Published in final edited form as:
Apoptosis. 2009 April ; 14(4): 478–500. doi:10.1007/s10495-008-0309-3.

Cell death pathways in Parkinson's disease: proximal triggers, distal effectors, and final steps

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Abstract

Parkinson's disease (PD) is a common neurodegenerative disease. Neuronal cell death in PD is still poorly understood, despite a wealth of potential pathogenic mechanisms and pathways. Defects in several cellular systems have been implicated as early triggers that start cells down the road towards neuronal death. These include abnormal protein accumulation, particularly of alpha-synuclein; altered protein degradation via multiple pathways; mitochondrial dysfunction; oxidative stress; neuroinflammation; and dysregulated kinase signaling. As dysfunction in these systems mounts, pathways that are more explicitly involved in cell death become recruited. These include JNK signaling, p53 activation, cell cycle re-activation, and signaling through bcl-2 family proteins. Eventually, neurons become overwhelmed and degenerate; however, even the mechanism of final cell death in PD is still unsettled. In this review, we will discuss cell death triggers and effectors that are relevant to PD, highlighting important unresolved issues and implications for the development of neuroprotective therapies.

Keywords

Parkinson's disease; Apoptosis; Neuroprotection; Alpha-synuclein

INTRODUCTION

Parkinson disease (PD) is the second most common neurodegenerative disorder, afflicting approximately 6 million people worldwide (1). The cardinal features of PD include resting tremor, rigidity, bradykinesia and postural instability. These symptoms are a result of the degeneration of the dopaminergic nigrostriatal pathway originating in the substantia nigra pars compacta (SNpc). While SNpc neurons are preferentially lost in PD, they are by no means the only neuronal population affected. Degeneration in other areas—locus ceruleus (where cell loss may exceed that seen in SNpc (2)), dorsal raphe nuclei, nucleus basalis of Meynert, postganglionic sympathetic neurons—likely contributes to many of the non-motor symptoms of PD, such as depression, dementia and autonomic dysfunction (3). Pathologically, in addition to neuronal loss, surviving neurons contain Lewy bodies, which are cytoplasmic, eosinophilic inclusions (1). Current treatments address the dopaminergic deficit, providing symptomatic benefit to many of the motor deficits. However, there are many symptoms that do not respond to dopaminergic therapy. In addition, there are no proven interventions to slow down the underlying degenerative process. The search for such neuroprotective treatments is a critical goal of PD research.

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In order to find neuroprotective strategies, a clear understanding of the mechanism of neuron death in PD is needed. In this article, we review the evidence regarding cell death in PD, organized around different pathogenic mechanisms. These mechanisms can be conceptualized as relatively early, proximal insults vs. late, distal effectors. Early pathways are not directly linked to the execution of cell death. In contrast, distal effectors are intimately involved in the death process, and their recruitment directly regulates the likelihood of cell death. Clearly, some of the mechanisms to be discussed do not fit nearly into this classification. Furthermore, the flow of signaling is not unidirectional; distal pathways can feedback and modulate proximal mechanisms.

For each mechanism, the evidence discussed will generally fall into 3 categories, each with its own strengths and weaknesses. First, analysis of post-mortem material from PD patients has the obvious advantage of relevance to the disease itself. However, most studies have focused on the SNpc, typically in late stage patients with severe loss of SNpc neurons. It is unclear what the relevance of findings in this setting is to earlier stages of disease. Second, the identification of multiple genes associated with familial forms of PD has been a major starting point for research regarding pathogenic mechanisms (Table 1). Given the clear association between these genes and the human disease, cellular and animal models based on these PD genes have enabled testing of pathogenic mechanisms in a more disease-relevant way. However, the degree to which rare monogenic forms of PD are representative of the more common sporadic PD is uncertain. Third, toxin-based models have been used extensively in PD research. These approaches are well defined and recapitulate many features of PD. However, the relationship between the toxin approach and *bona fide* PD is the most tenuous of all 3 approaches.

PROXIMAL EVENTS

Alpha-synuclein dysfunction and Lewy body formation

Alpha-synuclein (α-Syn) has emerged as a central protein in PD pathogenesis. Mutations in the α-Syn gene produce a rare autosomal dominant form of familial PD (4). α-Syn is a primary constituent of Lewy bodies (LBs), the defining pathological feature of all forms of PD (5). The notion that α-Syn accumulation and aggregation are essential to PD pathogenesis, and that inhibition of this process is a promising therapeutic approach, underlies much research in the field. It is important to emphasize, though, that this is an assumption, and we will explore its validity in this section.

While the normal functions of α-Syn are still unresolved, it is clear that α-Syn is closely linked with cellular membranes. In neurons, α-Syn is primarily presynaptic and associates closely with membrane structures such as synaptic vesicles and with lipid rafts (6). In addition, α-Syn binds to and inhibits phospholipase D (7). α-Syn knockout mice show subtle deficits in synaptic vesicle recycling and dopaminergic neurotransmission (8). Genetic screens in model organisms such as yeast and *C. elegans* have found that membrane trafficking components can modulate α-Syn-mediated cell death (9,10).

Several lines of evidence suggest that α-Syn is involved in neurodegenerative disease. In addition to its presence in LBs in PD, α-Syn aggregates are found in several other neurodegenerative diseases, which have been termed synucleinopathies (11). These include multiple system atrophy, dementia with Lewy bodies, and pure autonomic failure. As mentioned previously, mutations in α-Syn, namely A30P and A53T, cause rare forms of autosomal dominant PD (4,12). While the A30P mutation causes disease that resembles typical PD (13), the A53T mutation leads to a more aggressive phenotype with atypical features, such as myoclonus and early dysautonomia (14). In addition, the presence of increased copy numbers of the wild type (wt) α-Syn gene produces PD. Gene triplication causes atypical PD,

similar to A53T (15,16). Gene duplication causes milder symptoms, more typical of classic PD (17). Therefore, increased expression of even wild type α-Syn causes neurodegeneration, and the more severe phenotype resulting from gene triplication suggests a dose-response effect.

