propagated following the post-encephalitic outbreak of a variant of the syndrome. Yet for more than one hundred years, clinicians noted that patients with PD often had an affected relative (Gowers 1900). Further studies corroborated these suspicions with the identification and characterization of families that inherited PD in a Mendelian fashion (Bell & Clark 1926). In contrast, a number of studies utilizing twin registries demonstrated a low rate of concordance in monozygotic and dizygotic twins, indicative of a lack of genetic susceptibility in PD (Marttila et al. 1988, Ward et al. 1983). These early twin studies seemed to shift the

Table 1 Loci and genes associated with familial PD or implicated in PD[1]

Locus	Chromosome location	Gene	Inheritance pattern	Typical phenotype	Reference
PARK1 & PARK4	4q21–q23	*α-synuclein*	AD	Earlier onset, features of DLB common	Polymeropolous et al. 1997, Singleton et al. 2003
PARK2	6q25.2–q27	*parkin*	usually AR	Earlier onset with slow progression	Kitada et al. 1998
PARK3	2p13	unknown	AD, IP	Classic PD, sometimes dementia	Gasser et al. 1998
PARK5	4p14	*UCH-L1*	unclear	Classic PD	Leroy et al. 1998
PARK6	1p35–p36	*PINK1*	AR	Earlier onset with slow progression	Valente et al. 2004a
PARK7	1p36	*DJ-1*	AR	Earlier onset with slow progression	Bonifati et al. 2003
PARK8	12p11.2–q13.1	*LRRK2*[2]	AD	Classic PD	Funayama et al. 2002
PARK10	1p32	unknown	unclear	Classic PD	Hicks et al. 2002
PARK11	2q36–q37	unknown	unclear	Classic PD	Pankratz et al. 2003
NA	5q23.1–q23.3	*Synphilin-1*	unclear	Classic PD	Marx et al. 2003
NA	2q22–q23	*NR4A2*	unclear	Classic PD	Le et al. 2003

[1] Abbreviations: NA, not assigned; AD, autosomal dominant; AR, autosomal recessive; IP, incomplete penetrance; DLB, dementia with Lewy bodies.
[2] See note added in proof.

direction of PD research away from genetics, and the concomitant identification of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism (Langston et al. 1983) further downplayed any potential genetic component to PD. Despite these past assumptions that genetics played little role in PD, it became clear that there is a significant genetic component to disease (Dawson & Dawson 2003, Hicks et al. 2002, Marder et al. 1996, Sveinbjornsdottir et al. 2000). Indeed, there are at least 10 distinct genetic loci associated with PD, and mutations have been identified in four genes that definitively and unambiguously cause familial forms of PD (**Table 1**).

THE CONTRIBUTION OF GENES TO THE PATHOGENESIS OF PD

Although monogenic and sporadic forms of PD are clinically and pathologically distinct from each other, they tend to share many overlapping features that include, most importantly, parkinsonism with nigrostriatal DA degeneration (Hardy et al. 2003), which

perhaps implies that common pathogenic mechanisms may underlie disease. However, although the genes linked to different monogenic forms of PD do not necessarily fit into a common pathogenic pathway, they nevertheless promote our understanding of the specific molecular pathways that lead to DA neuronal degeneration in PD. This section discusses our current understanding of those gene products linked to monogenic forms of PD (PARK1, 2, 4, 5, 6, and 7), emphasizing, in particular, the normal function of each protein and how its dysfunction may contribute to disease pathogenesis.

α-Synuclein (OMIM 163890; PARK1; PARK4)

The first gene for familial PD was initially mapped to chromosome 4q21–q23 in an Italian American family (Contursi kindred) with more than 60 affected individuals spanning 5 generations with autosomal dominant disease inheritance (Polymeropoulos et al. 1996). An A53T missense mutation was isolated in affected individuals in the gene encoding the *α*-synuclein protein (Polymeropoulos et al.

1997). Subsequently, a second mutation in the *α-synuclein* gene (A30P) was found in a German family (Kruger et al. 1998), in addition to an E46K mutation in a Spanish family (Zarranz et al. 2004). Lastly, a genomic triplication of a region spanning the *α-synuclein* gene segregated with disease in the Iowa kindred (Singleton et al. 2003). Moreover, there is some suggestion that genetic variability in the *α-synuclein* promoter associates with sporadic PD, which implicates that variability of α-synuclein protein levels can predispose individuals to disease (Pals et al. 2004).

α-synuclein is a 140-amino-acid protein belonging to a family of related synucleins that include β- and γ-synuclein (Clayton & George 1998). Structurally, human α-synuclein consists of an N-terminal amphipathic region containing six imperfect repeats (with a KTKEGV consensus motif), a hydrophobic central region [containing the non-amyloid-β component (NAC) domain], and an acidic C-terminal region (**Figure 1**). α-synuclein is an intrinsically unstructured or natively unfolded protein but has significant conformational plasticity. For example,
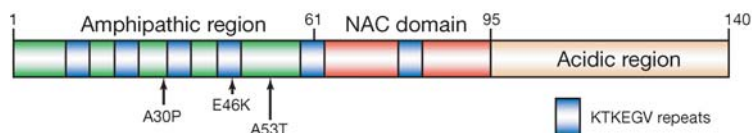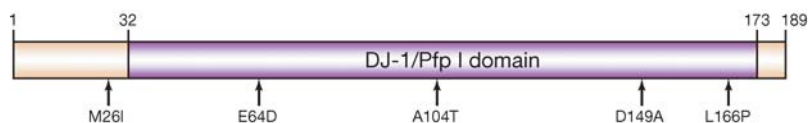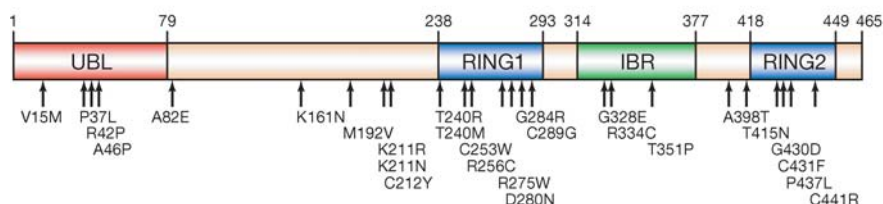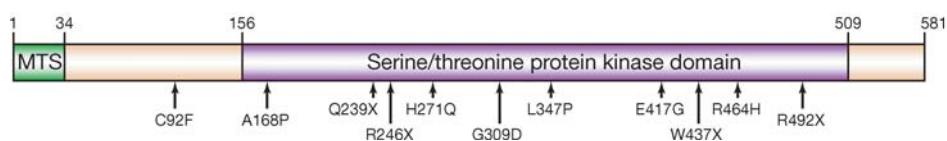
Figure 1

Domain architecture and familial mutations of proteins associated with PD. Protein domains of human (*A*) α-synuclein (140 aa), (*B*) DJ-1 (189 aa), (*C*) parkin (465 aa), and (*D*) PINK1 (581 aa). The position of amino acid missense mutations (*single letter code*) associated with familial forms of PD are indicated for each protein (*arrows*). Nonsense mutations are indicated by X, which denotes the introduction of a premature stop codon. Not all familial mutations are indicated for parkin. Refer to Mata et al. 2004 for detailed references to all reported parkin mutations. MTS, mitochondrial targeting sequence.

depending on the environment α-synuclein can remain unstructured, can form monomeric and oligomeric species, or can form amyloidogenic filaments (Uversky 2003). The physiological function of α-synuclein is unclear. α-synuclein is highly expressed throughout the mammalian brain and is enriched in presynaptic nerve terminals, where it can associate with membranes and vesicular structures (Irizarry et al. 1996, Kahle et al. 2000). Recent studies have shown that α-synuclein specifically associates with membrane microdomains known as lipid rafts, and this raft association may be required for its synaptic localization (Fortin et al. 2004). Analysis of mice with a targeted deletion of the α-synuclein gene suggest a role for α-synuclein in synaptic vesicle recycling and DA neurotransmission (Abeliovich et al. 2000). A role in synaptic vesicle recycling is further supported by observations in vitro that demonstrate that α-synuclein can bind to acidic phospholipid vesicles (Davidson et al. 1998) and can also bind to and inhibit the activity of mammalian phospholipase D (PLD) (Jenco et al. 1998). Furthermore, in cultured cells α-synuclein can regulate lipid metabolism by protecting lipid droplets from hydrolysis (Cole et al. 2002) and can regulate the size of presynaptic vesicular pools (Murphy et al. 2000). Studies in the model organism yeast further demonstrate that α-synuclein selectively associates with the plasma membrane, inhibits PLD activity, induces lipid droplet accumulation, and modulates vesicle trafficking (Outeiro & Lindquist 2003). Thus, α-synuclein may play an important role in regulating synaptic vesicle size and recycling with particular relevance to dopamine storage.

