What Causes Cell Death in Parkinson's Disease?

Amitabh Gupta, MD, PhD,[1] Valina L. Dawson, PhD,[1–4] and Ted M. Dawson, MD, PhD[1,2,4]

Currently, there is no proven neuroprotective or neurorestorative therapy for Parkinson's disease (PD). Several advances in the genetics of PD have created an opportunity to develop mechanistic-based therapies that hold particular promise for identifying agents that slow and even halt the progression of PD, as well as restore function. Here we review many of the advances in the last decade regarding the identification of new targets for the treatment of PD based on understanding the molecular mechanisms of how mutations in genes linked to PD cause neurodegeneration.

Ann Neurol 2008;64 (suppl):S3–S15

Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder with no proven neuroprotective or neurorestorative therapy. Identification or proof that a drug is neuroprotective or neurorestorative will herald a new era of PD treatment, much in the same way that L-dopa altered the course and management of PD. It is essential to understand the molecular and biochemical mechanisms of PD pathogenesis to develop neuroprotective and neurorestorative therapies. It is with this understanding that a fundamental basis for a mechanism-based rationale will be established that determines drug targets for modifying disease progression and tissue regeneration.

Human postmortem material, animal models, and genetic analyses have provided important clues to the cause of PD. In particular, the genetic approach has recently unraveled a series of proteins that, when mutated, can cause familial forms of PD. This is insofar important because this approach provides an entry into identifying the signaling pathways that go errant during the development of PD. Understanding the contribution of these signaling pathways to neurodegeneration in PD will undoubtedly allow for important access points for drug targeting. Importantly, understanding the interaction of the signaling pathways that are affected in the different forms of familial PD should help to identify crucial nodal points that may be prudent primary drug targets. In this article, key proteins mutated in PD are discussed in the context of their potential for drug development. Specifically, we start with the main proteins that cause genetic forms of PD (summarized in the Table) and conclude with proteins that play a significant role in sporadic disease that have come to the forefront mostly from nongenetic research avenues.

Gain-of-Function Mutations in Parkinson's Disease

Autosomal dominant PD is caused by mutations in α-synuclein and LRRK2 (leucine-rich repeat kinase 2), indicating gain-of-function mutations for both proteins.[1,2] Interestingly, α-synuclein is a major component of Lewy bodies and Lewy neurites, which constitute two of the main pathological hallmarks of PD.[3] This observation demonstrates how familial and sporadic forms of PD are molecularly interrelated, emphasizing the notion that insight into the pathogenesis of familial PD should critically advance our knowledge of sporadic PD (Fig). This, in turn, implies that drug therapies for familial PD should be applicable to the PD population at large, rather than be confined to a few rare cases.

α-Synuclein is a 140-amino acid protein that shows diffuse cellular localization, as it is found in the cytoplasm, perhaps in the cell nucleus, as well as associated with membrane structures.[4] α-Synuclein is thought to

From the Departments of [1]Neurology, [2]Neuroscience, [3]Physiology, and [4]Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD.

Received Mar 3, 2008, and in revised form Sep 30. Accepted for publication Oct 3, 2008.

Potential conflicts of interest: This article is part of a supplement sponsored by Boehringer Ingelheim (BI). A.G. has received honorarium from BI. V.L.D. has no relationship with BI. T.M.D. is a paid contributor. T.M.D. has consulted for BI in the past, but reports no consulting agreements with pharmaceutical companies at the time of publication of this manuscript.

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.21573

Address correspondence to Dr Dawson, Institute for Cell Engineering, Department of Neurology and Neuroscience, Johns Hopkins University School of Medicine, 733 North Broadway, Suite 731, Baltimore, MD 21205. E-mail: tdawson@jhmi.edu

Additional Supporting Information may be found in the online version of this article.

Published by Wiley-Liss, Inc., through Wiley Subscription Services

Table. **Genetic Forms of Parkinson's Disease**

Form	Pattern of Inheritance	Chromosome Region	Name of Gene	Gene Identified	Name of Protein	Function of Protein
Familial PD	AD	4q21-q22	*PARK1*	Yes	α-Synuclein	Synaptic protein
Young-onset PD	AR/AD	6q25.2-q27	*PARK2*	Yes	Parkin	Ubiquitin-protein ligase
Familial PD	AD	4q region	*PARK4*	Yes	Multiplication of α-synuclein chromosomal region	Excess α-synuclein protein
Young-onset PD	AR	1p35-p36	*PARK6*	Yes	PINK1	Mitochondrial stress-induced degeneration
Young-onset PD	AR	1p36	*PARK7*	Yes	DJ-1	Oxidative stress protection
Familial PD	AD	12p11.2-q13.1	*PARK8*	Yes	LRRK2	Protein
					Dardarin	Phosphorylation
Familial PD	AR	1p36	*PARK9*	Yes	ATP13A2	Lysosomal protein

Included are only those genetic forms of Parkinson's disease (PD) that have identified genes and are discussed in text.
AD = autosomal dominant; AR = autosomal recessive.
Modified from Fahn, and Jankovic. Principles and Practice of Movement Disorders. First Edition, 2007, by permission.

promote cellular toxicity in aggregate form,[5] although it is not clear whether the oligomeric or the polymeric form produces that effect. However, analogous to findings in the field of Alzheimer's disease, recent evidence suggests that oligomeric α-synuclein can promote cell death, whereas polymeric α-synuclein can be protective (the latter point is discussed separately in the section on sporadic disease). This observation has drawn attention to the possibility that reducing α-synuclein levels to avoid oligomer formation may constitute a disease-modifying treatment option, whereas interfering with conversion from oligomeric to polymeric α-synuclein may be disease promoting. Accordingly, RNA interference and interference with α-synuclein expression through selective transcriptional inhibition are two focused approaches that aim at preventing aggregation at the early stage.[6,7] Moreover, strategies that decrease the level of α-synuclein are attractive from the viewpoint that simple duplication or triplication of the wild-type α-synuclein causes autosomal dominant PD,[8] and polymorphisms in the α-synuclein promoter that alter the level of expression of α-synuclein determine one's relative risk for development of PD.[9,10] Consistent with this notion is also the observation that knock-out of α-synuclein dramatically protects against the loss of dopamine neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD.[11] Alternatively, inhibiting α-synuclein oligomers may be an important approach to preventing disease initiation or progression in PD.[12,13] In fact, immunoantibodies against oligomeric α-synuclein were developed by active and passive immunization protocols. In parallel to their ability to decrease aggregate formation, these antibodies decrease PD pathology in mice models,[14] implying that injections of antibodies in PD patients may constitute a viable neuroprotective treatment modality of the future. Currently, human trials are in preparation or in the beginning phases. Aside from immunoantibodies, the antibiotic rifampicin has also been shown to inhibit oligomerization of α-synuclein.[15–18] In a mouse model of multiple system atrophy, a synucleinopathy of glial cells that can present with parkinsonian features, rifampicin improved motor performance in association with decreased protein aggregation. These findings are promising because they provide a treatment alternative to the immunoantibodies, thereby allowing a comparison of the side-effect profile between the two approaches, as well as assessment of synergistic or additive effects in the setting of a combination therapy. Finally, it is known that α-synuclein is cleaved,[19] albeit mostly by as yet unidentified α-synucleinases. Given that cleavage of α-synuclein enhances its propensity to aggregate into toxic complexes, identifying the α-synucleinase(s) and developing drugs that inhibit their activity may provide great benefit to PD patients.

As the production of aggregated α-synuclein appears to contribute to neurodegeneration, a separate and independently valuable neuroprotective treatment approach is to facilitate clearance of these protein complexes. Accordingly, much research has been conducted on understanding what protein degradation pathways are involved in degrading wild-type and mutant α-synuclein.[4] α-Synuclein is ubiquitinated, but whether ubiquitination plays a role in the clearance of α-synuclein is not clear. The ubiquitin proteasome system (UPS) does appear to play a role in PD[20] because defects in the UPS are found in sporadic PD,[21] and