Overexpression of α-Syn produces cell death in many experimental systems. In several cell lines and primary neuronal cultures, overexpression of α-Syn causes apoptotic cell death and/or increased sensitivity to toxic insults (for example, refs 18-20). In some studies, only mutant α-Syn is associated with cell death (18), while others show equivalent toxicity from overexpression of wt and mutant α-Syn (20). Catecholaminergic cells are more sensitive to α-Syn overexpression (20). In *Drosophila*, overexpression of α-Syn in all neurons causes age-dependent, selective loss of the dopaminergic dorsomedial group (but not other dopaminergic neurons) with a corresponding loss of locomotor function, which can be rescued with dopaminergic agents (21,22). Wt, A53T and A30P α-Syn caused similar degrees of neuronal loss. Similar results were seen in *C. elegans* (23). Several lines of transgenic mice overexpressing either wild type or mutant α-Syn have been generated. Mice that express α-Syn pan-neuronally exhibit motor deficits, early lethality, and region-specific neuronal cell loss (24-26). The A53T mutation appears to cause the most cell loss in these lines; wt α-Syn overexpression caused neuronal loss in one study (26), but not another (25). Neuronal loss occurs primarily in spinal cord motor neurons, which likely accounts for the motor phenotype. The vulnerability of motor neurons (a population relatively spared in sporadic PD) is likely due to high transgene expression in these cells. Neuronal loss also occurs in lower brainstem nuclei and the neocortex, two regions that are affected in PD (27). Of note, SNpc neurons were not affected, even though there was produce robust expression in these cells. To further address this issue, transgenic mice were generated that overexpress wt or mutant α-Syn specifically in dopaminergic neurons, using the tyrosine hydroxylase (TH) promoter. One line, expressing an engineered double mutant with A30P and A53T, showed moderate age-dependent loss of dopaminergic neurons, with approximately 50% cell loss at 19 months (28,29). Another group found no evidence of SNpc cell loss in mice overexpressing either A30P or A53T (30). In contrast, viral transduction of either wt or mutant α-Syn directly into rat SNpc produces robust loss specifically of dopaminergic neurons (31).

Why is there relatively little pathology in dopaminergic SNpc cells in α-Syn transgenic mice, compared to the robust and selective degeneration of dopaminergic neurons in transgenic flies and worms? One possible explanation is that the presence of endogenous α-Syn might have a protective role. Neither *Drosophila* nor *C. elegans* has endogenous α-Syn. The protective effect of endogenous α-Syn is supported by the finding that overexpression of A53T α-Syn on an α-Syn-null background results in more robust motor neuron death in mice (32). This idea, however, does not explain why lentiviral expression of α-Syn in rats produces SNpc neuronal death. A possible explanation is that transgene expression level is higher in the lentiviral model. Finally, in some experimental systems, overexpression of wt α-Syn is protective (33). This is likely due to cell-type specific effects, and would be important to study further.

Genetic deletion of α-Syn does not support a physiologic role for α-Syn in cell death. There is normal developmental cell death in the SN of α-Syn-null mice (34). Furthermore, α-Syn -/- primary neurons are equally susceptible to cell death triggered by proteasome inhibition or trophic factor deprivation (34,35). One notable exception is the complete resistance of α-Syn-null mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity *in vivo* (36). Cultured midbrain neurons from these mice are resistant to MPP+ (the active metabolite of MPTP) but sensitive to rotenone, another complex I inhibitor. This suggests that α-Syn regulates the access of MPP+ to complex I, rather than a modulation of cell death pathways. Taken together, these data are consistent with the notion that α-Syn mutations and/or overexpression contribute to neuronal cell death via toxic gain of function, rather than loss of a protective action.

Multiple lines of evidence suggest that increased aggregation of α-Syn is critical for its capacity to promote cell death (reviewed in 37). Monomeric α-Syn is readily soluble. Oligomeric species can form under several conditions that are associated with PD and have been isolated from PD brain, confirming their occurrence *in vivo* (38). These oligomers, termed protofibrils, remain soluble. The protofibrils can then become amyloid-like fibrils, which are insoluble. Fibrillar α-Syn can then coalesce into Lewy bodies, along with numerous other associated proteins, such as ubiquitin. In addition, fibrillar α-Syn can form inclusions in neuronal processes, termed Lewy neurites. A great deal of evidence suggests that the soluble oligomeric protofibrils are the major toxic species. Both A30P and A53T α-Syn mutants increase the rate of protofibril formation *in vitro*, and A30P attenuates the conversion of oligomers into fibrils (39). Similarly, covalent modifications of α-Syn seen in PD, such as tyrosine nitration and Ser129 phosphorylation, block conversion of oligomers into fibrils (37). In some α-Syn overexpression models, cell death can be attenuated despite the persistence of insoluble α-Syn inclusions (31,40). Conversely, cell death can occur in the absence of fibrillar α-Syn (26). Other evidence, nevertheless, supports a role for fibrils in cell death. Transgenic mice with the most severe neuronal loss (A53T α-Syn expressed under the prion promoter) show fibrillar α-Syn inclusions (24). In sum, there is convincing evidence that both soluble oligomers and insoluble fibrils have the capacity to cause neuronal death.

While LBs are necessary for the pathological diagnosis of PD, are they necessary for cell death? Neuropathological evidence from monogenic forms of PD suggests the answer is no. Different patients with LRRK2 mutations can have distinct pathologies: typical LBs, tau inclusions or ubiquitin-positive cytoplasmic inclusions (41,42). Patients with parkin mutations have either pure nigral degeneration or typical LBs (43,44). Therefore, nigral degeneration can occur in the absence of LBs. In fact, evidence from *in vitro* experiments suggests that LBs may even be a protective response, aimed at sequestering harmful aggregated α-Syn species. LBs have several features in common with aggresomes, which are expanded centrosomes formed to eliminate misfolded proteins (45). In a non-neural cell line, α-Syn aggregation in aggresomes was correlated with improved cellular survival (46). Finally, a small molecule that enhances the formation of α-Syn inclusions is protective in a cell line overexpressing α-Syn (47).