α-synuclein is considered to play a central role in the pathophysiology of PD, in part, on the basis of the identification of mutations or triplications of the α-synuclein gene associated with familial PD (Kruger et al. 1998, Polymeropoulos et al. 1997, Singleton et al. 2003). Moreover, the identification of fibrillar forms of the α-synuclein protein as a major structural component of LBs in PD and other synucleinopathies provides compelling evidence that α-synuclein plays a major role in the pathogenesis of PD (Spillantini et al. 1998). Mutations in α-synuclein cause PD through a toxic gain-of-function mechanism consistent with the dominant inheritance pattern of mutations. The genomic triplication of α-synuclein leads to an approximate doubling of expression, thereby demonstrating that overexpression of wild-type α-synuclein is sufficient to cause disease. The effects of α-synuclein missense mutations are not so obvious. Both the A30P and A53T mutant proteins display an increased propensity to self-aggregate to form oligomeric species and LB-like fibrils in vitro compared with wild-type α-synuclein (Conway et al. 1998). α-synuclein oligomers are the precursors for higher-order aggregates, such as amyloid-like fibrils, which precipitate as the filamentous structures observed in LBs and Lewy neurites (**Figure 2**). Some investigators proposed that oligomeric fibrillization intermediates of α-synuclein termed protofibrils, rather than the fibrils themselves, may be the pathogenic cytotoxic moiety. For example, the A53T and A30P mutations both share the capacity to promote the oligomerization, but not fibrillization, of α-synuclein (Conway et al. 2000). Furthermore, these mutants may form annular protofibrils that resemble a class of

Monomer	Protofibril	Fibril	Lewy body
Natively unfolded	β-sheet rich oligomer	Amyloid-like	Fibrillar
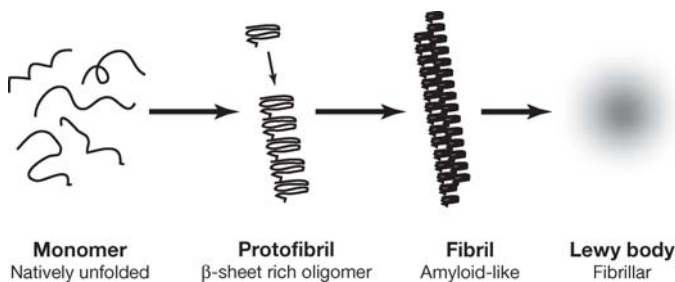
Figure 2

Schematic of α-synuclein fibrillogenesis. Natively unfolded or disordered α-synuclein monomers form β-sheet rich oligomers that comprise a transient population of protofibrils of heterogenous structure that may include spheres, chains, or rings. The protofibrils may give rise to more stable amyloid-like fibrils. α-synuclein fibrils eventually aggregate and precipitate to form LBs in vivo. Figure adapted from Lansbury & Brice 2002.

pore-forming bacterial toxin (Lashuel et al. 2002), which suggests that protofibrils might cause inappropriate permeabilization of cellular membranes. Catecholamines, particularly dopamine, can react with α-synuclein to form covalent adducts that slow conversion of protofibrils to fibrils (Conway et al. 2001), thus promoting protofibril accumulation. Soluble oligomeric forms of α-synuclein have recently been observed in human brain tissue (Sharon et al. 2003), which suggests that oligomeric species are physiologically relevant.

The notion of cytotoxic protofibrils is further supported by studies in one line of α-synuclein transgenic mice, whereby motoric impairment and loss of DA nerve terminals are observed in the presence of non-fibrillar α-synuclein inclusions (Masliah et al. 2000). However, the failure of transgenic mice overexpressing the protofibrillogenic A30P mutant α-synuclein to exhibit neurodegeneration (Lee et al. 2002) suggests that protofibrils may not be the primary cytotoxic moiety. Indeed, only when the A30P mutant forms both fibrils and inclusions is neurodegeneration observed in transgenic mice and flies (Feany & Bender 2000, Kahle et al. 2001, Neumann et al. 2002). Consistent with the cytotoxicity of fibrillar α-synuclein is the observation that β-amyloid promotes the formation of α-synuclein fibrillar inclusions in bigenic mice overexpressing mutant amyloid precursor protein and α-synuclein, leading to a more severe α-synuclein-related pathological and behavioral phenotype (Masliah et al. 2001). At this stage the relative contributions of α-synuclein protofibrils and fibrils to PD pathogenesis are incompletely understood, but both likely contribute to disease pathogenesis.

The mechanism by which wild-type α-synuclein aggregates in sporadic forms of PD is poorly understood. A number of factors enhance α-synuclein aggregation or fibrillization in different systems. Mitochondrial complex-I inhibitors such as rotenone and paraquat clearly lead to aggregation and accumulation of α-synuclein in vitro and in an-imal models (Betarbet et al. 2000; Manning-Bog et al. 2002; Sherer et al. 2002b, 2003), and other forms of oxidative and nitrosative stress also promote α-synuclein aggregation (Ischiropoulos & Beckman 2003). Oxidative damage may play a pertinent role in the aggregation of α-synuclein in sporadic PD because there is selective tyrosine nitration of α-synuclein in lesions in PD and other synucleinopathies (Giasson et al. 2000). Indeed, recent studies suggest that tyrosine nitration of α-synuclein may potentiate fibril formation of unmodified α-synuclein and may decrease the rate of degradation by the 20S proteasome and the cysteine protease calpain I (Hodara et al. 2004). α-synuclein protein levels also increase with aging in human substantia nigra (Li et al. 2004). The stabilization of α-synuclein with aging may be a significant factor in the pathogenesis of α-synucleinopathies because it could lead to the accumulation of pathogenic protein modifications, such as oxidative damage (Li et al. 2004). Proteasomal inhibition is also associated with increases in α-synuclein fibrillization with formation of insoluble inclusions in primary neuronal cultures and in vivo (McNaught et al. 2004, Rideout et al. 2004). Endogenous cofactors may play a role in modulating α-synuclein fibrillogenesis. Recent evidence suggests that interactions between α-synuclein and tau synergistically promote the fibrillization of both proteins in vitro and in animal models (Giasson et al. 2003), which is supported by the co-occurrence of α-synuclein and tau pathology in some monogenic forms of PD, in other neurodegenerative disorders, and in α-synuclein transgenic mice. β-amyloid can also enhance α-synuclein fibrillization in vivo, and pathology related to both proteins can coexist in neurodegenerative diseases such as the LB variant of Alzheimer's disease (Masliah et al. 2001). The interaction of α-synuclein with amyloidogenic proteins could be one mechanism that drives the formation of pathological fibrillar inclusions in human neurodegenerative diseases. Conversely, non-amyloidogenic proteins such as β-synuclein can have the

opposite effect to prevent α-synuclein fibrillization in vivo and may represent an endogenous negative regulator of fibrillization (Hashimoto et al. 2001). Mutations in the *β-synuclein* gene may predispose to dementia with LBs (DLB) (Ohtake et al. 2004). Posttranslational modifications may additionally contribute to the aggregation or fibrillization of α-synuclein because it is both selectively ubiquitinated and phosphorylated in lesions in sporadic PD and other synucleinopathies (Hasegawa et al. 2002).

Although the process of α-synuclein fibrillization may be the key pathogenic event in most forms of PD, the mechanism by which α-synuclein species exert their downstream neurotoxic effects is unclear. One proposed mechanism is through direct impairment of the ubiquitin-proteasome system (UPS). For example, pathogenic species of α-synuclein are more resistant to proteasomal degradation and can directly bind to 20/26S proteasomal subunits and impair proteolytic activity (Bennett et al. 1999, Lindersson et al. 2004, Snyder et al. 2003). Overexpression of mutant α-synuclein also sensitizes cultured cells to the toxicity associated with proteasome inhibitors, suggesting a prior level of proteasome impairment (Petrucelli et al. 2002, Tanaka et al. 2001). Thus, the reduced clearance of α-synuclein species and their direct inhibitory effect on proteasomal activity may contribute to disease pathogenesis. It is presently unclear whether α-synuclein turnover is physiologically regulated by the proteasome, although both ubiquitin-dependent and -independent mechanisms have been described (Tofaris et al. 2001, Webb et al. 2003). Furthermore, proteasomal inhibition produces LB-like α-synuclein inclusions in rodent models of PD and in cultured neurons (McNaught et al. 2004, Rideout et al. 2004). Alternative pathways of α-synuclein degradation have also been proposed including processing by the lysosome/autophagy pathway and by cytoplasmic proteases such as calpain I (Mishizen-Eberz et al. 2003, Webb et al. 2003). In addition to effects on the UPS,

α-synuclein overexpression in cultured cells, particularly of mutant forms, has been linked to mitochondrial deficits (Hsu et al. 2000), defective cellular trafficking (Gosavi et al. 2002), apoptosis (Lee et al. 2001b), impaired chaperone-mediated autophagy (Cuervo et al. 2004), and increased sensitivity to oxidative stress (Ko et al. 2000) and dopamine-mediated toxicity (Tabrizi et al. 2000). Thus, the mechanism of α-synuclein-mediated toxicity likely affects numerous critical cellular pathways and highlights the complexity of disentangling the primary pathogenic events from the secondary events.