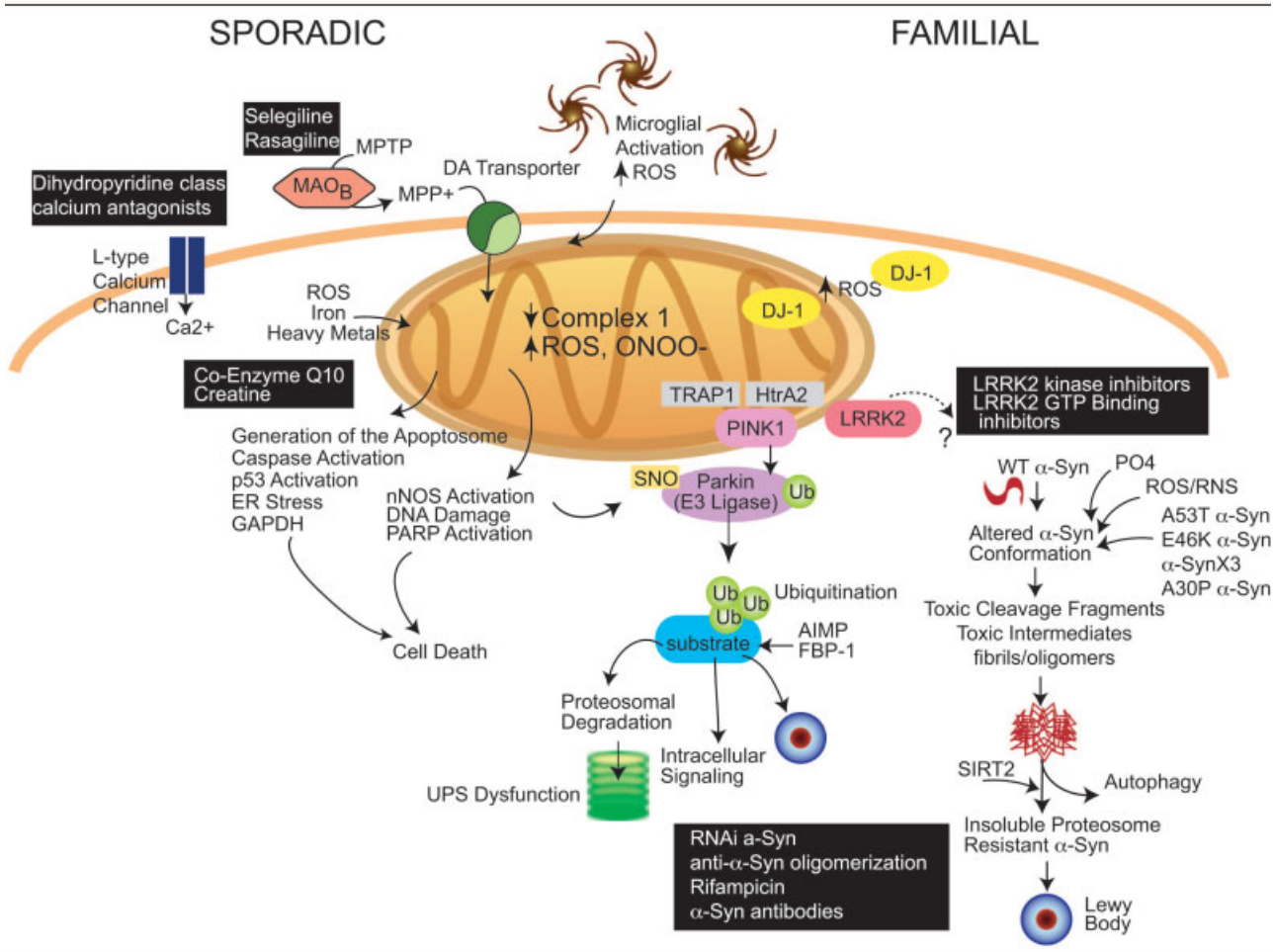

Fig. Molecular mechanisms of neurodegeneration in Parkinson's disease (PD). There are multiple potential pathways of cell death in PD. It is currently unclear whether these pathways converge to one overarching central cell death mechanism in PD, or whether they are independently and distinctly involved in different forms of genetic and sporadic PD. On the left are potential mechanisms and therapeutic targets for sporadic PD. Central to sporadic PD is mitochondrial complex I deficiency, which is modeled by MPTP and other complex I inhibitors. MPTP is converted by monoamine oxidase B (MAO_B) to 1-methyl-4-phenyl-pyridium (MPP+), where it is concentrated in dopamine neurons because of its high affinity for the dopamine (DA) transporter (DAT) followed by transport and concentration into the mitochondria because of its positive charge. Once in the mitochondria, MPP+ poisons the mitochondria by inhibiting complex I. Selegiline and rasagiline were initially used as neuroprotective agents because of their inhibition of MAO_B, but it is now thought that they may actually inhibit GAPDH-dependent cell death pathways downstream of complex I inhibition. Calcium-channel antagonists of the dihydropyridine class may rejuvenate DA neurons to the point where they use sodium channels for their characteristic pacing, thus rendering them resistant to toxic effects of complex I inhibition. Coenzyme Q10 and creatine have neuroprotective properties in animal models of PD through augmenting the function of mitochondria. Complex I inhibition leads to cell death through classic apoptotic pathways, endoplasmic reticulum (ER) stress, as well as neuronal nitric oxide synthase (nNOS) activation, DNA damage, and poly(ADP-ribose) polymerase (PARP) activation. Accompanying derangements in mitochondrial complex I is the subsequent activation of microglia. Strategies aimed at reducing the inflammatory process in PD holds particular promise. On the right are potential therapeutic opportunities for familial PD. DJ-1 appears to function in controlling the level of reactive oxygen species (ROS). Reducing oxidative stress or restoring DJ-1 function, or both, may be protective. PINK1 is a mitochondrial kinase, and disruption of PINK1 may lead to alterations in the function of TRAP1 and HtrA2, setting in motion cell death pathways. Thus, strategies aimed at inhibiting TRAP1 and/or HtrA2, as well as enhancing PINK1, may be beneficial. Parkin is an E3 ubiquitin (Ub) ligase, and strategies aimed at maintaining its catalytic activity are particularly attractive, as well as reducing the burden of toxic substrates such as aminoacyl-tRNA synthetase (ARS) interacting multifunctional protein type 2 (AIMP2) and the far upstream element-binding protein-1 (FBP-1), which are degraded by the ubiquitin proteasome system (UPS) in a parkin-dependent manner. S-nitrosylation (SNO) of parkin inhibits its activity, and interfering with this process may also be protective. PINK1 and parkin appear to function in a common genetic pathway with PINK1 acting upstream of parkin; thus, maintaining parkin function will, in theory, inhibit PINK1-dependent cell death pathways. LRRK2 (leucine-rich repeat kinase 2) kinase, GTP binding inhibitors, or both may be beneficial against cell death caused by LRRK2 mutations. Reducing ROS and reactive nitrogen species (RNS), modulating α-synuclein (α-syn) phosphorylation (PO_4), reducing α-synuclein levels through RNA interference or immunotherapy, reducing α-synuclein oligomerization, inhibiting α-synuclein cleavage, or enhancing the degradation of α-synuclein through inhibition of sirtuin 2 protein (SIRT2) are potential targets to reduce neurodegeneration by derangements in α-synuclein. WT = wild type.

the ubiquitin E3 ligase parkin[22] (see later) is mutated in some familial forms of PD. Hence, the notion is conveyed that UPS dysfunction results in abnormal protein accumulation and aggregation of proteins, which subsequently leads to cellular dysfunction and neurodegeneration in PD.[23] In this manner, α-synuclein may accumulate in dopaminergic cells and cause cell death. However, aside from the UPS, other protein degradation pathways may also play a role in PD. The autophagy pathway is one of those alternative routes, where proteins are processed within lysosomes. Such self-digestion can occur in a chaperone-dependent or -independent manner, with the former variant requiring the lysosomal receptor LAMP2A. Interestingly, the ill-defined ATP13A2 protein, an ATPase that localizes to the lysosome, is mutated in parkinsonism syndromes, including in the Kufor–Rakeb syndrome, and potentially in some juvenile and young-onset PD.[24–26] Given that in the disease state ATP13A2 redistributes to the endoplasmatic reticulum,[24] this protein may turn out to provide a first genetic link between lysosomal pathways and PD. In this context, it is notable that wild-type α-synuclein can be degraded by chaperone-mediated autophagy, whereas mutated α-synuclein binds to LAMP2A but is not taken up by the lysosome, thereby preventing its digestion.[27] Thus, the gain-of-function mutation of α-synuclein may impede chaperone-mediated autophagy–dependent self-degradation, suggesting the ensuing protein accumulation and toxicity to be a secondary and inevitable by-product.[28] Current research is focused on assessing why LAMP2A binding of mutant α-synuclein is not sufficient for degradation and how mutant α-synuclein may be degraded in the setting of PD. Some evidence suggests that mutant α-synuclein may also be degraded by autophagy, but potentially by the chaperone-independent pathway. Accordingly, promoting LAMP2A-mediated uptake of mutant α-synuclein and improving chaperone-independent autophagy in the dopaminergic cell may be two promising research avenues that should yield drug targets in the future. Consistent with this notion, lysosomal inhibitors are able to negate the effect of the immunoantibodies that target oligomeric α-synuclein.[14] These observations underscore the importance of the autophagy system in PD, regardless of which subtype is implemented for degrading the aggregate form of α-synuclein.[29]