There are several potential triggers for increased α-Syn aggregation. Simple overexpression of wt α-Syn predisposes towards its accumulation, which may be relevant to the causal relationship between increased α-Syn gene copy number and PD. More subtle alterations in α-Syn expression might also favor oligomerization and/or fibrillation (48,49). Several covalent modifications—Ser129 phosphorylation, C-terminal cleavage, tyrosine nitrosylation—of α-Syn can increase the likelihood of aggregation (37). While such alterations are more common in PD, it is unclear what regulates them, and whether they play causal roles in the disease. In addition, many of the proximal events in PD to be discussed in this review can cause α-Syn accumulation and/or inclusions. These include proteasomal dysfunction, alterations in autophagy, mitochondrial damage, and oxidative stress. Moreover, α-Syn accumulation and/or inclusions appear in turn to reciprocally promote such events. Thus, α-Syn may be involved in a self-amplifying pathological loop that promotes neuron degeneration. In terms of the potential mechanisms by which α-Syn can bring about cellular stress, α-Syn protofibrils can form pore-like structures that permeabilize membranes (50). Consistent with this, α-Syn overexpression can induce an increase in cytosolic catecholamine levels (51).

In summary, several conclusions can be drawn: 1) α-Syn overexpression is sufficient to produce neuronal cell death; 2) α-Syn is not necessary for cell death induced by many toxic insults, with the exception of MPTP; 3) α-Syn oligomers and/or fibrils appear to be involved in producing cell death; 4) Lewy bodies are neither necessary nor sufficient for cell death, and may even represent a protective response. These concepts have implications for the development of therapeutics aimed at α-Syn. In terms of evaluating disease-causing

mechanisms, it is important that, in sporadic PD, the pathogenic cascade almost always produces LBs. Therefore, any proposed etiologic mechanism should account for increased α-Syn accumulation. On the other hand, clearance of LBs *per se* is unlikely to alter cell death, and may even exacerbate neuronal injury if it produces an increase in intermediate forms of α-Syn. In fact, small molecules that enhance inclusion formation appear protective *in vitro* (47). Another approach might be reduction of endogenous α-Syn levels, for instance with siRNA. However, such an approach may be insufficient by itself to slow down disease progression, given that several PD-relevant cell death paradigms are unaffected by genetic deletion of α-Syn. Finally, therapies that reduce oligomers (and possibly fibrils) presently appear to be especially promising, and several experimental inhibitors of α-Syn aggregation already have been identified (52,53).

Ubiquitin-proteasome system (UPS)

Cells have evolved extensive mechanisms to deal with abnormally folded proteins, such as aggregated α-Syn. These mechanisms also regulate cellular levels of many normal proteins. The proteasome is a large multi-protein complex that degrades ubiquitin-tagged proteins. A series of conjugating enzymes, termed E1, E2 and E3 ligases, are responsible for tagging appropriate proteins with ubiquitin. E1 and E2 ligases prime ubiquitin for conjugation, which is then mediated by E3 ligase. There are numerous E3 ligases, each of which recognizes a group of target proteins.

Ample evidence suggests UPS dysfunction in PD. Lewy bodies contain multiple UPS components, such as ubiquitin and proteasomal subunits. In autopsy material, there is a 40% decrease in proteasome activity selectively in the SNpc (54). Proteasome activity also decreases with normal aging, particularly in the SNpc; this may contribute to the selective vulnerability of the SNpc (55).

A major boost to the notion that UPS dysfunction may be causal in PD came with the identification of mutations in the parkin gene in several families with autosomal recessive young-onset disease (56). Parkin is an E3 ligase, and PD-related mutations cause a decrease in ligase activity (57). Parkin has been identified in LBs (58). Nitrosylated forms of parkin are increased in the SNpc from sporadic PD, and this form has reduced ligase activity (59). Numerous parkin substrates have been identified *in vitro*, including Pael-R, synphilin, p38/JTV-1, cyclin E, CDC-rel1, FBP-1 and α-Syn (reviewed in 60). Overexpression of several of these putative substrates, including Pael-R, CDC-rel1 and synphilin, causes death of dopaminergic neurons (60). However, verification of parkin substrates *in vivo* has been difficult. In parkin-null mice, p38/JTV-1 and FBP1 show increased steady state levels only in midbrain/hindbrain, while Pael-R, CDC-rel1, cyclin E, and α-Syn levels are unchanged (61, 62). On the other hand, many of these substrates are increased in the SNpc and/or found in LBs in autopsy material from sporadic PD (63). Taken together, these data suggest that there is functional redundancy in the degradation of parkin substrates, and that, even in sporadic PD, the activity of both parkin and redundant E3 ligases are diminished.

In general, parkin has a positive effect on neuronal survival. Parkin overexpression is neuroprotective in several paradigms, including proteasomal inhibition, overexpression of mutant α-Syn, MPTP treatment and dopamine toxicity (63). Moreover, parkin overexpression protects from neuron death induced by several potential parkin substrates (60). However, parkin deletion does not exacerbate MPTP or α-Syn toxicity *in vivo* (64,65), even though parkin overexpression protects against both of these insults. In contrast, parkin null mice are more sensitive to the toxic effects of the over-expression of either Pael-R or tau (66,67).

Parkin is especially interesting because it appears to lie at the crossroads of several pathways that are relevant for PD pathogenesis. As will be discussed in later sections, parkin may link proteasomal dysfunction with mitochondrial integrity and the ER stress pathway.

The UPS has also been closely linked with α-Syn. Overexpression of either wt or mutant α-Syn in PC12 cells causes inhibition of proteasome activity, likely by direct binding of α-Syn oligomers to the proteasome itself (18,68). In turn, UPS inhibition contributes to α-Syn accumulation and aggregation into cytoplasmic inclusions (35,69).