A role for dopamine in mediating α-synuclein toxicity is an attractive notion that would account for the relatively selective degeneration of DA neurons in PD. In cultured cells, the toxic effects of α-synuclein appear to be selective for DA neurons, which requires endogenous dopamine production and is mediated by reactive oxygen species (ROS) (Xu et al. 2002). Furthermore, α-synuclein can interact with and enhance the activity of the dopamine transporter, thereby accelerating cellular dopamine uptake and dopamine-induced apoptosis (Lee et al. 2001a). Overexpression of mutant α-synuclein can also downregulate the vesicular monoamine transporter 2, which perhaps promotes enhanced levels of cytoplasmic dopamine and increased oxidative stress (Lotharius et al. 2002). Thus, one effect of toxic α-synuclein species could be to increase cytosolic dopamine levels, which, owing to the high oxidative potential of dopamine metabolism, could promote oxidative stress and ensuing DA neuronal cell death. However, degeneration observed in *α-synuclein*-linked PD (PARK1 and PARK4) is not limited to DA neurons or even to catecholaminergic neurons but is widespread and may affect other neurotransmitter systems. This mechanism would account only for loss of DA neurons in PD, and α-synuclein toxicity may affect distinct neuronal populations through alternate mechanisms. Future research will help to delineate the precise mechanism of α-synuclein toxicity in specific

neuronal populations that will hopefully give rise to an ordered pathway of events that lead to neuronal degeneration in PD.

Parkin (OMIM 602544; PARK2)

A large region spanning chromosome 6q25.2–q27 was initially linked to a rare form of autosomal recessive juvenile-onset parkinsonism (AR-JP), in consanguineous Japanese families (Matsumine et al. 1997). Subsequently, a homozygous deletion spanning a microsatellite marker was identified in an affected case, and the adjacent gene was cloned and named *parkin* (Kitada et al. 1998). Mutations in the *parkin* gene are relatively common in familial PD, with mutations found in 50% of familial early-onset cases compatible with recessive inheritance and 10% of all early-onset PD cases (Lucking et al. 2000). A genome-wide scan in families with early-onset PD revealed significant linkage only to *parkin*, thereby demonstrating a unique role for the *parkin* gene in the development of early-onset PD (Scott et al. 2001). However, mutations have also been described in apparent sporadic cases with a clinical presentation indistinguishable from late-onset idiopathic PD (Lucking et al. 1998). A wide variety of *parkin* mutations have been described in PD cases, ranging from deletions of a single nucleotide to hundreds of thousands of nucleotides, in addition to genomic multiplications and missense mutations (Mata et al. 2004, West & Maidment 2004). Most *parkin*-linked disease is inherited in an autosomal recessive fashion; however, there are several descriptions of families with *parkin* mutations and disease segregating in a mode incompatible with recessive inheritance, and some evidence indicates that haploinsufficiency in the *parkin* gene predisposes to PD (Farrer et al. 2001).

The *parkin* gene encodes a 465-amino-acid protein with a modular structure that contains an N-terminal ubiquitin-like (UBL) domain, a central linker region, and a C-terminal RING domain comprising two RING finger motifs separated by an in-between-RING (IBR) domain (**Figure 1**). Like many other RING finger–containing proteins, parkin can function as an E3 ubiquitin protein ligase (Shimura et al. 2000, Zhang et al. 2000). E3 ligases are an important part of the cellular machinery that covalently tags target proteins with ubiquitin (Glickman & Ciechanover 2002) (**Figure 3**). Ubiquitination of proteins results from the successive actions of ubiquitin-activating (E1), conjugating (E2), and ligase (E3) enzymes, respectively. Subsequent cycles add additional ubiquitin molecules to a previously ligated ubiquitin, resulting in the formation of a poly-ubiquitin chain containing four or more ubiquitin molecules. Such poly-ubiquitinated proteins are specifically recognized by the 26S proteasome and are subsequently targeted for degradation. The ubiquitination machinery can also tag proteins with single ubiquitin molecules or with alternatively linked poly-ubiquitin chains that do not signal proteasomal degradation but are implicated in numerous nondegradative cellular processes (**Figure 3**). E3 ligases typically confer substrate specificity to the ubiquitination process by simultaneously interacting with E2-ubiquitin and the substrate protein to catalyze the transfer of ubiquitin from E2 to a substrate lysine residue. Parkin interacts with the E2 enzymes, UbcH7 and UbcH8 (Shimura et al. 2000, Zhang et al. 2000), as well as with the endoplasmic reticulum–associated E2s, UBC6 and UBC7 (Imai et al. 2001). Parkin generally tends to interact with E2s and substrate proteins through its RING domain. The UBL domain of parkin can interact with subunits of the 19S cap of the 26S proteasomal complex, such as Rpn10, which most likely mediates transfer of poly-ubiquitinated substrates to the proteasome (Sakata et al. 2003). Parkin may function as part of a larger protein complex. The interaction of parkin with an SCF-like (Skp1-Cullin-F-box protein) complex, or with a complex containing Hsp70 and CHIP, can enhance its E3 ligase activity and its neuroprotective capacity (Imai et al. 2002, Staropoli et al. 2003). Most
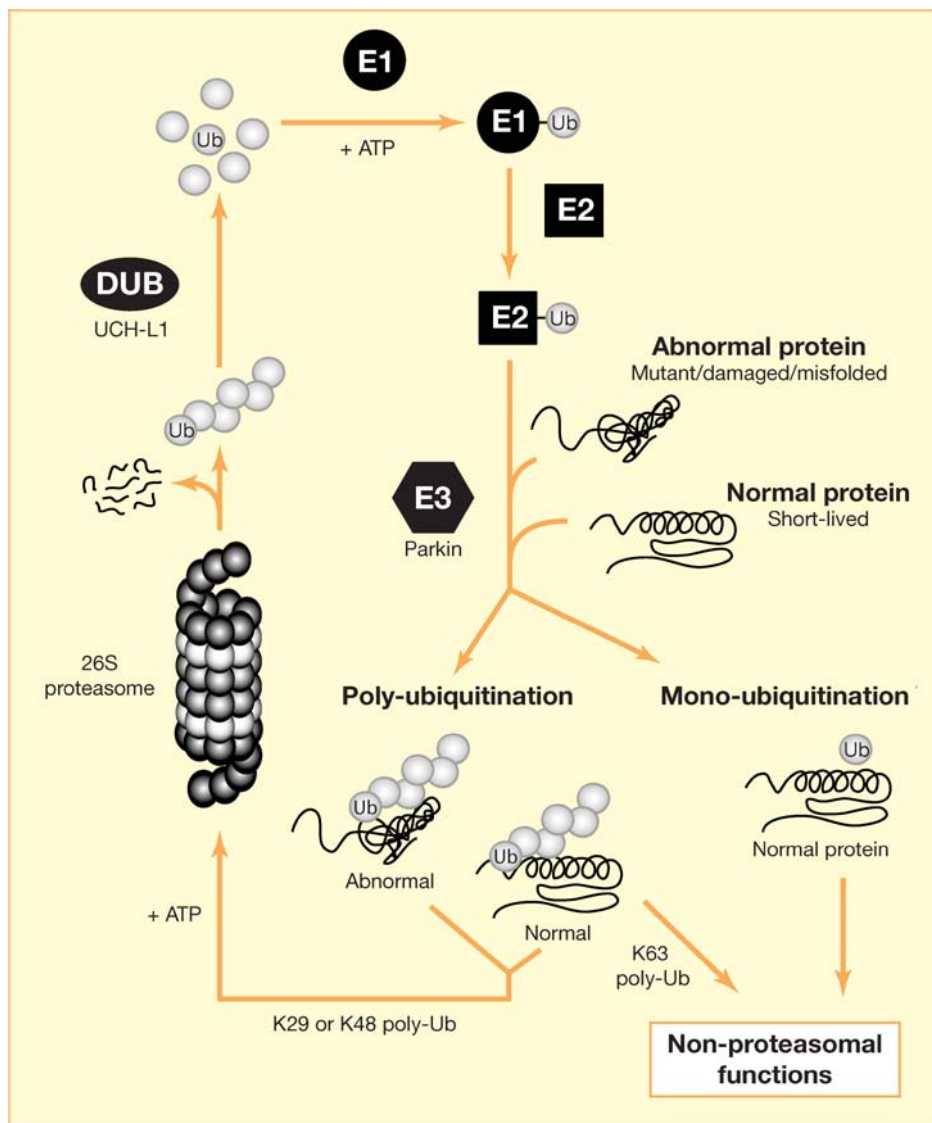
Figure 3