Several cellular targets of α-synuclein have been found. The protein is thought to be an important component of the presynaptic protein scaffold that regulates dopamine release. In fact, inhibiting α-synuclein or proteins that interact with it at the synapse alters dopaminergic transmission and synaptic function.[30–32] This provides a direct link between α-synuclein function and PD, given that dopamine depletion is a hallmark of the disease. Furthermore, aggregated α-synuclein impacts on various membrane structures of the cell. It can form porelike complexes in the plasma membrane that alter membrane excitability and calcium permeability,[33,34] affect the integrity of lysosomal and endosomal compartments, and inhibit vesicular trafficking from the endoplasmic reticulum to the Golgi apparatus in a Rab-dependent manner.[35] α-Synuclein also functions as a chaperone to maintain the integrity of the presynaptic terminal because α-synuclein overexpression rescues the neurodegenerative phenotype of cysteine-string protein knock-out mice.[36] Despite the progress made in delineating the various cellular functions that mutant α-synuclein may affect, it is not yet understood how much each of these perturbances contributes to overall neurodegeneration. Similarly, it is unknown what precise molecular properties of α-synuclein mediate these toxic effects. An attractive hypothesis suggests that oligomeric mutant α-synuclein may integrate into cellular protein complexes, much like prion proteins, to disrupt physiological protein-protein interactions. It is speculated that the PD mutations trigger changes in secondary protein structures of α-synuclein that promote such toxic protein binding behavior. Questions such as these are under intensive investigation, considering that their resolution will greatly help to prioritize cellular targets for neuroprotective and neurorestorative treatments.

LRRK2 is the other protein mutated in an expected gain-of-function manner. Similarly to the case with α-synuclein, there is evidence that knowledge about pathogenesis of familial PD will translate effectively into understanding sporadic PD. Because LRRK2 is commonly mutated in sporadic cases, with estimates ranging up to 4%, there is great enthusiasm in the research community that understanding LRRK2 function and contribution to PD pathogenesis will benefit a wide spectrum of PD patients.[1]

Possessing a molecular weight of 280kDa, LRRK2 constitutes a large protein that is localized to the cytoplasm, associated with cellular membranes that include the outer mitochondrial membrane.[37,38] LRRK2 is arranged in multiple protein modules, including GTPase Roc and kinase domains. Based on the module analysis, it is thought that LRRK2 belongs to the ROCO protein family, a novel group of the Ras/GTPase superfamily. In addition, the kinase domain appears to have homology to the mitogen-activated protein kinase kinase kinases of the mixed lineage kinase subclass.[39]

Despite the structural evidence of defined domains with known functions, the overall function of LRRK2 is not well understood. Much data, however, points to the possibility that the gain-of-function is linked to increased kinase activity. Indeed, individual LRRK2 mutations found in PD families, including the G2019S and I2020T mutations, show increased intrinsic kinase activity, when assessed with myelin basic protein as

substrate.[38,40] Moreover, increased LRRK2 kinase activity appears to go hand in hand with disease-segregating genetic mutations.[39] Reduction in kinase activity of mutant LRRK2 reduces neurotoxicity,[39,41,42] consistent with the notion that toxicity is linked to enhanced kinase activity. The neurotoxicity is, at least in part, reflected in apoptosis that is associated with prominent phospho-tau–positive inclusions with lysosomal characteristics and a reduction in neurite length and branching.[43] Altogether then, it appears that increased or abnormal phosphorylation of critical LRRK2 substrates is involved in mutant LRRK2 neurodegeneration.

Despite the support for the model of increased kinase activity, some line of research urges caution about such conclusion because kinase activity depends on the substrates tested. No definitive LRRK2 phosphorylation substrates have been identified. However, when using the cytoskeletal proteins moesin, ezrin, and radixin as potential substrates, instead of myelin basic protein, the picture of LRRK2 kinase activity becomes rather complex.[44] Whereas the G2019S mutation continued to demonstrate increased kinase activity, the I2020T mutation differed from previous data by showing a reduced substrate phosphorylation. Furthermore, seven other PD mutations of LRRK2 showed either reduced or unchanged kinase activity. Hence, as authentic and physiologically relevant LRRK2 substrates are discovered, the PD mutations of LRRK2 may differentially affect its kinase activity on selective subsets of substrates. Implying a more complex picture then, specific PD mutations may affect LRRK2-dependent phosphorylation pathways differently to cause disease pathogenesis.[45]

The principal link between LRRK2 kinase activity and PD pathogenesis directs the research focus on the mechanisms that regulate LRRK2 kinase activity. In this light, the kinase activity of LRRK2 appears to be regulated by its GTPase domain. GTP binding is critical for the kinase activity of LRRK2,[46] and enhanced GTP binding appears to promote neuronal toxicity, just like increased LRKK2 kinase activity does.[39] Recent structural evidence suggests that the GTPase domain may function as a dimer, with the PD-associated pathogenic residues R1441 and I1371 being positioned at the interface of the two monomers to critically contribute to dimer stability.[47] Moreover, the PD mutation R1441C/G disrupts GTP hydrolysis.[48,49] Hence, judging from the effect of the PD mutations on the GTPase domain of LRRK2, GTP binding may positively regulate LRRK2 kinase activity and neurotoxicity in PD, whereas GTP hydrolysis may counteract this effect, with residue 1441 contributing to dimer stability to promote GTPase activity. Additional studies are needed to better dissect the relation between the GTPase domain of LRRK2 and PD pathogenesis, in particular, studies that address how GTP hydrolysis regulates kinase activity.

Irrespective of the exact mechanism of LRKK2 kinase regulation, such research poses a great opportunity for the development of new drugs for PD. One avenue involves the modulation of GTPase activity, thereby perhaps fine tuning the kinase activity back to the reference range rather than just eliminating it. In this way, key aspects of the physiological function of LRRK2 may be preserved, which would allow for an effective treatment. Alternatively, even selective inhibition of aberrant LRRK2 kinase as a whole offers a tremendous potential, judging from successes with kinase inhibitors in other fields of medicine, such as the use of the c-Abl kinase inhibitor for the treatment of specific cancer types.[50] Lastly, given that the chaperone activity of heat shock protein (HSP90) can stabilize LRRK2,[51] regulating HSP90 or other LRRK2 stabilizers may yet provide a separate level of control for the kinase activity.

Recently, evidence indicates that some PD families may have mutations in the LRRK2 protein that are not localized to the kinase or GTPase domains. In particular, two disease-causing mutations were found in the N-terminal domain.[52] Unless these mutations have long-ranging effects on kinase or GTPase activity, this observation suggests that mechanisms distinct from altered kinase activity may account for PD pathogenesis in some PD families. How mutations in the N-terminal region cause disease is currently unknown, owing in part to a limited functional understanding of that area. Nonetheless, the notion that PD mutations may be situated across the entire protein lends support to nonkinase mechanisms, likely implemented through interacting proteins of the various LRRK2 domains. It may even be possible to speculate that some of these LRRK2 mutations may not reflect a gain-of-function state of LRRK2, as phosphorylation-independent pathways may be affected in a cell-selective manner.

Given the key role of LRRK2 in PD, strong efforts to identify LRRK2 interaction partners have been made. These efforts are now beginning to yield promising protein candidates. The discovery that parkin can molecularly interact with LRRK2 is of particular interest[53] because parkin causes autosomal recessive PD (see later). Given that parkin is part of the UPS and LRRK2 is associated with the outer mitochondrial membrane, it may be considered that the molecular connection between these two proteins involves mitochondrial LRRK2 substrates that require carefully regulated degradation by the UPS. In any event, discovering the way in which LRRK2 intersects with the cellular signaling network will provide an understanding of how LRRK2 kinase activity drives neurodegeneration and, in extension, which protein interactions may represent effective drug target points. Restoration

of physiological LRRK2 interactions or inhibition of abnormal LRRK2 interactions by drugs that regulate LRRK2 conformation may yield promising therapeutic results.

Loss-of-Function Mutations in Parkinson's Disease

Autosomal recessive PD is caused by mutations in parkin, PINK1, and DJ-1, indicating that each of these proteins likely loses its physiological function in the disease state. Parkin is an E3 ubiquitin ligase, which catalyzes the final step of protein ubiquitination to allow for successful targeting to and degradation by the UPS.[54–56] Loss of function of parkin may result in protein accumulation as a consequence of lack of ubiquitination. Subsequent neurodegeneration may be caused by toxic effects of aggregated protein.[57] Interestingly, PD patients with parkin mutations, in general, do not have any large inclusions, such as Lewy bodies. This may suggest that large-scale protein aggregation does require ubiquitination and processing beyond the parkin step.[58,59] Furthermore, the toxicity may be mediated by small oligomeric proteins rather than by large polymeric protein complexes. The oligomeric structures may not include α-synuclein, however, because mice mutant for α-synuclein (overexpressed A53T mutation of α-synuclein) did not change their phenotype in the background of a loss-of-function parkin mutation.[60] Hence, parkin and α-synuclein may mediate independent mechanisms of PD pathogenesis, which is consistent with the absence and presence of Lewy bodies in parkin and α-synuclein mice, respectively. To some extent then, these results point out pathogenetic and molecular differences between some of the PD families and argue for a therapeutic approach that may need tailoring to specific PD subtypes.