Further supporting a role for UPS in PD, pharmacologic inhibition of this system recapitulates some features the disease. In cell culture, UPS inhibitors such as lactacystin produce α-Syn-positive inclusions and apoptotic cell death preferentially in dopaminergic cells (69-71). Localized injection of UPS inhibitors into the striatum has a similar effect (72). Apoptosis caused by UPS inhibitors is associated with activation of many of the downstream cell death pathways seen in other toxin models of PD, including JNK, p53, cdk family members, cytochrome c release, and caspase 3 cleavage (ref 73; see sections below for more details). Interestingly, proteasome inhibition can also be protective in some paradigms, such as MPP+ treatment of cultured mesencephalic neurons or 6-hydroxydopamine (6OHDA) treatment of PC12 cells (74,75). These differences may be dependent on the dose of inhibitor, length of inhibition, or the toxic insult itself. Nevertheless, the majority of evidence suggests that UPS dysfunction contributes to neuronal death in PD.

In terms of potential therapeutics, activation of the UPS appears to be desirable in PD. *In vitro*, enhancement of UPS activity reduces markers of cellular senescence (76), suggesting this approach may have promise in aging-related conditions. Small molecule UPS activators are just starting to be developed (77). Nevertheless, given the central role of UPS function in cell function, there is the possibility for significant side effects by global UPS enhancers. Activating specific E3 ligases, such as parkin, may be a more promising approach, as might selective activation of the UPS in neurons affected by PD or selective delivery of parkin.

Autophagy/lysosomal pathway (ALP)

In addition to the UPS, the autophagy/lysosomal pathway (ALP) is an equally important degradation pathway that may play a significant role in neurodegeneration. There are three arms of the autophagy pathway: macroautophagy, chaperone-mediated autophagy (CMA), and microautophagy (reviewed in 78,79). Macroautophagy involves the degradation of large debris, including protein aggregates and organelles such as mitochondria. A large, double-membrane bound vesicle, called an autophagosome, forms around the material to be degraded. Multiple subsequent fusion events with different membrane compartments ensue. Finally, the autophagosome fuses with a lysosome, allowing lysosomal enzymes and the acidic environment to degrade the contents. Several components of the macroautophagy machinery have been identified, including members of the Atg family that play various roles in the generation and development of autophagosomes. CMA involves direct lysosomal targeting and degradation of soluble, cytoplasmic proteins that have a specific target sequence. CMA plays an important role in α-Syn turnover, and possibly its pathogenic effects (80). Microautophagy, which involves direct uptake of nutrients into lysosomes, is relatively poorly understood.

What is the evidence for autophagy/lysosome involvement in neurodegeneration in general, and PD in particular? There are ultrastructural features suggestive of autophagy in the SNpc of PD brains (81,82). In general, both macroautophagy and CMA diminish with aging (79). In mouse models, selective deletion of Atg proteins (either Atg5 or Atg7) in neurons causes neurodegeneration with formation of ubiquitin-positive cytoplasmic aggregates, suggesting that basal ALP is important for neuronal function (83,84). However, the anatomic distribution

of cell loss in these mice is quite different from that seen in PD—the most cell loss occurs in Purkinje cells (which are spared in PD), while the SNpc did not appear to be particularly vulnerable.

Certain genes related to PD encode lysosomal proteins. A rare hereditary form of parkinsonism, Kufor-Rakeb syndrome (PARK9), is due to mutations in the lysosomal ATPase ATP13A2 (85). Kufor-Rakeb syndrome has several features that are atypical in sporadic PD, such as rapid progression and early dementia. Another lysosomal enzyme, glucocerebrosidase (GBA), has also been implicated in PD. GBA mutations cause Gaucher disease, an autosomal recessive lysosomal storage disease. Epidemiological studies suggest that heterozygous carriers of GBA mutations are at increased risk of PD (86,87).

ALP is important for the regulation of α-Syn. Different forms of α-Syn are degraded by distinct mechanisms. Soluble forms (either monomeric or oligomeric) can be degraded by either the UPS or CMA (80,88). In contrast, insoluble forms of α-Syn are broken down by macroautophagy (89). Also, biochemical studies suggest a critical role for cathepsin D, a lysosomal protease, in cleaving α-Syn to form C-terminally truncated species that are more prone to aggregate (90). Conversely, α-Syn also affects ALP function. Mutant forms of α-Syn inhibit CMA (80). This is analogous to the inhibitory effect mutant α-Syn has on the UPS. Wild type α-Syn only affects its own degradation by CMA, not global CMA function. However, α-Syn-dopamine adducts block the degradation of other CMA substrates, suggesting a possible mechanism whereby dopaminergic cells are more susceptible to α-Syn toxicity (91).

Autophagy may also play a role in the pathogenic effects of mutant LRRK2. Expression of mutant LRRK2 in neurons causes shorter, less branched processes (92). In a cell culture system, mutant LRRK2 also causes an increase in markers of autophagy. Using both genetic and pharmacological manipulations, the authors showed that increased autophagy was necessary for the effect on neurite morphology (93). It is unclear what role autophagy plays in LRRK2-associated neuronal death (see below).

ALP also plays a role in toxin models of PD. In a PC12 cell model, reducing autophagy via beclin-1 siRNA enhances cell death caused by the proteasome inhibitor lactacystin (94). MPP + induces autophagy in neuronal cell lines and primary neuronal cultures (95). In this setting, autophagy appears to promote cell death, since knockdown of Atg proteins protects cells against MPP+. The underlying signaling pathways involved in MPP+-induced autophagy appear to be different than autophagy induced by other factors. Beclin-1 plays an important role in macroautophagy caused by starvation, a well-studied inducer of ALP. On the other hand, MPP+-induced macroautophagy does not involve beclin-1, but relies on ERK2 activation (96). The phosphorylation and mitochondrial localization of ERK2 appears to regulate macroautophagy of mitochondria (mitophagy) *in vitro*, a process that occurs after treatment with either MPP+ or 6OHDA (97). The *in vivo* relevance of this mechanism is highlighted by the presence of phospho-ERK2 in association with mitochondria in PD brain (81). It is also interesting to note that the effect of mutant LRRK2 on autophagy and neurite morphology (discussed above) can be blocked by a MEK inhibitor (93), which is consistent with a role for ERK2 in this setting.