The ubiquitin-proteasome system. Ubiquitin (Ub) monomers are activated by the Ub-activating enzyme (E1) and are then transferred to a Ub-conjugating enzyme (E2). Normal or abnormal target proteins are recognized by a Ub protein ligase (E3), such as parkin, which mediates the transfer of Ub from the E2 enzyme to the target protein. The sequential covalent attachment of Ub monomers to a lysine (K) acceptor residue of the previous Ub results in the formation of a poly-Ub chain. Poly-Ub chains linked through K29 or K48 signal the target protein for degradation through the 26S proteasome in an ATP-dependent manner, resulting in the generation of small peptide fragments. The resulting poly-Ub chains are recycled to free Ub monomers by deubiquitinating (DUB) enzymes, such as UCH-L1, for subsequent rounds of ubiquitination. The addition of Ub also has other diverse roles. Normal proteins can be singly or multiply mono-ubiquitinated, or poly-ubiquitinated with K63-linked chains, which leads to nonproteasomal functions that include DNA repair, endocytosis, protein trafficking, and transcription (Glickman & Ciechanover 2002).

familial-associated mutations in parkin are considered to be loss-of-function and tend to impair the interaction of parkin with E2s or substrates and reduce or abolish parkin's E3 ligase activity or expression. Hence, parkin mutations are thought to result, in general, in the improper targeting of its substrates for proteasomal degradation leading to their potentially neurotoxic accumulation. For this reason, great importance has been placed on the identification of protein substrates of parkin and their possible role in DA neuron loss in PD.

A number of putative substrates have been reported for parkin on the basis of in vitro and cell culture experiments, including CDCrel-1 (Zhang et al. 2000), synphilin-1 (Chung et al. 2001b), a rare *O*-glycosylated form of α-synuclein (Shimura et al. 2001), the parkin-associated endothelin receptor-like receptor (Pael-R) (Imai et al. 2001), synaptotagmin XI (Huynh et al. 2003), cyclin E (Staropoli et al. 2003), the p38 subunit of the aminoacyl-tRNA synthetase complex (Corti et al. 2003), and α/β-tubulin (Ren et al. 2003). The large number of substrates identified is somewhat surprising because typically E3 ligases demonstrate a high level of specificity for one or a small number of substrates. Parkin substrates are diverse, widely distributed, and initially appear to have little in common, and it remains to be seen which, if any, of these putative substrates are relevant in vivo. Some of parkin's substrates have been implicated in enhancing neuronal cell death or toxicity, thus reinforcing a potential role in neuronal dysfunction in PD. CDCrel-1 is a synaptic vesicle–associated protein implicated in regulating neurotransmitter release and can specifically inhibit dopamine release (Dong et al. 2003). Overexpression of CDCrel-1 in SNc DA neurons of rats by virus-mediated gene transfer induces dopamine-dependent neurodegeneration (Dong et al. 2003). When overexpressed in cultured cells, Pael-R tends to become unfolded and insoluble, inducing the unfolded protein response that eventually leads to cell death (Imai et al. 2001). Further-

more, panneuronal expression of Pael-R in *Drosophila* causes age-dependent selective degeneration of DA neurons (Yang et al. 2003). Similarly, overexpression of the p38 subunit results in aggresome-like inclusion formation and/or cell death, depending on the cell type (Corti et al. 2003). The deleterious accumulation of such toxic substrates in the absence of parkin in AR-JP patients may be one mechanism that eventually leads to dysfunction and death of susceptible neurons.

Some parkin substrates also have a tendency to become insoluble and form cytoplasmic inclusions when overexpressed in cells. For example, both Pael-R and p38 form insoluble inclusions in cells, and both have been detected in LBs in sporadic PD together with synphilin-1 and synaptotagmin XI (Corti et al. 2003, Huynh et al. 2003, Imai et al. 2001, Murakami et al. 2004, Wakabayashi et al. 2000). Synphilin-1 interacts with α-synuclein and coexpression of both proteins in cultured cells leads to the formation of insoluble LB-like cytoplasmic inclusions (Engelender et al. 1999). Although parkin can interact with and ubiquitinate synphilin-1, it can also localize to and ubiquitinate α-synuclein/synphilin-1 LB-like inclusions (Chung et al. 2001b). Furthermore, parkin can protect against the toxicity induced by α-synuclein/synphilin-1 overexpression following proteasome inhibition (Chung et al. 2004). A direct role for synphilin-1 in PD is suggested by the identification of a R621C mutation in the *synphilin-1* gene in two apparently sporadic PD patients of German origin (Marx et al. 2003). Overexpression of the R621C mutant reduced the number of inclusions formed following proteasome inhibition, compared with wild-type synphilin-1, and also sensitized cells to staurosporine-induced cell death (Marx et al. 2003). These findings support a causative role for R621C mutant synphilin-1 in PD and suggest that inclusion formation may be a protective event. Parkin, synphilin-1, and α-synuclein are therefore intimately linked in a common biochemical pathway that may contribute to the biogenesis of LBs and may

play an important role in the pathogenesis of PD.

On the basis of strong evidence that loss of function of parkin is the underlying cause of AR-JP, mice with a targeted deletion of the *parkin* gene have been generated to model this form of PD. Surprisingly, parkin knockout mice do not develop a PD-like behavioral or neuropathological phenotype. Two of the knockouts show subtle behavioral deficits, mild alterations of dopaminergic and glutamatergic neurotransmission, and abnormalities in dopamine metabolism (Goldberg et al. 2003, Itier et al. 2003). A third line of knockout mice shows a reduced number of noradrenergic neurons in the locus coeruleus, accompanied by a marked reduction of the norepinephrine-dependent acoustic startle response (Von Coelln et al. 2004b). Furthermore, none of the putative parkin substrates have so far been reported to accumulate in the brains of parkin knockout mice, which has cast doubt on the authenticity of these substrates as well as the contribution of parkin to their turnover by the proteasome. Proteomic analysis has instead revealed reduced levels of several proteins involved in mitochondrial oxidative phosphorylation and protection from oxidative stress in the ventral midbrain of parkin knockout mice (Palacino et al. 2004). This was accompanied by decreases in mitochondrial respiratory capacity and age-dependent increases of oxidative damage. This suggests an unexpected role for parkin in the regulation of normal mitochondrial function. Recent studies in *Drosophila* models of PD support this notion. *Drosophila* parkin null mutants exhibit reduced lifespan, locomotor defects, and male sterility (Greene et al. 2003, Pesah et al. 2004). The locomotor defects derive from apoptotic cell death of muscle subsets, whereas male sterility results from a spermatid individualization defect. The earliest manifestation of muscle degeneration and defective spermatogenesis is mitochondrial pathology, and there are also accompanying signs of increased oxidative stress. The observation of mitochondrial defects and increased oxidative stress in parkin-deficient mice and flies in the absence of DA neuron loss suggests that mitochondrial impairment may be a primary pathogenic event in AR-JP that may eventually trigger selective neuronal degeneration. The absence of DA neuron loss in these models could suggest that neuronal degeneration proceeds over a prolonged time scale exceeding that obtainable in animal models or may require a further pathogenic "hit" to precipitate neuronal death.

The mechanism by which parkin confers neuroprotection, or specifically, promotes the survival of DA neurons, is a central unanswered question. Consistent with a role in maintaining mitochondrial integrity, overexpression of parkin in cultured cells confers resistance to stimuli that promote mitochondria-dependent apoptosis (Darios et al. 2003). Furthermore, a small proportion of parkin is localized to the outer membrane of mitochondria in cells (Darios et al. 2003). In cultured cells, parkin overexpression may confer protection against dopamine-mediated toxicity possibly by decreasing oxidative stress through an undetermined mechanism, thus potentially linking parkin to the survival of DA neurons (Jiang et al. 2004). Parkin can also confer protection against kainate-induced excitotoxicity in primary neuronal cultures, presumably by suppressing cyclin E accumulation (Staropoli et al. 2003). Similarly, parkin can also suppress the ensuing cell death induced by overexpression of Pael-R or the p38 subunit in cultured cells, possibly by ubiquitinating and promoting their degradation (Corti et al. 2003, Imai et al. 2001). In cell culture, overexpression of parkin protects against the toxicity induced by proteasomal inhibition and overexpression of mutant α-synuclein (Petrucelli et al. 2002). Furthermore, in *Drosophila*, parkin overexpression can protect against the selective loss of DA neurons induced by neuronal expression of α-synuclein or Pael-R (Yang et al. 2003). In the α-synuclein *Drosophila* model, parkin overexpression leads to a sharp reduction

in the abundance of α-synuclein-positive LB-like inclusions, which suggests that parkin may act to clear specifically aberrant α-synuclein deposits. Because parkin does not directly interact with native α-synuclein, alterations in the structure of α-synuclein, either through post-translational modifications (including phosphorylation, O-glycosylation, ubiquitination, or nitration) or conformational changes (oligomers or fibrils), may promote an interaction of both proteins at some level, possibly by converging on a common molecular pathway. In this respect it is intriguing that parkin can suppress the toxic effects of α-synuclein species apparently through rescuing impaired proteasome function (Petrucelli et al. 2002). The surprisingly large number of putative parkin substrates, together with the capacity of parkin to confer cellular protection against a diversity of toxic insults, suggests that parkin may represent a multipurpose neuroprotectant (Feany & Pallanck 2003).