Recent studies have identified distinct parkin substrates. One of them, the adaptor protein Eps15, implicates parkin in proteasome-independent ubiquitination, because parkin was shown to promote epidermal growth factor (EGF) signaling by delaying epidermal growth factor receptor (EGFR) endocytosis.[61] This effect was mediated by parkin-dependent ubiquitination of Eps15, which resulted in decreased binding of Eps15 to EGFR, thereby delaying EGFR internalization and degradation. Prolonged EGF signaling, in turn, extended PI(3)K-Akt signaling. This result implies that parkin may be centrally involved in modulating ubiquitin-dependent degradation of proteins, but in many ways other than merely regulating proteasome activity. Furthermore, given the crucial role of Akt in neuronal survival, it is plausible that loss of parkin function in PD may correlate with reduced Akt activity to explain decreased cell survival. Two other substrates that have been identified are aminoacyl-tRNA synthetase (ARS) interacting multifunctional protein type 2 (AIMP2) (p38/JTV-1) and the far upstream element-binding protein-1 (FBP-1). They appear to be authentic parkin substrates because they accumulate in parkin-deficient mice and in autosomal recessive PD caused by parkin mutations.[62,63] Such confirmatory data are valuable and missing for other proposed parkin substrates. Interestingly, AIMP2 is selectively toxic to dopaminergic neurons.[63] Both proteins are currently being investigated for their baseline functions and their functional changes in the absence of parkin.

Other proteins that bind to parkin are not necessarily substrates but, in fact, modulate parkin function. In that capacity, they are heavily studied, not lastly because of interest in developing novel therapeutics. One of those proteins is HSP70. It is intriguing that parkin interacts, albeit indirectly, with Hsp70,[64] given the significance of the heat shock response in PD pathogenesis. In animal models of PD, overexpression of Hsp70 ameliorates disease progression,[65] and drugs that activate the heat shock response, such as geldanamycin, prevent neurodegeneration.[66] Now, the interaction between parkin and Hsp70 begins to unravel how the protective effect of the HSPs may be exerted in PD. Understanding the functional consequences of the parkin-Hsp70 interaction should aid in designing more specific therapeutic strategies that compensate for the fallouts of a compromised parkin-Hsp70 interaction in the disease state. Recently, the protein BAG5 was found in a complex with parkin and Hsp70.[67] By inhibiting Hsp70, BAG5 could promote sequestration and suppression of parkin. BAG5 was also shown to directly inhibit parkin, as reflected by decreased ubiquitination activity and decreased suppression of UPS dysfunction and cell death. Although the mechanism by which BAG5 exerts its inhibitory effect is not understood, it has been speculated that the RING finger domain of parkin is blocked. Independently, the E4-like protein CHIP was shown to dissociate parkin from Hsp70. This promoted ubiquitination of parkin substrates, such as the Pael receptor, that otherwise would induce cell stress.[64] Hence, targeting BAG5, CHIP, or other yet unknown modulators of the parkin-Hsp70 interaction should yield therapeutic benefit in PD. Alternatively, parkin is modulated by *S*-nitrosylation,[68,69] and developing drugs that regulate such modification may prove valuable. It has been demonstrated that nitrosylation inhibits parkin E3 ligase activity and thereby causes UPS dysfunction, which is consistent with increased nitrosylation of parkin in PD.[68,69] Novel drugs that inhibit parkin nitrosylation may therefore be able to delay or even reverse neurodegeneration in PD.

In contrast with parkin data that support the importance of UPS dysfunction in PD pathogenesis, the discovery of mutations in PINK1 and DJ-1 underscores a critical role for mitochondrial dysfunction in the dis-

ease state. PINK1, which stands for PTEN (phosphatase and tensin homologue)-induced putative kinase 1, encodes a putative serine/threonine kinase that localizes to the mitochondria.[70] Several lines of evidence suggest that it is part of a signaling network that regulates the mitochondrial response to various cellular stressors. Consistent with that notion, animal models of mutant PINK1 and inhibition of PINK1 expression in cell systems both demonstrate mitochondria with altered structure and defective function.[71–73] These experiments have established at the same time that the PINK1 mutation is a loss-of-function mutation. Interestingly, PINK1 dysfunction can be rescued by overexpressing parkin, but not vice versa. This indicates a genetic interaction between the two genes and places PINK1 upstream of parkin in the signaling hierarchy.[71,72] Current research is focusing on elucidating the molecular nature of this cross talk, entertaining the possibility that PINK1 and parkin may be components of a linear signaling pathway.[74] Similar to the molecular connection between LRRK2 and parkin, the signaling interaction between PINK1 and parkin may be mediated through mitochondrial PINK1 substrates that require degradation by the UPS. This notion is insofar attractive to drug development because it implies that certain key nodal points in signaling exist that connect several of the familial PD genes. Hence, targeting these nodal points should simultaneously improve multiple cellular defects during PD pathogenesis and be of great value to PD treatment across a wide range of PD patients.

Given the loss-of-function nature of the PINK1 mutation, it is hypothesized that the loss of phosphorylation of PINK1 substrates leads to mitochondrial dysfunction and triggers or promotes neurodegeneration. Thus, much effort has been invested in defining PINK1 interaction partners. TRAP1 and HtrA2 represent the two main PINK1 interactors that have been identified so far.[75,76] TRAP1 is a mitochondrial chaperone that is activated under oxidative stress. Under such conditions, PINK1 phosphorylates TRAP1, which decreases cytochrome *c* release from the mitochondria into the cytoplasm, and thereby dampens an important mitochondrial trigger for apoptotic signaling pathways. HtrA2, a mitochondrial protein with serine protease activity, is also involved in stress signaling pathways. Notably, on activation of MEKK3-p38, PINK1 phosphorylates HtrA2 to increase its proteolytic activity and confer resistance to mitochondrial stress.[77] The targets of proteolytic activity and the type of apoptotic pathway that these targets regulate are not currently known. Nonetheless, the findings on TRAP1 and HtrA2 both elucidate how PINK1 is intricately placed in mitochondrial stress-sensing pathways that protect the cell from oxidative stress. Conversely, loss of PINK1 function in PD may promote neurodegeneration, because protective responses of the mitochondria to oxidative stress are impaired, which results in mitochondrial dysfunction and aberrant activation of cell death pathways.

Current research is trying to determine whether the signaling pathways that involve TRAP1 and HtrA2 intersect. Along the same line, it is being evaluated whether the cell death pathways that these proteins impact on are related or even the same. Such knowledge is required for developing treatment approaches that affect oxidative stress responses. In case of signaling intersection, a nodal point in signaling may be identified that allows for a drug target with a broad protective range. In this context, it is interesting to note that HtrA2 not only molecularly interacts with PINK1, but like PINK1 also appears to be genetically identified as causing a familial form of PD. HtrA2 mutations were shown to be linked to the PD-13 locus, unlike PINK1 inherited in an autosomal dominant pattern, and suggested to affect the serine protease function.[78] Consistent with the notion that the proteolytic function is reduced by these mutations, mice that harbor familial HtrA2 mutations have a PD phenotype that is similar to HtrA2 knock-out mice.[79,80] Curiously, the PINK1 phosphorylation site is adjacent to the familial mutations, indicating that perhaps distinct mutations in the protease domain cause different alterations in protease activity that affect separate groups of effector proteins. However, despite this promising evidence for a genetic role of HtrA2 in PD, caution has to be exercised in light of recent data that point to significant frequencies of the HtrA2 mutations in control populations.[70] Hence, further research requires sorting out these contrasting results to better assess the role of HtrA2 in PD. Perhaps some mutations will turn out to reflect benign polymorphisms, whereas other mutations in HtrA2 may be disease promoting. By extension, on a molecular level, some reductions in the proteolytic function of HtrA2 may be compensated for, whereas others may be pathological. Moreover, it appears that PINK1 is topologically organized in a way that it can phosphorylate only physiological PINK1 substrates that are outside the mitochondria in the cytosol; thus, HtrA2 and TRAP may not be regulated by PINK1 phosphorylation *in vivo*.[81]

Aside from PINK1 and HtrA2, another mitochondrial protein of great interest is DJ-1. Some evidence suggests that DJ-1 functions as an atypical peroxiredoxin-like peroxidase that scavenges mitochondrial peroxide (H_2O_2) through oxidation of its Cysteine 106.[82] Moreover, and consistent with DJ-1 being involved in regulating oxidative stress, DJ-1 was found to stabilize the antioxidant master regulator Nrf2. DJ-1 prevents Nrf2 binding to Keap1, which would otherwise promote Nrf2 degradation by the UPS.[83] Hence, DJ-1 aids in maintaining Nrf2 protein levels, which

secures the efficient transcription of antioxidative proteins to preserve an adequate stress response. The exact molecular mechanism by which DJ-1 exerts its effect on Nrf2 is not yet known, because physical interactions with the involved proteins were not observed. Nonetheless, just like with PINK1 and HtrA2, DJ-1 appears to demonstrate a property to mount a response to oxidative stress, which may also arise from the mitochondria.[84] In contrast with these two proteins, however, the protective response of DJ-1 appears to be targeted to a separate component of the oxidative stress. It is possible that, in this way, all three proteins coordinate their functions to protect the cell from oxidative damage.