The above experiments highlight the confusing relationship between autophagy and neuronal cell death. In some settings, autophagy is protective, whereas it seems to promote cell death in others (e.g. MPP+ treatment) (79). It should be noted that many studies demonstrating “autophagic cell death” require highly artificial conditions, such as blockade of apoptotic pathways. In short, the mere presence of autophagic vesicles in the setting of neuronal death does not indicate whether autophagy is enabling or suppressing cell death. It thus remains to be seen whether ALP is protective or destructive in the context of PD. It may be that both

possibilities pertain to the disease. That is, at some point in PD pathology, it may be that forms of ALP are protective and that dysfunction of the response contributes to cell death. In addition, excessive ALP responses may also contribute to neuron degeneration and death. If this is the case, then potential therapies based on regulating ALP would have to carefully balance the potentially therapeutic and deleterious effects of such an approach.

ER stress/ unfolded protein response (UPR)

In addition to the UPS and ALP, cells utilize another pathway, the unfolded protein response (UPR) or ER stress pathway, in an attempt to deal with increasing amounts of misfolded or abnormal proteins. The UPR is a multifaceted response program that uses different mechanisms to reduce the load of aberrant proteins (reviewed in 98). First, protein translation is reduced via phosphorylation and activation of the ER sensor kinase PERK and consequent phosphorylation and inhibition of eIF2-alpha, thus reducing the amount of new proteins that would need to be folded. Second, expression of genes involved in protein folding is induced by activation of the ATF6 transcription factor. Third, ER associated degradation of misfolded proteins involves transport of misfolded proteins out of the ER into the cytoplasm for proteasomal degradation. This arm is mediated by activation of the ER sensor proteins PERK, IRE1 and the transcriptional regulator XBP. With prolonged activation of the UPR, however, other pathways are activated that promote cell death. The transcription factor CHOP is induced after sustained ER stress. CHOP enhances cell death via mechanisms that include downregulation of Bcl-2 expression. In rodent cells, ER stress can activate caspase 12, which can activate downstream caspases. In humans, caspase 4 may play an analogous role, although this area is still unresolved.

What is the evidence that UPR plays a role in PD? Recently, one study demonstrated the presence of ER stress *in vivo* (99). Phospho-PERK and phospho-eIF2-alpha were detected in the SNpc of PD patients, but not controls. Many model studies have linked PD-related gene products and toxins with the UPR. In cell culture, mutant α-Syn and toxins such as 6-OHDA and MPP+ induce UPR (100-103). Blocking UPR, either with a chemical inhibitor or caspase 12 RNAi, was partially protective in the α-Syn model (103). In contrast, PERK-null primary neurons were more sensitive to 6OHDA (102), presumably because the cells were unable to mount an appropriate protective response to the presence of misfolded proteins. One possible explanation for the difference in roles of the UPR in responses to 6OHDA and mutant α-Syn is that each triggers distinct arms of the UPR that have differential effects on survival.

Examination of *in vivo* rodent 6-OHDA and MPTP models revealed up-regulation of the pro-apoptotic transcription factor CHOP, but not of BiP expression or alternate splicing of XBP, two additional markers of UPR (104). CHOP null mice showed protection from 6-OHDA, but not from MPTP (104). These findings underline the differences between various PD models, but do support a potential role for CHOP in the disease.

Parkin might play a particularly important role in linking ER stress to proteasomal degradation. Parkin expression is increased by ER stress (105), and parkin overexpression is protective against ER stress induced cell death (106). One parkin substrate, Pael-R, is particularly prone to misfolding, and insoluble Pael-R accumulates in neurons of patients with PD (107). As mentioned previously, Pael-R overexpression is toxic to dopaminergic cells *in vivo*, and parkin expression is protective in this context.

Overall, much like the UPS and ALP, present findings suggest that the ER stress pathway can be either pro-or anti-survival in the context of PD. This is exemplified, for example, by the opposite effect of UPR downregulation on cell death produced by mutant α-Syn vs. 6OHDA. As with the other degradative pathways, the degree and duration of ER stress and UPR activation likely plays a key role. Certain facets of the response, such as increased expression

of chaperones, would seem to be beneficial by diminishing the cellular load of misfolded proteins. In this regard, expression of the chaperone Hsp70 can mitigate neuronal death in many PD models (108,109). Other ER stress responses, such as inhibition of translation, might only be protective for a brief time and lead to degeneration if prolonged. Still other aspects, such as induction of CHOP and activation of caspases, appear likely to be harmful. Conversely, impairment of the ER stress/UPR (due, for example, to aging, accumulation of aggregated proteins such as α-Syn or to genetic factors) would put neurons at risk. Thus, the contributions of ER stress and the UPR to neuronal survival and death need to be further clarified. It therefore remains to be seen whether and how manipulation of the ER stress pathway might be utilized for therapeutic treatment of PD.

Mitochondrial dysfunction

Mitochondrial impairment is an attractive candidate to explain neurodegeneration (reviewed in 110). Neurons are highly metabolically active and dependent upon aerobic metabolism for energy. Mitochondria accumulate mutations with aging. Mitochondrial dysfunction can lead to insufficient ATP production and the generation of ROS. Furthermore, mitochondria are critical for the regulation of apoptosis.