UCH-L1 (OMIM 191342; PARK5; Neuron-Specific PGP9.5)

Using a candidate gene-screening approach to identify new mutations in familial PD cases, a heterozygous I93M mutation in the gene encoding ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) was identified in an affected sibling pair in a German family (Leroy et al. 1998). However, the transmitting parent was asymptomatic, suggesting that either the I93M variant is nonpathogenic or that it causes disease with incomplete penetrance. An additional heterozygous M124L variant was described in an affected individual; however, this variant failed to segregate with disease, suggesting that rare polymorphisms in *UCH-L1* may not be pathogenic (Farrer et al. 2000). Additional mutations have not been identified despite extensive genetic screening (Lincoln et al. 1999). Thus, the involvement of the *UCH-L1* gene in familial PD is contentious. A common nonsynonymous polymorphism (S18Y) in the *UCH-L1* gene was first reported as underrep-resented in a European cohort of cases and controls (Maraganore et al. 1999), and a number of subsequent studies either confirmed or failed to replicate the original results. A meta-analysis of the literature provided some evidence to corroborate a potential protective effect of the S18Y variant in PD, which thereby suggests that genetic variability in the *UCH-L1* gene plays a role in the development of late-onset idiopathic PD (Maraganore et al. 2004).

UCH-L1 is a highly abundant, neuron-specific protein that belongs to a family of deubiquitinating enzymes that are responsible for hydrolyzing polymeric ubiquitin chains to free ubiquitin monomers (Wilkinson et al. 1989). UCH-L1 might additionally function as a dimerization-dependent ubiquitin protein ligase (Liu et al. 2002) and can apparently maintain ubiquitin homeostasis by promoting the stability of ubiquitin monomers in vivo (Osaka et al. 2003). UCH-L1 has been localized to LBs in sporadic PD (Lowe et al. 1990). The mechanism by which *UCH-L1* mutations cause PD is poorly understood. The I93M mutation decreased the in vitro hydrolytic activity of UCH-L1 (Leroy et al. 1998), which originally suggested that this form of PD results from a partial loss of function. However, this idea may be an oversimplified interpretation because the I93M mutation shows incomplete penetrance, and mutant mice lacking functional UCH-L1 (the gracile axonal dystrophy mouse) do not develop a parkinsonian phenotype (Leroy et al. 1998, Saigoh et al. 1999). Some investigators have proposed that UCH-L1 may recycle free ubiquitin by cleaving ubiquitinated peptides that are products of the proteasomal degradation of polyubiquitinated proteins (**Figure 3**). Thus, reduced UCH-L1 hydrolytic activity caused by the I93M mutation might impair the overall efficiency of the UPS by reducing the availability of free ubiquitin monomers, thereby leading to potentially deleterious protein accumulation (Leroy et al. 1998). Although a direct role in the UPS is an intriguing possibility, the function of UCH-L1 in vivo remains

to be clarified. UCH-L1 can also promote the accumulation of α-synuclein in cultured cells presumably through the addition of Lys63-linked poly-ubiquitin chains that are not associated with proteasomal degradation (Liu et al. 2002). The potentially protective S18Y polymorphic variant of UCH-L1 has reduced ligase activity but exhibited comparable hydrolase activity to wild-type enzyme and does not promote α-synuclein accumulation (Liu et al. 2002). Thus, both the ubiquitin ligase and hydrolase activities of UCH-L1 may play a normal role in the UPS and may be relevant to the pathogenesis of PD.

PINK1 (OMIM 608309; PARK6; PTEN-Induced Putative Kinase 1)

A genome-wide homozygosity screen performed on a large Sicilian family with four early-onset PD patients revealed a shared 12.5 cM region on chromosome 1p35–p36 (Valente et al. 2001). Additional unrelated families with positive linkage to this region were described (Valente et al. 2002), and mutations in the *PINK1* gene were identified (Valente et al. 2004a). Initial screens for *PINK1* mutations in early-onset familial cases revealed a number of novel mutations; however, these mutations are less common than are alterations in the *parkin* gene in early-onset PD cases (Hatano et al. 2004, Valente et al. 2004b). Genetic variation in the *PINK1* gene did not influence the onset of idiopathic PD in a large European cohort of cases and controls (Healy et al. 2004).

PINK1 is a 581-amino-acid protein that contains a mitochondrial targeting sequence at its N-terminus and a highly conserved protein kinase domain similar to serine/threonine kinases of the Ca^{2+}-calmodulin family (Valente et al. 2004a) (**Figure 1**). Accordingly, overexpressed PINK1 is localized to mitochondria in cultured cells (Valente et al. 2004a). Studies of PINK1 are at an early stage, and currently, little is known about the physiological function of PINK1. Although PINK1 is considered to be a mitochondrial protein

kinase, the kinase activity of PINK1 has not yet been demonstrated, and as such, no putative mitochondrial substrates or interacting proteins have been identified. Mutations in *PINK1* are thought to cause PD through loss of function of PINK1 activity with most mutations clustering in or around the putative kinase domain (Hatano et al. 2004; Valente et al. 2004a,b), which perhaps suggests that the loss of PINK1 kinase activity directly causes PD. Initial studies in cultured cells suggest that PINK1 may afford some protection against mitochondrial dysfunction and apoptosis induced by proteasomal inhibition, although the mechanism for this action is not understood (Valente et al. 2004a). The G309D mutation, identified in a Spanish family with PD, impairs this protective effect of PINK1, and homology modeling reveals that this mutation is located in the ADP binding site of PINK1 and may therefore interfere with ADP binding and kinase activity (Bossy-Wetzel et al. 2004, Valente et al. 2004a). Thus, the loss of the putative kinase activity of PINK1 likely affects mitochondrial function. It has been suggested that PINK1 phosphorylates mitochondrial proteins, in response to cellular stress, to prevent mitochondrial dysfunction (Valente et al. 2004a), although alternatively an inability to normally phosphorylate mitochondrial proteins could actually lead to mitochondrial dysfunction. Although such a mechanism remains to be formally demonstrated, what is clear instead is that a probable role for PINK1 in mitochondrial function links for the first time a primary defect of mitochondria to the molecular pathogenesis of PD. This may have important implications for sporadic forms of PD in which deficits in mitochondrial function have long been proposed.

DJ-1 (OMIM 602533; PARK7)

Homozygosity mapping in a family with early-onset parkinsonism and multiple consanguinity loops demonstrated significant evidence for linkage on chromosome 1p36 (van Duijn et al. 2001). Mutations within the gene

encoding DJ-1 were identified in both an Italian and Dutch family (Bonifati et al. 2003). Additional mutations, including splice site alterations, missense mutations, and small deletions were identified in a number of other familial PD cases (Bonifati et al. 2004). However mutations are extremely rare in early-onset PD cases, present in perhaps less than 1% of PD cohorts (Lockhart et al. 2004). To date, evidence suggests that genetic variability within the *DJ-1* gene does not contribute to the onset of idiopathic PD (Morris et al. 2003).

The *DJ-1* gene encodes a highly conserved protein of 189 amino acids that belongs to the DJ-1/ThiJ/PfpI superfamily (**Figure 1**). DJ-1 is ubiquitously and abundantly expressed in most mammalian tissues, including the brain, where it is localized to both neurons and glia (Bandopadhyay et al. 2004, Olzmann et al. 2004). DJ-1 does not appear to be localized to LBs in sporadic PD and other synucleinopathies but does colocalize with tau-positive inclusions in a number of neurodegenerative tauopathies and with α-synuclein-positive glial inclusions in multiple system atrophy (Neumann et al. 2004, Rizzu et al. 2004), which suggests that DJ-1 may play a diverse role in seemingly distinct neurodegenerative diseases. Furthermore, insoluble forms of DJ-1 are dramatically increased in the brains of sporadic PD patients (Moore et al. 2005) perhaps also implicating DJ-1 in sporadic forms of this disease. The crystal structure of human DJ-1 has been resolved and reveals a flavodoxin-like fold similar to the bacterial protease PH1704 and the stress-inducible molecular chaperone Hsp31, from *Escherichia coli* and yeast (Tao & Tong 2003, Wilson et al. 2003). The crystal structure also shows that DJ-1 exists as a dimer in solution, which has been confirmed in cultured cells (Miller et al. 2003, Moore et al. 2003b). A putative active site has been identified near the dimer interface with similarities to the active site catalytic triad (Cys-His-Asp/Glu) of cysteine proteases, involving residues Cys106, His126, and perhaps Glu18, although these residues do not show an orientation favorable

for proton transfer that is typical of cysteine protease catalysis (Tao & Tong 2003). Consistent with these observations DJ-1 may possess chaperone-like activity as well as weak proteolytic activity in vitro against synthetic model substrates (Lee et al. 2003, Olzmann et al. 2004). At present no substrates have been definitively identified for DJ-1.