A separate line of investigation suggests that DJ-1 is involved in dopamine signaling. Based on knock-out studies in mice, it was proposed that DJ-1 may regulate dynamic dopaminergic transmission in the nigrostriatal neuronal network, with particular affinity to the D2 receptor system.[85] In line with that function, DJ-1 has been found to promote the transcription of tyrosine hydroxylase. Precisely, DJ-1 inhibits the sumoylation of the transcriptional corepressor protein associated splicing factor (PSF), which prevents sumoylation-dependent recruitment of histone deacetylase 1 (HDAC-1) to the tyrosine hydroxylase promoter complex.[86] This mechanism implies that DJ-1 acts as a transcriptional coactivator by inhibiting PSF-mediated transcriptional repression by histone deacetylases. Given that DJ-1 physically interacts with PSF, it further implies that DJ-1 localization extends to the cell nucleus. It is plausible that such action of DJ-1 may explain, at least in part, the phenotype observed in the knock-out mice.[85]

Yet another avenue of research has placed DJ-1 directly into the cell death pathway. DJ-1 can interact with the protein Daxx in the nucleus to prevent Daxx from entering the cytoplasm, where it otherwise would activate apotosis-regulating signal kinase (ASK)-dependent apoptotic cell signaling.[87] In that respect, cell stressors that promote Daxx-mediated neurodegeneration are counteracted. Interestingly, given the multitude of functions that have been associated with DJ-1,[88] drug development for promoting DJ-1 function in PD patients may appear a challenging undertaking, because the spectrum of unwanted side effects may be significant.

Sporadic Parkinson's Disease

A prominent type of defect seen in sporadic PD is mitochondrial dysfunction.[89,90] Important evidence supporting this dysfunction is derived from the observation that inhibitors of complex I of the mitochondrial electron chain transport system can cause sporadic PD.[91] Moreover, mutations in the mitochondrial DNA polymerase gamma (POLG) is associated with sporadic PD and certain forms of hereditary parkinsonism illustrating the central role of mitochondria in the pathogenesis of PD.[92–95] Neuronal dysfunction caused by complex I inhibitors is complex and involves activation of most, if not all, cell death pathways, because an inadequate electron chain transport system produces significant mitochondrial dysfunction. The cell death pathways include caspase-dependent and -independent signaling, necrotic mechanisms, and neuronal injury that results from glial cell–induced inflammation.[96] Perhaps somewhat surprising, the neurodegeneration caused by some of these agents is reasonably selective to dopamine neurons, which explains the prominent PD symptoms in affected patients.

The pesticide rotenone and MPTP are two prominent examples of complex I inhibitors.[97] The discovery of MPTP, in particular, provided a good model for PD in patients, animals, and cell culture systems alike.[98] In addition, MPTP models have been used effectively to develop and test novel drugs for PD. In this way, several crucial candidates for neuroprotection were identified, which are currently being assessed in drug trials with PD patients. MPTP must be converted to 1-methyl-4-phenyl-pyridium (MPP+) by monoamine oxide B (MAO-B) to produce dopaminergic neurodegeneration.[99] Accordingly, inhibition of MAO-B has been a long-standing target point for drug development. Selegiline and rasagiline are such MAO-B inhibitors that are being rigorously tested for their neuroprotective effects in PD patients. Some findings indicate that their potential for neuroprotection may relate to inhibition of GAPDH translocation.[100] Preliminary data appear promising, although interpretation of the data is confounded by the observation that these drugs also provide symptomatic relief.[101] As such, an isolated neuroprotective effect for the MAO inhibitors has not been demonstrated to date. Other compounds that have shown protection against MPTP neurotoxicity are coenzyme Q10 and creatine.[102,103] Coenzyme Q10 is proposed to maintain the integrity of the mitochondrial electron transport system as part of its mechanism of action, whereas the exact mechanism for creatine is not yet fully understood. These two agents are also thought to preserve mitochondrial function and are currently in various stages of investigation as potential neuroprotective agents in PD. Initial reports have failed to demonstrate a clear neuroprotective or disease-slowing effect in PD patients.

Another contributor to sporadic PD is oxidative stress. Superoxide anion, nitric oxide, and hydroxyl radicals can inflict neuronal damage in multiple ways, which includes damaging mitochondria that subsequently activates cell death pathways. Notably, additional compromises of the cell defense systems against oxidative stress, such as mitochondrial complex I inhibition or even aging, may provide increased suscepti-

bility to neurodegeneration. This may begin to explain the onset of sporadic PD in the middle age and the progressive nature of the disease. Inflammation also appears to play a role in PD,[104] and α-synuclein that is oxidatively damaged through nitration of its tyrosine residues can bypass immunological tolerance, setting in motion adaptive immune responses that exacerbate PD.[105] Prudently then, assessing medication that aims at curbing oxidative stress, inflammation, and modulating immune function bears some prospect of neuroprotective potential. However, this theoretically promising approach has not yet yielded significant results in animal models or drug development.

Dopamine agonists are an important class of drugs that are being tested for their neuroprotective quality, because their hydroxylated benzyl ring structure confers antioxidative properties.[106,107] Moreover, they appear to reduce dopamine turnover and hence free radical generation through their action on presynaptic autoreceptors. Consistent with their suggested antioxidant function, dopamine agonists protect against 6-hydroxydopamine, MPP+, or systemic administration of MPTP, although the exact mechanism of their action in these cellular or animal models is not yet understood.[108] Nonetheless, two clinical trials with dopamine agonists have demonstrated a delay in the progression of imaging markers that are thought to reflect nigral striatal cell density. The trial findings are encouraging because they indicate the possibility of neuroprotection, although a clear relation between imaging markers and clinical outcome has not been established. Hence, a careful clinical assessment of the PD patients is needed to justifiably correlate delayed progression of imaging markers with delayed progression of PD. To date, such a correlation has not been shown convincingly in any trial.

What has always been poorly understood is why only certain neuronal cell populations are affected in PD. This seminal matter of selective neuronal vulnerability has recently received some unusual insight.[109] Dopaminergic neurons in the substantia nigra of the mouse possess pacemaker activity, which is present since birth and thought to orchestrate voluntary motor control.[110] A few weeks after birth, these neurons replace their sodium ion channels with 1.3 L-type calcium channels to maintain pacemaker activity. Interestingly, application of calcium antagonists to chemical PD models (MPTP, rotenone, 6-hydroxydopamine) demonstrated survival of dopaminergic substantia nigra neurons, which was associated with a reversal to sodium-channel–regulated pacemaker activity.[111] This observation suggests that the selective vulnerability in PD may be related to unusual calcium ion-channel activity in the affected neuronal population, with increased calcium influx into the cell triggering neuronal dysfunction and cell death. Spared neurons in PD, in turn, may not have similar plasma membrane permeability for calcium, which allows escape from neuronal dysfunction even in the presence of the same cell stressors that dopaminergic neurons face. Alternatively, as seen in the ventral tegmental area neurons that are not affected in PD but have abundant L-type calcium channels, effective calcium sequestration by calcium-binding proteins may provide sufficient resistance to calcium-induced cell stress. Indeed, the calcium-binding protein calbindin is expressed at much greater levels in the ventral tegmental area neurons than in dopaminergic substantia nigra neurons.

Consistent with the notion that the mouse data may be transferable to PD patients, a retrospective epidemiological analysis demonstrated that patients taking the dihydropyridine class of calcium antagonists have a reduced incidence of PD.[112] Although prospective trials with PD patients are needed to confirm these results, calcium antagonists have been moved to the forefront of new neuroprotective PD drugs. The calcium antagonists used in the mouse models and epidemiological analysis were preferentially targeting L-type calcium ion channels, but interestingly enough had a greater affinity for the 1.1 to 1.2 than the 1.3 L-type channel. Hence, there appears to be still room for improved efficacy, which has sparked great interest to develop specific 1.3 L-type calcium channel inhibitors to maximize the benefit-to-side-effect ratio. However, improving the drug selectivity for 1.3 L-type calcium channels may not be as straightforward as anticipated, given that knock-out mice for the 1.3 L-type calcium channel present with deafness.[113] It will be a challenge to design drugs where increased selectivity does not produce a serious side effect, whereas decreasing the side effect profile overall.