In PD, several lines of evidence suggest a mitochondrial complex I deficit. There is a reduction in complex I activity in the SNpc of PD patients (111,112). However, there is a large range, and the majority of patients have levels that fall in the control range. Cybrids made from mtDNA from sporadic PD SNpc have reduced complex I activity, implying that the complex I defect comes from alterations in mitochondrial DNA (mtDNA) (113). Despite considerable effort at detection, there are no inherited mutations in mtDNA that have been associated with PD. Interestingly, PD patients have a higher overall rate of mtDNA mutations compared to controls (114,115).

Several nuclear-encoded PD genes can be linked to mitochondrial function. PINK1 and parkin appear to act in the same pathway, regulating mitochondrial function. PINK1 is a kinase that is found in mitochondria (116). A small amount of parkin resides in the mitochondrial outer membrane, although the majority of the protein is cytoplasmic (117). Both parkin (118,119) and PINK1 (120,121) knockout mice have reduced mitochondrial respiratory function with normal (or minimally changed) mitochondrial morphology and no signs of neurodegeneration (with the exception of one parkin-null line with mild neuronal loss in the locus ceruleus (122)). However, neurons from both mutant animal types show evidence of increased oxidative stress (120,123). The phenotype of PINK1 (124) or parkin (125) deletion is more dramatic in *Drosophila*, with markedly abnormal mitochondria and degeneration of flight muscles. There is also a small subset of dopaminergic neurons that display age-dependent degeneration in parkin-null flies (126). Notably, parkin and PINK1 interact genetically, with parkin downstream of PINK1 (124,127). Functionally, parkin and PINK1 act in a pathway that favors mitochondrial fission (128,129). The regulation of mitochondrial fusion and fission has become increasingly recognized as an important factor in mitochondrial function and neurological disease (130). The role of mitochondrial fission and fusion in PD should be explored more fully.

Toxins that act on mitochondria mimic several features of PD. MPTP was accidentally identified in an attempt by drug users to synthesize a meperidine analog. Several of them developed acute parkinsonism after intravenous use of the compound (131). *In vivo*, MPTP is converted to MPP+ by MAO-B in glial cells (1). Then, MPP+ is transported into neurons by catecholamine transporters, accounting in part for the specificity of the insult (132). MPP+ is a potent complex I inhibitor, resulting in production of ROS and ATP depletion (133). After its isolation, MPTP was found to induce a parkinsonian state in mice and primates, and MPTP remains the gold standard of toxin-based PD models (1). Systemic administration of MPTP

recapitulates several features of PD: preferential loss of dopaminergic SNpc neurons, with greater impairment of projections to the putamen vs. caudate nucleus; relative sparing of VTA neurons; a greater degree of loss of nigrostriatal projections than nigral cell bodies; and post-translational modifications of α-Syn similar to those seen in PD, such as nitration. Shortcomings of the MPTP model include a lack of *bona fide* Lewy bodies and sparing of other neuronal populations typically affected in PD, such as the locus ceruleus. An important point is that the regimen of MPTP dosing is a critical determinant of the type of cell death observed in SNpc neurons (134). The so-called acute regimen (multiple injections over the course of 1 day) causes necrotic cell death, while the subacute regimen (daily injection for several days) causes apoptotic cell death.

Rotenone is an insecticide that is also a complex I inhibitor. Chronic intravenous administration of rotenone in rats leads to relatively selective degeneration of dopaminergic SNpc neurons, moderate loss of locus ceruleus neurons, α-Syn-positive fibrillar inclusions, and behavioral changes (135). Others, though, have found that rotenone treatment also depletes striatal neurons, which are spared in PD, and also causes tau-positive inclusions in cortical neurons (136). Unfortunately, rotenone administration does not affect mice, preventing the use of genetically modified animals that has been so powerful in addressing mechanistic questions.

Advances in tissue-specific gene deletion have allowed for interesting new rodent models of PD based on mitochondrial dysfunction. Reduction of respiratory chain function in dopaminergic neurons, via tissue specific deletion of mitochondrial transcription factor A (TFAM), causes progressive loss of SNpc neurons with α-Syn-negative inclusions (137). Crossing α-Syn null mice with the tissue-specific TFAM null mice does not significantly modify the neurodegenerative phenotype, suggesting that α-Syn does not play a role in the neurodegeneration seen in this model. The TFAM (MitoPark) transgenic model supports a role for mitochondrial dysfunction in PD and should be important for testing potential ameliorative strategies.

Especially provocative is recent work that calls for a re-appraisal of the role of complex I in the development of PD and the effect of PD toxins (138). Ndufs4 is a nuclear encoded complex I subunit that is essential for complex I activity. Ndufs4 -/- mice were generated; as expected, cultured neurons from these mice had no detectable complex I activity. However, dopaminergic mesencephalic neurons from wild type and Ndufs null mice were equally susceptible to MPP + and rotenone treatment. Furthermore, there did not appear to be an increase in oxidative stress or cell death in neurons derived from Ndufs-null mice. Finally, the authors reported that tissue specific deletion of Ndufs in dopaminergic neurons produces no discernable phenotype in mice up to 9 months of age. These findings contrast with several other studies that have supported a role for complex I inhibition as the mechanism of action of MPTP and rotenone (139,140). For example, expression of a rotenone-insensitive NADH dehydrogenase from yeast, NDI1, protects dopaminergic neurons from rotenone and MPTP toxicity *in vitro* and *in vivo* (141, 142). The most straightforward interpretation of these apparently conflicting results is that complex I inhibition is necessary, but not sufficient, for cell death caused by PD toxins. To this end, there are reports of other potential activities of MPTP and rotenone, such as microtubule destabilization (143,144).

Future work should address the role of complex I, as well as the relative contributions of ATP depletion, oxidative stress, and dysregulation of pro-apoptotic mitochondrial proteins in neuronal death caused by mitochondrial dysfunction. The MitoPark and Ndufs-null mice offer opportunities to test the role of mitochondrial dysfunction in PD-relevant cell death paradigms. Genetic approaches to enhancing mitochondrial function would also prove useful in the study of PD pathogenesis. For example, TFAM overexpressing mice show reduced age-related oxidative stress and respiratory chain dysfunction (145). These mice would be useful in

examining the ability of mitochondrial enhancement to protect against various PD-relevant stressors. In terms of therapeutics, augmentation of mitochondrial function appears to be a potentially promising approach. Indeed, two enhancers of mitochondrial respiratory chain function, coenzyme Q10 and creatine, are each undergoing advanced clinical testing in PD. However, stimulating mitochondrial function *in vivo* may be complex, and combining multiple mitochondrial enhancers with different mechanisms of action may maximize the benefit from this approach.