The physiological function of DJ-1 is unclear although many lines of evidence suggest that DJ-1 may function as an anti-oxidant protein or as a sensor of oxidative stress. For example, DJ-1 demonstrates an acidic shift in isoelectric point in cultured cells following oxidative stress owing mainly to oxidation of cysteine residues, particularly Cys106, which can be converted to a cysteine sulfinic acid (Cys-SO_2H) (Canet-Aviles et al. 2004, Mitsumoto et al. 2001). DJ-1 can also eliminate hydrogen peroxide in vitro by oxidizing itself suggesting that it may function, in part, as a direct scavenger of ROS (Taira et al. 2004). In cultured cells, overexpression of DJ-1 protects against oxidative injury whereas knockdown of DJ-1 by short interfering RNA enhances the susceptibility to oxidative stress (Taira et al. 2004). A recent study has shown that oxidative stress promotes cysteine sulfinic acid-driven mitochondrial localization of DJ-1 and subsequent protection against mitochondria-dependent cell death (Canet-Aviles et al. 2004). Oxidative stress also promotes an interaction of DJ-1 with parkin in cultured cells (Moore et al. 2005), perhaps linking both proteins in a common neuroprotective pathway. Thus, DJ-1 may play a critical role in both sensing and conferring protection against a range of oxidative stressors. DJ-1 may also confer protection against endoplasmic reticulum stress, proteasomal inhibition, and the toxicity induced by overexpression of Pael-R (Yokota et al. 2003). These diverse cellular insults all share the capacity to induce protein misfolding and aggregation perhaps suggesting that DJ-1 is a component of the UPS and may confer protection by functioning as a molecular chaperone or protease to refold or promote

the degradation of misfolded or aggregated proteins. The possibility that DJ-1 possesses dual enzymatic function i.e., chaperone and protease activities, should not be discounted at this early stage. However, the precise mechanism by which DJ-1 confers neuroprotection awaits further clarification. Familial-associated mutations in *DJ-1* are considered to cause PD through a loss-of-function mechanism, consistent with the recessive inheritance pattern in two *DJ-1*-linked families with PD (Bonifati et al. 2003). The manner in which missense mutations cause loss of DJ-1 function are beginning to be clarified. The L166P mutation, identified in an Italian kindred with PD, drastically destabilizes the DJ-1 protein by promoting the unfolding of its C-terminal region, leading to a loss of dimerization, and subsequently enhancing its degradation by the proteasome (Miller et al. 2003, Moore et al. 2003b, Olzmann et al. 2004). Parkin, CHIP and Hsp70 may play a role in these events since they interact robustly with DJ-1 harboring the L166P mutation (Moore et al. 2005). The L166P mutation also impairs the neuroprotective function of DJ-1 against a range of oxidative stimuli in cultured cells, probably as a direct consequence of its instability (Taira et al. 2004). Other missense mutations may similarly reduce the ability of DJ-1 to protect against selective forms of oxidative stress (Takahashi-Niki et al. 2004), and such mutations also share the capacity to reduce DJ-1 dimerization (Moore et al. 2005). Further clarifying the properties of such mutations may provide novel insight into the neuroprotective function of DJ-1.

COMMON PATHWAYS UNDERLYING THE PATHOGENESIS OF PD

Prior to the identification of genes underlying monogenic forms of PD, both mitochondrial dysfunction and oxidative stress were considered to play a prominent role in the pathogenesis of sporadic PD. Both genetic and non-genetic studies have further implicated these pathways, but they have also highlighted protein mishandling due to UPS dysfunction as a major pathway leading to neuronal degeneration in PD. This section reviews accumulating evidence that mitochondrial dysfunction, oxidative stress, and impairment of the UPS may represent the principal molecular pathways that commonly underlie the pathogenesis of both sporadic and familial forms of PD.

Mitochondrial Dysfunction and Oxidative Stress

Post-mortem studies have consistently implicated oxidative damage in the pathogenesis of PD, and in particular, oxidative damage to lipids, proteins, and DNA has been observed in the SNc of sporadic PD brains (Jenner 2003). Oxidative stress is considered to compromise the integrity of vulnerable neurons and thus to contribute to neuronal degeneration. The source of this increased oxidative stress is unclear but may include mitochondrial dysfunction, increased dopamine metabolism that can yield excess hydrogen peroxide and other ROS, an increase in reactive iron, and impaired antioxidant defense pathways (Jenner 2003). Mitochondria are exposed to a highly oxidative environment, and the process of oxidative phosphorylation is associated with the production of ROS. Much evidence suggests a major role for mitochondrial dysfunction in the pathogenesis of PD, and in particular, defects in mitochondrial complex-I (complex-I) of the respiratory chain. A complex-I defect could most obviously contribute to neuronal degeneration in PD through decreased ATP synthesis as well as damage caused by excess production of ROS. There are consistent findings of decrements in complex-I activity in the SNc of sporadic PD patients (Schapira et al. 1990), although the cause of this is unknown. Complex-I activity is also reduced in cytoplasmic hybrid (cybrids) cell lines that contain mitochondrial DNA (mtDNA) from sporadic PD patients, which indicates that

deficits in complex-I can be stably transmitted, although it is unclear whether such defects in mtDNA arise somatically or are due to inherited mutations (Swerdlow et al. 1996). Rare maternal patterns of inheritance of PD in some families are consistent with the notion of mitochondrial inheritance (Wooten et al. 1997). Indeed, cybrid cell lines derived from maternal descendents of these families similarly exhibit reduced complex-I activity, increased ROS production, and increased radical scavenging enzyme activities (Swerdlow et al. 1998). Genetic evidence that alterations in complex-I activity play a role in the pathogenesis of sporadic PD is provided in part by the observation that a single nucleotide polymorphism in the gene encoding the NADH dehydrogenase 3 enzyme of complex-I, causing an amino acid change from threonine to alanine, leads to a significantly reduced risk of developing PD in Caucasian populations (van der Walt et al. 2003). However, as yet no specific disease-related mutations have been detected in sporadic PD in mitochondrial or nuclear genes that encode complex-I proteins, and pathogenic mutations in mtDNA also have not been identified.

Several epidemiological studies suggest that pesticides and other environmental toxins that inhibit complex-I are involved in the pathogenesis of sporadic PD (Sherer et al. 2002a). MPTP inhibits complex-I and replicates most features of PD in humans and in animal models (Dauer & Przedborski 2003). MPTP was identified as a contaminant of the manufacture of a synthetic opiate, and drug users who accidentally injected MPTP developed a syndrome resembling PD (Langston et al. 1983). The selectivity of MPTP for DA neurons is due to its conversion in astrocytes by monoamine oxidase B to the active metabolite, 1-methyl-4-phenyl pyridinium (MPP$^+$), which is taken up by DA neurons via the dopamine transporter, where it inhibits complex-I and ultimately leads to cell death (Dauer & Przedborski 2003). In aged nonhuman primates, MPTP treatment produces intracellular proteinaceous in-

clusions resembling immature LBs that are filamentous and contain α-synuclein (Forno et al. 1988). Coadministration of the widely used herbicide paraquat (1,1′-dimethyl-4,4′-5 bipyridinium) and the fungicide maneb (manganese ethylenepistithiocarbamate) leads to the pronounced and selective loss of nigrostriatal DA neurons in mice (Thiruchelvam et al. 2000). Paraquat is a complex-I inhibitor with structural similarity to MPP$^+$ and, when administered alone, can also induce selective degeneration of DA neurons together with upregulation and aggregation of α-synuclein in the SNc of mice (Manning-Bog et al. 2002, McCormack et al. 2002). Chronic systemic complex-I inhibition caused by exposure to rotenone, a common insecticide and fish poison, induces parkinsonism in rats, including selective nigrostriatal DA degeneration and the formation of LB-like intraneuronal filamentous inclusions containing α-synuclein and ubiquitin (Betarbet et al. 2000, Sherer et al. 2003). In contrast to MPTP and paraquat, rotenone is not selectively taken up by DA neurons but can still induce selective DA neuronal degeneration, which implies that DA neurons are especially vulnerable to deficits in complex-I. These findings suggest that deficits in complex-I may be central to the pathogenesis of sporadic PD and imply that environmental factors may contribute to PD pathogenesis.