A perhaps less expected mechanism of how sporadic PD may come about involves histone deacetylation by the sirtuin protein family (SIRTs). SIRT2 has received the most attention. Its activity appears to be neurotoxic in PD, as inhibition of SIRT2 by the compound AGK2 diminished α-synuclein–mediated toxicity in primary midbrain cultures, as well as in a *Drosophila* model of PD.[114] Curiously, SIRT2 inhibition also results in larger α-synuclein aggregate size in cell culture,[115] pointing to the possibility that polymeric α-synuclein may be neuroprotective and oligomeric α-synuclein the primary neurotoxic form. The mechanism by which inhibition of SIRT2 may confer neuroprotection is poorly understood, but promoting the formation of enlarged inclusion bodies may be one mechanism.[116] SIRT2 deacetylates α-tubulin and histones.[117] Because histone deacetylation is required for autophagy,[118] SIRT2 may be neurotoxic by virtue of regulating protein clearance, including α-synuclein protein turnover and aggregate size. Because autophagy involves lysosomal recruitment to aggregated cellular proteins that are associated with the microtubule orga-

nizing center, inhibition of SIRT2-mediated deacetylation of histones and α-tubulin may play a role in neuroprotection.

Aside from SIRT2, the sirtuin family member SIRT1 has also been investigated in PD. Interestingly, in contrast with SIRT2, increased activity of SIRT1 showed a delay in α-synuclein toxicity,[119] indicating that different sirtuin proteins may have complex and at times opposing effects on neurodegeneration in PD.[120] Although SIRT2 may promote α-synuclein to exist in the oligomeric form, it is currently being investigated whether SIRT1 may dissemble oligomeric α-synuclein into monomers. These findings emphasize that although drug targeting of the sirtuins may warrant important therapeutic consideration in PD, much attention has to be paid to selective drug targeting and type of regulation, given the complex interactions of histone deacetylases during neurodegeneration.

Based on the research findings, considerable intersection between sporadic and familial PD can be appreciated. It may be argued that a commonality is particularly seen with respect to mitochondrial dysfunction. As outlined previously, several proteins mutated in familial PD, including PINK1, HtrA2, and DJ-1, play a role in dampening oxidative stress by upholding an appropriate mitochondrial stress response. Thus, what connects sporadic and familial PD is the observation that increased production of reactive oxygen species, decreased stress responses, and complex I inhibition all cause dysfunction of the mitochondrial electron chain transport system; when severe enough, such mitochondrial dysfunction reaches a threshold that activates the various cell death pathways. It is not surprising then that inhibition of excitotoxicity from oxygen radicals also protects against MPTP neurotoxicity. In fact, loss of parkin function has been implicated in neuronal death in the MPTP model.[67] Furthermore, the detrimental *in vivo* role of BAG5 was tested in this mouse model, where the degeneration of the substantia nigra in response to MPTP was markedly increased when BAG5 was expressed selectively in that area. In support of the critical role of mitochondrial integrity in sporadic and familial PD is also the finding that calcium antagonists may be neuroprotective in PD patients. Because increased intracellular calcium may trigger neurodegeneration, a link to mitochondrial function is not unlikely, given that mitochondria are effective buffers of cellular calcium.[121] Furthermore, increased intracellular calcium can induce oxidative stress, which likely mounts mitochondrial stress responses that likely involve the proteins PINK1, HtrA2, and DJ-1. Altogether, these findings are yet another example where pathogenetic insight into sporadic and familial PD can be cross-fertilizing in many aspects. It may not come as a surprise when novel drugs, derived independently from research in sporadic and familial PD, will act synergistically or additive with respect to restoring mitochondrial function in either category of PD patients.

Conclusions

As we enter the 21st century, it is expected that we will identify and have substantial proof of neuroprotective therapies for PD. It is yet to be determined whether the MPTP or related toxin models will be predictive for neuroprotective therapies for PD because no proven therapy for PD has yet arisen from these models. The most promising neuroprotective therapies will probably emerge from discoveries and testing of hypotheses that are based on insights from the genetic causes of PD. Indeed, since the dawn of the 21st century, enormous advances have been made in identifying how the proteins affected in familial PD are incorporated into signaling pathways to exert their cellular functions. However, it remains to be determined whether these pathways have common nodal points that are compromised in all forms of sporadic and genetic PD, or whether these pathways represent distinct entities that are compromised individually in the various forms of idiopathic PD. Perhaps agents that are protective in both the genetic and MPTP models might yield the greatest therapeutic reward. Based on the extent of progress that has been made over the last few years alone, the continuous pursuit of mechanism-based studies will undoubtedly make the day come when we will be able to offer patients neuroprotective treatment options.

References

1. Gasser T. Update on the genetics of Parkinson's disease. Mov Disord 2007;22(suppl 17):S343–S350.
2. Hardy J, Cui H, Cookson MR, et al. Genetics of Parkinson's disease and parkinsonism. 2006;60:389–398.
3. Goedert M. Alpha-synuclein and neurodegenerative diseases. Nat Rev Neurosci 2001;2:492–501.
4. Lee VM, Trojanowski JQ. Mechanisms of Parkinson's disease linked to pathological alpha-synuclein: new targets for drug discovery. Neuron 2006;52:33–38.
5. Cookson MR, van der Brug M. Cell systems and the toxic mechanism(s) of alpha-synuclein. Exp Neurol 2008;209:5–11.
6. Sapru MK, Yates JW, Hogan S, et al. Silencing of human alpha-synuclein in vitro and in rat brain using lentiviral-mediated RNAi. Exp Neurol 2006;198:382–390.
7. Fountaine TM, Wade-Martins R. RNA interference-mediated knockdown of alpha-synuclein protects human dopaminergic neuroblastoma cells from MPP(+) toxicity and reduces dopamine transport. J Neurosci Res 2007;85:351–363.
8. Singleton A, Gwinn-Hardy K. Parkinson's disease and dementia with Lewy bodies: a difference in dose? Lancet 2004;364: 1105–1107.
9. Fuchs J, Tichopad A, Golub Y, et al. Genetic variability in the SNCA gene influences alpha-synuclein levels in the blood and brain. Faseb J 2008;22:1327–1334.
10. Winkler S, Hagenah J, Lincoln S, et al. alpha-Synuclein and Parkinson disease susceptibility. Neurology 2007;69: 1745–1750.