Oxidative stress

The presence of oxidative stress in PD is indisputable. Numerous studies have confirmed increased levels of several markers of oxidative damage in the SN of PD patients: DNA damage, lipid peroxidation, protein oxidation, less reduced glutathione, and increased iron deposition (reviewed in 110). The key question has been whether oxidative stress is an early indicator or a late consequence of neuronal distress. One argument that favors a proximal role for oxidative stress is the association of mutations in DJ1 with autosomal recessive PD (146). DJ1 plays a critical role in the response to oxidative stress, through its role as an atypical peroxiredoxin-like peroxidase (147). DJ1 exhibits antioxidant activity by scavenging hydrogen peroxide. Oxidized DJ1 cannot be reduced, which may explain why oxidized DJ1 is more prevalent in PD brain (as well as in AD) (148). While DJ1-null mice and flies do not exhibit neuronal loss, they are more sensitive to oxidative stressors (149,150). Therefore, at least in rare familial cases, enhanced sensitivity to oxidative stress appears sufficient to produce PD.

Oxidative stress can result from defects in several systems, such as mitochondrial dysfunction, increased calcium, and inflammatory responses (110). One potential source of ROS that deserves special consideration is dopamine (DA) itself. The oxidative potential of dopamine has been posited as a possible reason for the selective susceptibility of SNpc dopaminergic neurons. DA is a highly reactive molecule that can be oxidized and subsequently react with cellular components to form adducts or to form more toxic ROS (110). One such derivative of DA, 6OHDA, is used to experimentally lesion dopaminergic neurons. Normally DA is effectively sequestered in synaptic vesicles, but cytosolic dopamine can be toxic to neurons. As discussed earlier, DA-α-Syn adducts potently inhibit chaperone-mediated autophagy (91). Furthermore, in some experimental systems—intrastriatal injection of lactacystin, and α-Syn overexpression in primary dopaminergic midbrain neurons—DA depletion has a protective effect (20). Several points, however, argue against a key role for dopamine *per se* as an important contributor to nigral cell death. In acute MPTP treatment, genetic deletion of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, does not alter neuronal death (151). Also, other dopaminergic populations, such as the neighboring ventral tegmental area as well as dopaminergic hypothalamic neurons, are resistant to degeneration in PD (1). Conversely, other neuronal groups, both catecholaminergic (locus ceruleus) and non-catecholaminergic (nucleus basalis), are significantly affected in the disease.

In addition to oxidative damage, PD autopsy material shows evidence of extensive nitrative stress. Nitration of tyrosine residues on α-Syn leads to enhanced aggregation and reduced proteasomal degradation (37). S-nitrosylated parkin is increased in PD brain (59). This form of parkin has reduced enzymatic activity and less neuroprotective function. Vulnerable neurons in PD and AD brains show increased levels of S-nitrosylated protein disulfide isomerase (PDI), a molecular chaperone (152). S-nitrosylation of PDI diminishes its neuroprotective activity. Finally, nitric oxide (NO) reacts with ROS, such as superoxide, to form peroxynitrite, a very labile and reactive toxic species (110). The source of increased NO is unclear. There is increased astrocytic expression of inducible nitric oxide synthase (iNOS) in PD, and activated microglia can produce NO as well. Of note, NOS inhibitors can protect against MPTP treatment

in animals, and mice that are null for iNOS or neuronal NOS are more resistant to MPTP (153).

Overall, it appears that oxidative (and nitrative) stress can be linked both proximally and distally to nearly every pathogenic mechanism posited to play a role in PD. Moreover, ROS can have a significant amplifying effect on pathways that favor cell death. For these reasons, despite the difficulty in definitively placing it within the pathogenic cascade, oxidative stress would appear to be an attractive target for PD therapy. In this regard, a large clinical study, DATATOP, demonstrated that vitamin E had no effect on the progression of PD. In addition, even though oxidative stress has been advanced as critical for several other neurodegenerative diseases, no anti-oxidants have proven effective in clinical trials for such disorders. Given the strong evidence supporting a role for oxidative damage in PD, it may be that present clinical trial failures are more attributable to defects in specific agents, not the underlying rationale. Rather than using small molecule anti-oxidants, a more promising approach may be the upregulation of endogenous anti-oxidant systems. In addition, the use of multiple anti-oxidants or combination with drugs targeted to other factors in the disease may increase the likelihood of producing a significant neuroprotective effect.

Calcium dysregulation

Ionic calcium (Ca) is a critical signaling component in neurons, and its cytoplasmic levels are tightly regulated. Ca can be pumped out to the extracellular space or buffered in intracellular compartments, mainly the ER and mitochondria. The neurotoxic effects of Ca are well known. The most well studied trigger for this is glutamate excitotoxicity via NMDA receptors. Excessive Ca leads to cell death via multiple potential pathways, including mitochondrial dysfunction, oxidative stress, ER dysfunction, and dysregulated signaling via Ca-dependent enzymes. In PD, SNpc neurons might be subject to excessive glutamate signaling via projections from overactive STN neurons. In some (but not all) studies, glutamate receptor blockers attenuate MPTP toxicity in animals (110).