How might the gene products linked to monogenic forms of PD be associated with mitochondrial dysfunction observed in sporadic forms of the disease? Current evidence suggests an intriguing connection between α-synuclein and mitochondria. Complex-I inhibition both in vitro and in vivo consistently leads to the accumulation of LB-like α-synuclein-positive inclusions, which suggests that α-synuclein aggregation is a downstream consequence of mitochondrial dysfunction and might be an effector of neuronal cell death (Betarbet et al. 2000, Forno et al. 1988, Manning-Bog et al. 2002). This idea is supported in part by the observation that cybrid lines derived from members of

the Contursi kindred with PD fail to manifest complex-I deficiency (Swerdlow et al. 2001). Furthermore, α-synuclein knockout mice are resistant to the neurotoxic effects of MPTP, whereas α-synuclein transgenic mice show enhanced toxicity (Dauer et al. 2002, Song et al. 2004), which thus implies that α-synuclein is required for mediating the deleterious downstream effects of complex-I inhibition. α-synuclein itself may further contribute to mitochondrial dysfunction induced by complex-I inhibition because α-synuclein transgenic mice develop enhanced SNc mitochondrial pathology following exposure to MPTP, compared with wild-type mice (Song et al. 2004). Overexpression of mutant α-synuclein can sensitize cultured cells to mitochondrial-dependent apoptosis (Tanaka et al. 2001), which suggests that mutant forms of α-synuclein can also impair mitochondrial function.

Other gene products linked to monogenic forms of PD also appear to be implicated in mitochondrial function. On the basis of studies in mice and *Drosophila*, parkin may have an unexpected role in the regulation of normal mitochondrial function, perhaps linking mitochondria with UPS function (Greene et al. 2003, Palacino et al. 2004). Mitochondrial dysfunction is probably the leading source of increased oxidative and nitrosative stress observed in the brains of sporadic PD patients (Ischiropoulos & Beckman 2003, Jenner 2003). Such stress can promote the *S*-nitrosylation of parkin through its RING domain both in vitro and in cultured cells, and this *S*-nitrosylation can impair parkin's ubiquitin ligase activity and its neuroprotective function (Chung et al. 2004). Furthermore, parkin is selectively *S*-nitrosylated in brains from MPTP-treated mice in a nitric oxide–dependent manner and in patients with sporadic PD and DLB (Chung et al. 2004). Thus, inhibition of parkin's E3 ligase activity by *S*-nitrosylation could contribute to the pathogenesis of PD and related disorders by impairing the ubiquitination of parkin substrates. Because the level of *S*-nitrosylated proteins

is increased in PD and DLB brains in general (Chung et al. 2004), *S*-nitrosylation of proteins due to oxidative and nitrosative stress may play a prominent role in disease pathogenesis. DJ-1 may play a role in mitochondrial function because a proportion of DJ-1 is normally localized to mitochondria, whereas oxidative stress induced by complex-I inhibition can enhance DJ-1 mitochondrial localization (Canet-Aviles et al. 2004). Finally, PINK1, a putative mitochondrial kinase, is the first gene to directly link mitochondria to PD, and accordingly, PINK1 can partially protect against mitochondrial dysfunction induced by proteasome inhibition (Valente et al. 2004a). Future analysis of post-mortem brain tissue from monogenic forms of PD and genetic animal models, including DJ-1 or PINK1 knockout mice, may help to clarify further the molecular pathway linking PD with mitochondrial dysfunction and oxidative stress.

Impairment of the Ubiquitin-Proteasome System

Emerging evidence suggests that impairment of the UPS and protein mishandling may also underlie the molecular pathogenesis of familial and sporadic forms of PD (Giasson & Lee 2003, Moore et al. 2003a). Consistent with this notion there are both structural and functional deficits in the 20/26S proteasome in the SNc of sporadic PD patients (McNaught et al. 2002, 2003). Systemic exposure of rats to naturally occurring and synthetic proteasome inhibitors closely recapitulates many key features of PD, including progressive parkinsonism, selective neurodegeneration of the nigrostriatal pathway and specific brainstem nuclei, and the formation of LB-like intracytoplasmic inclusions containing α-synuclein and ubiquitin (McNaught et al. 2004). If these studies are independently replicated and confirmed, it suggests that perhaps proteasomal dysfunction may be a common end point that precipitates DA neuronal degeneration in PD. The accumulation and aggregation of potentially cytotoxic proteins,

including α-synuclein, in LBs in DA neurons in sporadic PD strongly suggest generalized protein mishandling and subsequent proteolytic stress, which perhaps implies impaired UPS function (Chung et al. 2001a). In *Drosophila*, transgenic or pharmacologically induced overexpression of molecular chaperones rescues the motoric and pathological features induced by transgenic overexpression of normal or mutant α-synuclein (Auluck & Bonini 2002, Auluck et al. 2002), which further suggests a role for protein mishandling in PD. Parkin overexpression has similar effects in *Drosophila* models of PD (Yang et al. 2003). A role for chaperones and other components of the UPS, including UCH-L1, proteasomal subunits, and ubiquitin, in sporadic PD is also supported by their presence in LBs in postmortem brain tissue (Auluck et al. 2002, Forno 1996, Ii et al. 1997, Lowe et al. 1990).

Perhaps the most compelling evidence supporting a role for the UPS in the pathogenesis of PD is the association of *parkin* with familial forms of this disease. Disease-linked mutations in parkin are thought to cause defects in normal UPS function with subsequent proteolytic stress due to aberrant protein accumulation, perhaps leading to the eventual demise of DA neurons (Chung et al. 2001a, Von Coelln et al. 2004a). On the basis of structural comparison with Hsp31, DJ-1 may also participate in the UPS as a redox-sensitive molecular chaperone to alleviate protein misfolding by interacting with early unfolding intermediates (Quigley et al. 2003). As discussed previously, α-synuclein species may be associated with UPS dysfunction through binding and inhibiting the 20/26S proteasome (Snyder et al. 2003), and mutated or aggregated forms of α-synuclein may also overwhelm the degradative capacity of the proteasome, leading to further impairment (Bence et al. 2001, Petrucelli et al. 2002). The consistent presence of fibrillar α-synuclein as a major component of LBs in PD (Spillantini et al. 1998), and the formation of LB-like inclusions containing α-synuclein following proteasome inhibition in vivo (McNaught et al. 2004), tends

to support this notion. LBs are a pathological hallmark of sporadic and some familial forms of PD and indicate the involvement of protein mishandling in disease pathogenesis, although we do not know whether LB formation is a primary or secondary event. The role of LB formation in PD is the subject of some controversy with both pathogenic and protective mechanisms being proposed (Bence et al. 2001, Chung et al. 2001a). Recent studies suggest that the biogenesis of LBs may be akin to the formation of aggresomes (Olanow et al. 2004, Tanaka et al. 2004). Aggresomes are cytoprotective proteinaceous inclusions formed at the centrosome to sequester and aid in the degradation of excess, possibly deleterious proteins (Kopito 2000). One might hypothesize that the formation of LBs is in direct response to proteolytic stress due to proteasomal impairment (Chung et al. 2001a). Parkin has been implicated in promoting LB formation because the majority of patients with *parkin* mutations lack LB pathology (Mori et al. 1998). Alternatively, parkin-mediated neurodegeneration may proceed through mechanisms distinct from those that cause classic PD with LBs, or parkin may be downstream of α-synuclein aggregation, thus bypassing the formation of LBs. The idea that LBs are protective is supported in part by the observation that *parkin*-linked AR-JP patients, which lack LBs, exhibit an earlier onset and more aggressive disease compared with patients with classic PD with LBs (Hardy et al. 2003). The occurrence of LBs in other monogenic forms of PD, including PARK 5, 6, and 7, is not yet known, but future neuropathological analysis may shed light on the significance of LB formation in PD.