11. Dauer W, Kholodilov N, Vila M, et al. Resistance of alpha-synuclein null mice to the parkinsonian neurotoxin MPTP. Proc Natl Acad Sci U S A 2002;99:14524–14529.
12. Amer DA, Irvine GB, El-Agnaf OM. Inhibitors of alpha-synuclein oligomerization and toxicity: a future therapeutic strategy for Parkinson's disease and related disorders. Exp Brain Res 2006;173:223–233.
13. Hashimoto M, Rockenstein E, Mante M, et al. An antiaggregation gene therapy strategy for Lewy body disease utilizing beta-synuclein lentivirus in a transgenic model. Gene Ther 2004;11:1713–1723.
14. Masliah E, Rockenstein E, Adame A, et al. Effects of alpha-synuclein immunization in a mouse model of Parkinson's disease. Neuron 2005;46:857–868.
15. Kapurniotu A. Targeting alpha-synuclein in Parkinson's disease. Chem Biol 2004;11:1476–1478.
16. Li J, Zhu M, Rajamani S, et al. Rifampicin inhibits alpha-synuclein fibrillation and disaggregates fibrils. Chem Biol 2004;11:1513–1521.
17. Ono K, Hirohata M, Yamada M. Anti-fibrillogenic and fibril-destabilizing activities of anti-Parkinsonian agents for alpha-synuclein fibrils in vitro. J Neurosci Res 2007;85:1547–1557.
18. Xu J, Wei C, Xu C, et al. Rifampicin protects PC12 cells against MPP+-induced apoptosis and inhibits the expression of an alpha-synuclein multimer. Brain Res 2007;1139: 220–225.
19. Li W, West N, Colla E, et al. Aggregation promoting C-terminal truncation of alpha-synuclein is a normal cellular process and is enhanced by the familial Parkinson's disease-linked mutations. Proc Natl Acad Sci U S A 2005;102: 2162–2167.
20. Sherman MY, Goldberg AL. Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. Neuron 2001;29:15–32.
21. Giasson BI, Lee VM. Are ubiquitination pathways central to Parkinson's disease? Cell 2003;114:1–8.
22. Kitada T, Asakawa S, Hattori N, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 1998;392:605–608.
23. McNaught KS, Olanow CW, Halliwell B, et al. Failure of the ubiquitin-proteasome system in Parkinson's disease. Nat Rev Neurosci 2001;2:589–594.
24. Ramirez A, Heimbach A, Grundemann J, et al. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. Nat Genet 2006;38:1184–1191.
25. Di Fonzo A, Chien HF, Socal M, et al. ATP13A2 missense mutations in juvenile parkinsonism and young onset Parkinson disease. Neurology 2007;68:1557–1562.
26. Ning YP, Kanai K, Tomiyama H, et al. PARK9-linked parkinsonism in eastern Asia: mutation detection in ATP13A2 and clinical phenotype. Neurology 2008;70:1491–1493.
27. Cuervo AM, Stefanis L, Fredenburg R, et al. Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. Science 2004;305:1292–1295.
28. Martinez-Vicente M, Cuervo AM. Autophagy and neurodegeneration: when the cleaning crew goes on strike. Lancet Neurol 2007;6:352–361.
29. Lee HJ, Khoshaghideh F, Patel S, Lee SJ. Clearance of alpha-synuclein oligomeric intermediates via the lysosomal degradation pathway. J Neurosci 2004;24:1888–1896.
30. Abeliovich A, Schmitz Y, Farinas I, et al. Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. Neuron 2000;25:239–252.
31. Ihara M, Yamasaki N, Hagiwara A, et al. Sept4, a component of presynaptic scaffold and Lewy bodies, is required for the suppression of alpha-synuclein neurotoxicity. Neuron 2007; 53:519–533.
32. Ihara M, Tomimoto H, Kitayama H, et al. Association of the cytoskeletal GTP-binding protein Sept4/H5 with cytoplasmic inclusions found in Parkinson's disease and other synucleinopathies. J Biol Chem 2003;278:24095–24102.
33. Furukawa K, Matsuzaki-Kobayashi M, Hasegawa T, et al. Plasma membrane ion permeability induced by mutant alpha-synuclein contributes to the degeneration of neural cells. J Neurochem 2006;97:1071–1077.
34. Tsigelny IF, Bar-On P, Sharikov Y, et al. Dynamics of alpha-synuclein aggregation and inhibition of pore-like oligomer development by beta-synuclein. FEBS J 2007;274:1862–1877.
35. Cooper AA, Gitler AD, Cashikar A, et al. Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. Science 2006;313:324–328.
36. Chandra S, Gallardo G, Fernandez-Chacon R, et al. Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. Cell 2005;123:383–396.
37. Biskup S, Moore DJ, Celsi F, et al. Localization of LRRK2 to membranous and vesicular structures in mammalian brain. Ann Neurol 2006;60:557–569.
38. West AB, Moore DJ, Biskup S, et al. Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. Proc Natl Acad Sci U S A 2005;102: 16842–16847.
39. West AB, Moore DJ, Choi C, et al. Parkinson's disease-associated mutations in LRRK2 link enhanced GTP-binding and kinase activities to neuronal toxicity. Hum Mol Genet 2007;16:223–232.
40. Gloeckner CJ, Kinkl N, Schumacher A, et al. The Parkinson disease causing LRRK2 mutation I2020T is associated with increased kinase activity. Hum Mol Genet 2006;15:223–232.
41. Greggio E, Jain S, Kingsbury A, et al. Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. Neurobiol Dis 2006;23:329–341.
42. Smith WW, Pei Z, Jiang H, et al. Kinase activity of mutant LRRK2 mediates neuronal toxicity. Nat Neurosci 2006;9: 1231–1233.
43. MacLeod D, Dowman J, Hammond R, et al. The familial Parkinsonism gene LRRK2 regulates neurite process morphology. Neuron 2006;52:587–593.
44. Jaleel M, Nichols RJ, Deak M, et al. LRRK2 phosphorylates moesin at threonine-558: characterization of how Parkinson's disease mutants affect kinase activity. Biochem J 2007;405: 307–317.
45. Farrer MJ. Lrrk2 in the limelight! Neurology 2007;69: 1732–1733.
46. Ito G, Okai T, Fujino G, et al. GTP binding is essential to the protein kinase activity of LRRK2, a causative gene product for familial Parkinson's disease. Biochemistry 2007;46: 1380–1388.
47. Deng J, Lewis PA, Greggio E, et al. Structure of the ROC domain from the Parkinson's disease-associated leucine-rich repeat kinase 2 reveals a dimeric GTPase. Proc Natl Acad Sci U S A 2008;105:1499–1504.
48. Lewis PA, Greggio E, Beilina A, et al. The R1441C mutation of LRRK2 disrupts GTP hydrolysis. Biochem Biophys Res Commun 2007;357:668–671.
49. Li X, Tan YC, Poulose S, et al. Leucine-rich repeat kinase 2 (LRRK2)/PARK8 possesses GTPase activity that is altered in familial Parkinson's disease R1441C/G mutants. J Neurochem 2007;103:238–247.

50. Reiter A, Walz C, Cross NC. Tyrosine kinases as therapeutic targets in BCR-ABL negative chronic myeloproliferative disorders. Curr Drug Targets 2007;8:205–216.
51. Wang L, Xie C, Greggio E, et al. The chaperone activity of heat shock protein 90 is critical for maintaining the stability of leucine-rich repeat kinase 2. J Neurosci 2008;28:3384–3391.
52. Nichols WC, Elsaesser VE, Pankratz N, et al. LRRK2 mutation analysis in Parkinson disease families with evidence of linkage to PARK8. Neurology 2007;69:1737–1744.
53. Smith WW, Pei Z, Jiang H, et al. Leucine-rich repeat kinase 2 (LRRK2) interacts with parkin, and mutant LRRK2 induces neuronal degeneration. Proc Natl Acad Sci U S A 2005;102: 18676–18681.
54. Imai Y, Soda M, Takahashi R. Parkin suppresses unfolded protein stress-induced cell death through its E3 ubiquitin-protein ligase activity. J Biol Chem 2000;275:35661–35664.
55. Shimura H, Hattori N, Kubo S, et al. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet 2000;25:302–305.
56. Zhang Y, Gao J, Chung KK, et al. Parkin functions as an E2-dependent ubiquitin- protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1. Proc Natl Acad Sci U S A 2000;97:13354–13359.
57. von Coelln R, Dawson VL, Dawson TM. Parkin-associated Parkinson's disease. Cell Tissue Res 2004;318:175–184.
58. Chung KK, Zhang Y, Lim KL, et al. Parkin ubiquitinates the alpha-synuclein-interacting protein, synphilin-1: implications for Lewy-body formation in Parkinson disease. Nat Med 2001;7:1144–1150.
59. Engelender S, Kaminsky Z, Guo X, et al. Synphilin-1 associates with alpha-synuclein and promotes the formation of cytosolic inclusions. Nat Genet 1999;22:110–114.
60. von Coelln R, Thomas B, Andrabi SA, et al. Inclusion body formation and neurodegeneration are parkin independent in a mouse model of alpha-synucleinopathy. J Neurosci 2006;26: 3685–3696.
61. Fallon L, Belanger CM, Corera AT, et al. A regulated interaction with the UIM protein Eps15 implicates parkin in EGF receptor trafficking and PI(3)K-Akt signalling. Nat Cell Biol 2006;8:834–842.
62. Ko HS, Kim SW, Sriram SR, et al. Identification of far upstream element-binding protein-1 as an authentic Parkin substrate. J Biol Chem 2006;281:16193–16196.
63. Ko HS, von Coelln R, Sriram SR, et al. Accumulation of the authentic parkin substrate aminoacyl-tRNA synthetase cofactor, p38/JTV-1, leads to catecholaminergic cell death. J Neurosci 2005;25:7968–7978.
64. Imai Y, Soda M, Hatakeyama S, et al. CHIP is associated with Parkin, a gene responsible for familial Parkinson's disease, and enhances its ubiquitin ligase activity. Mol Cell 2002;10: 55–67.
65. Auluck PK, Chan HY, Trojanowski JQ, et al. Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. Science 2002;295:865–868.
66. Auluck PK, Bonini NM. Pharmacological prevention of Parkinson disease in Drosophila. Nat Med 2002;8:1185–1186.
67. Kalia SK, Lee S, Smith PD, et al. BAG5 inhibits parkin and enhances dopaminergic neuron degeneration. Neuron 2004; 44:931–945.
68. Chung KK, Thomas B, Li X, et al. S-nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function. Science 2004;304:1328–1331.
69. Yao D, Gu Z, Nakamura T, et al. Nitrosative stress linked to sporadic Parkinson's disease: S-nitrosylation of parkin regulates its E3 ubiquitin ligase activity. Proc Natl Acad Sci U S A 2004;101:10810–10814.
70. Valente EM, Abou-Sleiman PM, Caputo V, et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 2004;304:1158–1160.
71. Clark IE, Dodson MW, Jiang C, et al. Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. Nature 2006;441:1162–1166.
72. Park J, Lee SB, Lee S, et al. Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. Nature 2006;441:1157–1161.
73. Exner N, Treske B, Paquet D, et al. Loss-of-function of human PINK1 results in mitochondrial pathology and can be rescued by parkin. J Neurosci 2007;27:12413–12418.
74. Tan JM, Dawson TM. Parkin blushed by PINK1. Neuron 2006;50:527–529.
75. Pridgeon JW, Olzmann JA, Chin LS, Li L. PINK1 Protects against oxidative stress by phosphorylating mitochondrial chaperone TRAP1. PLoS Biol 2007;5:e172.
76. Plun-Favreau H, Klupsch K, Moisoi N, et al. The mitochondrial protease HtrA2 is regulated by Parkinson's disease-associated kinase PINK1. Nat Cell Biol 2007;9:1243–1252.
77. Alnemri ES. HtrA2 and Parkinson's disease: think PINK? Nat Cell Biol 2007;9:1227–1229.
78. Strauss KM, Martins LM, Plun-Favreau H, et al. Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. Hum Mol Genet 2005;14:2099–2111.
79. Jones JM, Datta P, Srinivasula SM, et al. Loss of Omi mitochondrial protease activity causes the neuromuscular disorder of mnd2 mutant mice. Nature 2003;425:721–727.
80. Martins LM, Morrison A, Klupsch K, et al. Neuroprotective role of the Reaper-related serine protease HtrA2/Omi revealed by targeted deletion in mice. Mol Cell Biol 2004;24: 9848–9862.
81. Zhou C, Huang Y, Shao Y, et al. The kinase domain of mitochondrial PINK1 faces the cytoplasm. Proc Natl Acad Sci U S A 2008;105:12022–12027.
82. Andres-Mateos E, Perier C, Zhang L, et al. DJ-1 gene deletion reveals that DJ-1 is an atypical peroxiredoxin-like peroxidase. Proc Natl Acad Sci U S A 2007;104:14807–14812.
83. Clements CM, McNally RS, Conti BJ, et al. DJ-1, a cancer- and Parkinson's disease-associated protein, stabilizes the antioxidant transcriptional master regulator Nrf2. Proc Natl Acad Sci U S A 2006;103:15091–15096.
84. Dodson MW, Guo M. Pink1, Parkin, DJ-1 and mitochondrial dysfunction in Parkinson's disease. Curr Opin Neurobiol 2007;17:331–337.
85. Goldberg MS, Pisani A, Haburcak M, et al. Nigrostriatal dopaminergic deficits and hypokinesia caused by inactivation of the familial Parkinsonism-linked gene DJ-1. Neuron 2005;45: 489–496.
86. Zhong N, Kim CY, Rizzu P, et al. DJ-1 transcriptionally upregulates the human tyrosine hydroxylase by inhibiting the sumoylation of pyrimidine tract-binding protein-associated splicing factor. J Biol Chem 2006;281:20940–20948.
87. Junn E, Taniguchi H, Jeong BS, et al. Interaction of DJ-1 with Daxx inhibits apoptosis signal-regulating kinase 1 activity and cell death. Proc Natl Acad Sci U S A 2005;102: 9691–9696.
88. Jin J, Li GJ, Davis J, et al. Identification of novel proteins associated with both alpha-synuclein and DJ-1. Mol Cell Proteomics 2007;6:845–859.
89. Schapira AH. Mitochondrial dysfunction in Parkinson's disease. Cell Death Differ 2007;14:1261–1266.
90. Thomas B, Beal MF. Parkinson's disease. Hum Mol Genet 2007;16(spec no 2):R183–R194.
91. Dawson TM, Dawson VL. Molecular pathways of neurodegeneration in Parkinson's disease. Science 2003;302:819–822.