CONCLUSIONS

Mitochondrial dysfunction, oxidative stress, and impairment of the UPS may underlie the molecular pathogenesis of familial and sporadic PD, and these pathways may be linked together at multiple levels (**Figure 4**). This
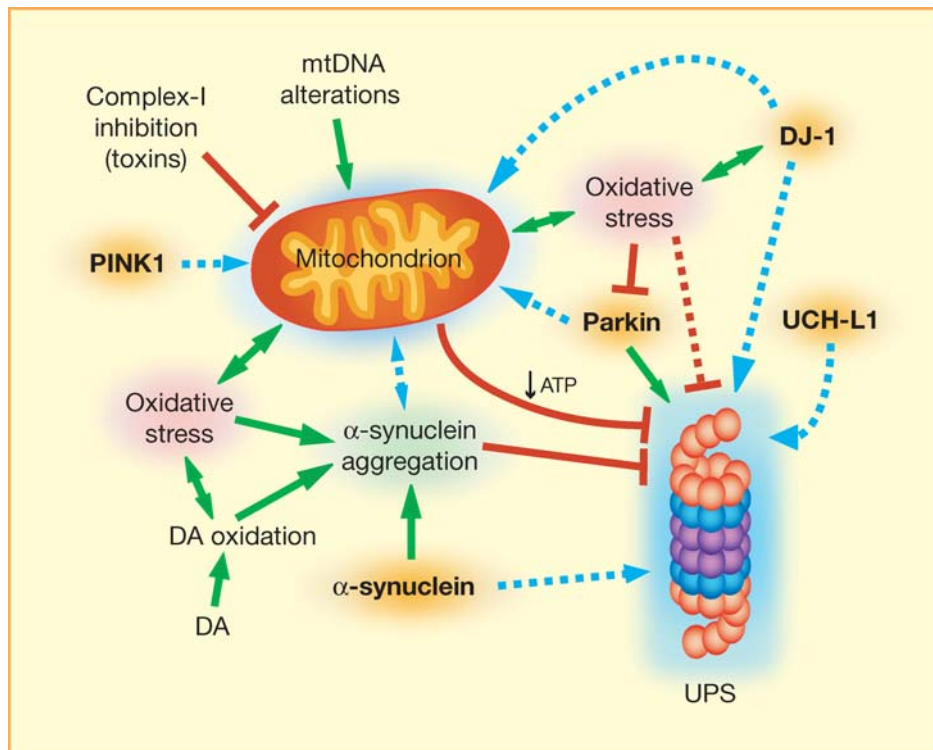
Figure 4

Common pathways underlying PD pathogenesis. Mutations in five genes encoding *α-synuclein*, *parkin*, *UCH-L1*, *PINK1*, and *DJ-1* are associated with familial forms of PD through pathogenic pathways that may commonly lead to deficits in mitochondrial and UPS function. PINK1, parkin, and DJ-1 may play a role in normal mitochondrial function, whereas parkin, UCH-L1, and DJ-1 may be involved in normal UPS function. *α*-synuclein fibrillization and aggregation is promoted by pathogenic mutations, oxidative stress, and oxidation of cytosolic dopamine (DA), leading to impaired UPS function and possibly mitochondrial damage. *α*-synuclein may normally be degraded by the UPS. Some environmental toxins and pesticides can inhibit complex-I and lead to mitochondrial dysfunction, whereas alterations in mitochondrial DNA (mtDNA) may influence mitochondrial function. Impaired mitochondrial function leads to oxidative stress, deficits in ATP synthesis, and *α*-synuclein aggregation, which may contribute to UPS dysfunction. Oxidative and nitrosative stress may also influence the antioxidant function of DJ-1, can impair parkin function through *S*-nitrosylation, and may promote dopamine oxidation. Excess dopamine metabolism may further promote oxidative stress. Mitochondrial and UPS dysfunction, oxidative stress, and *α*-synuclein aggregation ultimately contribute to the demise of DA neurons in PD. Red lines indicate inhibitory effects, green arrows depict defined relationships between components or systems, and blue dashed arrows indicate proposed or putative relationships.

idea is supported by the fact that *α*-synuclein, parkin, and DJ-1 share the capacity to influence both mitochondrial and UPS function, perhaps providing the beginnings of a common pathway involved in neuronal degeneration in PD. However, it is currently unclear whether dysfunction of mitochondria and the UPS tends to converge on a common pathway

or factor, whether they are part of the same pathway, or whether they cause PD through distinct mechanisms. Because the aggregation of *α*-synuclein is clearly downstream from complex-I inhibition and such aggregation can also inhibit or overwhelm proteasomal function, a putative model for the pathogenesis of sporadic PD emerges from these

AR-JP: autosomal recessive juvenile-onset parkinsonism

DA: dopaminergic

DLB: dementia with Lewy bodies

LB: Lewy body

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

PD: Parkinson's disease

Pael-R: parkin-associated endothelin receptor-like receptor

PINK1: PTEN-induced putative kinase 1

ROS: reactive oxygen species

SNc: substantia nigra pars compacta

UCH-L1: ubiquitin carboxyl-terminal hydrolase L1

UPS: ubiquitin-proteasome system

observations. If complex-I inhibition is central to PD pathogenesis, it would set in motion a series of events that lead to α-synuclein aggregation, increased oxidative stress, and deficits in ATP synthesis, all of which can impair normal UPS function. Inhibition of the UPS would lead to the accumulation of proteins otherwise targeted for degradation, some of which may be cytotoxic; that in combination with oxidative damage would ultimately lead to the demise of DA neurons. Parkin, UCH-L1, and DJ-1 may be involved in maintaining normal UPS function, whereas PINK1, together with parkin and DJ-1, may regulate normal mitochondrial function; disease-linked mutations in these genes would lead to a similar set of events precipitating in the demise of DA neurons. However, this pathway of events is by no means straightforward because proteasomal inhibition alone may potentially cause a PD-like phenotype including α-synuclein aggregation (McNaught et al. 2004), and furthermore, proteasomal inhibition can also reciprocally impair mitochondrial function (Sullivan et al. 2004). These observations suggest a large degree of cross-talk between mitochondria and the UPS, and dysfunction in either or both systems may lead to the common end point of DA neuronal degeneration. Future studies of the monogenic forms of PD and their identified gene products, together with experimental animal models of complex-I or UPS dysfunction, will help to determine whether common or distinct molecular pathways contribute to the pathogenesis of familial and sporadic PD.

NOTE ADDED IN PROOF

Recently, two studies have identified the gene associated with PARK8-linked PD. The PARK8 locus was originally identified by parametric two-point analysis of a large Japanese family with autosomal dominant parkinsonism, which yielded significant linkage to chromosome 12p11.2–q13.1, and haplotype analysis further reduced the linked region to a 13.6-cM interval (PARK8, OMIM 607060) (Funayama et al. 2002). Several Caucasian families consistent with autosomal dominant parkinsonism also demonstrated linkage to the PARK8 region, suggesting that genetic variation in PARK8 may be a significant cause of autosomal dominant PD (Zimprich et al. 2004a). Upon screening candidate genes within the linked region, heterozygous mutations within the leucine-rich repeat kinase 2 (*LRRK2*) gene were identified in a number of families (Paisán-Ruíz et al. 2004, Zimprich et al. 2004a). In particular, Paisán-Ruíz et al. (2004) identified two different mutations that segregated with disease in four Basque families (R1396G) and in one large family from the United Kingdom (Y1654C). Subsequent analysis of a population of apparently unrelated Spanish PD patients revealed that approximately 8% of cases carried the R1396G mutation (Paisán-Ruíz et al. 2004). Zimprich et al. (2004b) identified mutations in the same two amino acid residues of the *LRRK2* gene in two large families of German-Canadian (Y1699C) and probable English (Western Nebraska kindred; R1441C) origin; the difference in numbering resulted from the inclusion of a 45 amino acid sequence that may correspond to exon 6. Three distinct mutations (I1122V and I2020T, and the putative splice site variant L1114L [3342A > G]) as well as the R1441C variant were identified in four families by analysis of an additional 32 families with typical late-onset PD, compatible with dominant transmission. These initial findings suggest that mutations in the *LRRK2* gene are, to date, the most common identified genetic cause of late-onset PD.

The *LRRK2* gene contains 51 exons that are predicted to encode a 2527 amino acid protein that has been named dardarin (Paisán-Ruíz et al. 2004). The predicted LRRK2/dardarin protein contains numerous highly conserved domains, including multiple leucine-rich repeats, a Ras-like/small GTPase superfamily domain, a tyrosine kinase-like domain, and a WD40 domain. Mutations

in LRRK2/dardarin tend to be distributed throughout these functional domains, and thus it is currently unclear which domains are relevant for neurodegeneration. Although most PARK8-linked families described to date exhibit a clinical phenotype of classic PD, neuropathology in those affected individuals examined so far ranges from pure nigral degeneration without LBs to nigral degeneration associated with brainstem LBs typical of PD, widespread LBs consistent with DLB, or neurofibrillary tau-positive tangles suggestive of tauopathy (Funayama et al. 2002, Wszolek et al. 2004, Zimprich et al. 2004b). At present,

it is unclear how mutations in LRRK2 cause PD, but it will be of particular interest to determine whether LRRK2/dardarin is related to other gene products or molecular pathways associated with PD. It is tempting to speculate that the putative kinase activity of LRRK2/dardarin (and perhaps that of PINK1) may be involved in the phosphorylation of proteins implicated in PD pathogenesis, such as the phosphoproteins α-synuclein and tau, possibly leading to alterations in their expression, turnover, processing, conformation, cellular localization, or protein-protein interactions.

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