92. Davidzon G, Greene P, Mancuso M, et al. Early-onset familial parkinsonism due to POLG mutations. Ann Neurol 2006;59: 859–862.
93. Hudson G, Schaefer AM, Taylor RW, et al. Mutation of the linker region of the polymerase gamma-1 (POLG1) gene associated with progressive external ophthalmoplegia and Parkinsonism. Arch Neurol 2007;64:553–557.
94. Luoma PT, Eerola J, Ahola S, et al. Mitochondrial DNA polymerase gamma variants in idiopathic sporadic Parkinson disease. Neurology 2007;69:1152–1159.
95. Pagnamenta AT, Taanman JW, Wilson CJ, et al. Dominant inheritance of premature ovarian failure associated with mutant mitochondrial DNA polymerase gamma. Hum Reprod 2006;21:2467–2473.
96. Dawson TM, Dawson VL. Neuroprotective and neurorestorative strategies for Parkinson's disease. Nat Neurosci 2002; 5(suppl):1058–1061.
97. Jenner P. Parkinson's disease, pesticides and mitochondrial dysfunction. Trends Neurosci 2001;24:245–247.
98. Betarbet R, Sherer TB, MacKenzie G, et al. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci 2000;3:1301–1306.
99. Vila M, Przedborski S. Targeting programmed cell death in neurodegenerative diseases. Nat Rev Neurosci 2003;4: 365–375.
100. Hara MR, Thomas B, Cascio MB, et al. Neuroprotection by pharmacologic blockade of the GAPDH death cascade. Proc Natl Acad Sci U S A 2006;103:3887–3889.
101. Schapira AH. Future directions in the treatment of Parkinson's disease. Mov Disord 2007;22:S385–S391.
102. Beal MF. Coenzyme Q10 as a possible treatment for neurodegenerative diseases. Free Radic Res 2002;36:455–460.
103. Matthews RT, Ferrante RJ, Klivenyi P, et al. Creatine and cyclocreatine attenuate MPTP neurotoxicity. Exp Neurol 1999;157:142–149.
104. McGeer PL, McGeer EG. Glial reactions in Parkinson's disease. Mov Disord 2008;23:474–483.
105. Benner EJ, Banerjee R, Reynolds AD, et al. Nitrated alpha-synuclein immunity accelerates degeneration of nigral dopaminergic neurons. PLoS ONE 2008;3:e1376.
106. Schapira AH. Neuroprotection and dopamine agonists. Neurology 2002;58:S9–S18.
107. Bonuccelli U, Pavese N. Role of dopamine agonists in Parkinson's disease: an update. Expert Rev Neurother 2007;7: 1391–1399.
108. Scheller D, Chan P, Li Q, et al. Rotigotine treatment partially protects from MPTP toxicity in a progressive macaque model of Parkinson's disease. Exp Neurol 2007;203:415–422.
109. Sulzer D, Schmitz Y. Parkinson's disease: return of an old prime suspect. Neuron 2007;55:8–10.
110. Grace AA, Onn SP. Morphology and electrophysiological properties of immunocytochemically identified rat dopamine neurons recorded in vitro. J Neurosci 1989;9:3463–3481.
111. Chan CS, Guzman JN, Ilijic E, et al. 'Rejuvenation' protects neurons in mouse models of Parkinson's disease. Nature 2007; 447:1081–1086.
112. Surmeier DJ. Calcium, ageing, and neuronal vulnerability in Parkinson's disease. Lancet Neurol 2007;6:933–938.
113. Striessnig J, Koschak A, Sinnegger-Brauns MJ, et al. Role of voltage-gated L-type Ca2+ channel isoforms for brain function. Biochem Soc Trans 2006;34:903–909.
114. Outeiro TF, Kontopoulos E, Altmann SM, et al. Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of Parkinson's disease. Science 2007;317:516–519.
115. Bodner RA, Outeiro TF, Altmann S, et al. Pharmacological promotion of inclusion formation: a therapeutic approach for Huntington's and Parkinson's diseases. Proc Natl Acad Sci U S A 2006;103:4246–4251.
116. Garske AL, Smith BC, Denu JM. Linking SIRT2 to Parkinson's disease. ACS Chem Biol 2007;2:529–532.
117. Inoue T, Hiratsuka M, Osaki M, Oshimura M. The molecular biology of mammalian SIRT proteins: SIRT2 in cell cycle regulation. Cell Cycle 2007;6:1011–1018.
118. Iwata A, Riley BE, Johnston JA, Kopito RR. HDAC6 and microtubules are required for autophagic degradation of aggregated huntingtin. J Biol Chem 2005;280:40282–40292.
119. Okawara M, Katsuki H, Kurimoto E, et al. Resveratrol protects dopaminergic neurons in midbrain slice culture from multiple insults. Biochem Pharmacol 2007;73:550–560.
120. Dillin A, Kelly JW. Medicine. The yin-yang of sirtuins. Science 2007;317:461–462.
121. Beal MF. Excitotoxicity and nitric oxide in Parkinson's disease pathogenesis. Ann Neurol 1998;44:S110–S114.