Recently, a different theory has come forward that features Ca dysregulation as a key driver of SNpc vulnerability (154). Dopaminergic SNpc neurons have spontaneous pacemaker activity that is driven, in adults, by Cav1.3, an L-type dihydropyridine-sensitive Ca channel (155). Therefore, adult SNpc neurons must cope with a very high, oscillating cytoplasmic Ca burden. It has been suggested that this situation puts SNpc neurons at particular risk. Significantly, blockade of the Cav1.3 channels with the dihydropyridine isradipine is protective in the MPTP model, implying that high levels of cytoplasmic Ca are central to MPTP-induced cell death. Intriguingly, neurons in the locus ceruleus, which are affected in PD, also possess pacemaker activity mediated by Cav1.3 channels (154). Thus, excessive Ca might contribute to the selective degeneration of other neuronal populations in PD. It will be interesting to see if other vulnerable populations, e.g. dorsal vagal nucleus, raphe nucleus, olfactory neurons, also express Cav1.3. However, all of the affected neuronal populations in PD do not have pacemaker function. Therefore, it is likely that Ca dysregulation is an exacerbating factor that enhances the underlying pathogenic defect(s) in PD.

The idea of using Cav1.3 blockers to protect vulnerable neurons from excessive cytoplasmic Ca, without markedly affecting their function, has generated considerable interest. One major reason is that dihyropyridines have been used extensively as anti-hypertensives in humans. In fact, an epidemiological study suggests that chronic use of calcium channel blockers, but not other classes of antihypertensives, reduces the risk of PD (156). However, the effect was roughly equivalent with either dihydropyridines or non-dihydropyridine agents, even though the latter do not block Cav1.3. Nonetheless, clinical trials with isradipine, a dihydropyridine that has good brain penetration, have been initiated.

Altered kinase signaling-focus on LRRK2 and Akt

Kinases are involved in essentially all cellular functions, and abnormalities of several kinases have been linked to PD. In this section, we will focus on two kinases: LRRK2 and Akt. LRRK2 was identified as the PARK8 locus, associated with autosomal dominant PD that is clinically very similar to sporadic PD (157,158). Mutations in LRRK2 are the most common known cause of monogenic PD; in certain populations, such as North African Arabs, LRRK2 mutations account for nearly 40% of all PD cases (159). LRRK2 is a large multi-domain member of the Roco protein family that possesses both Ser/Thr kinase and GTPase activities (160). The most common PD-associated mutation in LRRK2 is Gly2019Ser, located in the kinase domain. Most (though not all) studies suggest that disease-linked mutations cause an increase in kinase activity, either by affecting the kinase domain itself or by reducing GTPase activity (161,162). LRRK2 is primarily cytosolic, but appears to associate with various membrane structures.

Expression of mutant LRRK2 *in vitro* causes neuronal death; a functional kinase domain is required for this effect (163). Interestingly, mutant LRRK2 causes tau-positive, α-Syn-negative inclusions and apoptosis in affected neurons (92). This finding is interesting given the association of LRRK2 mutations with tauopathies in some patients. In addition, mutant LRRK2 also alters neurite morphology, causing shorter, less branched processes (92). In contrast, LRRK2 knockdown causes longer neurites, fitting with a gain-of-function effect of the mutations. In *Drosophila*, expression of either wild type or Gly2019Ser human LRRK2 causes preferential loss of dopaminergic neurons, with the latter producing a more severe phenotype (164). Given the frequency of LRRK2-associated PD, this represents a promising model for further research. Several labs have generated LRRK2 transgenic mice, though no reports have been published yet. If the activity of LRRK2 mutants is responsible or required for its promotion of disease, as it currently appears, this opens the door to development of LRRK2 inhibitors for potential therapeutics.

Akt is a serine/threonine kinase central to multiple cellular functions, including survival, proliferation, and growth (reviewed in 165,166). Receptor activation by many different growth factors results in Akt activation. Akt has several downstream effects relevant for cellular survival: these include inhibition of JNK signaling; reduced expression of the pro-apoptotic gene Bim, via inhibition of FOXO transcription factors; inhibition of p53 signaling; and activation of mTOR signaling by inhibition of TSC2, a negative regulator of mTOR. mTOR activation in turn has several downstream effects that affect cell survival including inhibition of autophagy; further stimulation of Akt activation by directly phosphorylating Akt at serine 473; and increasing protein translation, which can increase levels of anti-apoptotic proteins, e.g. Bcl-XL. Expression of a constitutively active form of Akt in nigral neurons, using adeno-associated virus, significantly protected the neurons from 6OHDA toxicity (167). Importantly, nigrostriatal projections were also protected. Such findings are consistent with the idea that activation of Akt by growth factors such as GDNF may at least in part mediate their protective actions in PD models (168).

A link between PD-mimetic toxins and Akt signaling is the protein RTP801/REDD1. RTP801 is dramatically upregulated relatively quickly after exposure to several toxins, including 6OHDA, MPP+ and rotenone (169). In addition, neuromelanin-containing neurons in the SNpc of PD patients, but not in controls, contain elevated levels of RTP801. RTP801 is sufficient to induce neuron death and is necessary for toxin-induced cell death in PC12 cells and primary sympathetic neurons. RTP801 blocks mTOR signaling by releasing TSC2 from inhibition, allowing TSC2 in turn to reduce mTOR activity (170,171). As a result, neuronal Akt activity goes down, due to a reduction in mTOR-dependent Akt phosphorylation (172). As expected from this model, constitutively active Akt overrides cell death caused by RTP801 overexpression. In sum, RTP801 mediates cell death in response to PD-related toxins, at least

in part via Akt inhibition. In support of the relevance of these observations to PD, phospho-Akt levels are substantially depleted in SN neurons of post-mortem PD brains compared with controls (172). Taken together, such findings indicate that RTP801 and Akt may represent potential therapeutic targets for treatment of PD.

Recently, an intriguing link between LRRK2 and the Akt/mTOR pathway has been described (173). In *Drosophila*, LRRK2 genetically interacts with several members of the Akt/mTOR pathway. Specifically, the authors find that LRRK2 can directly phosphorylate 4E-BP, and that 4E-BP plays a critical role in dopaminergic degeneration caused by LRRK2. 4E-BP binds and inhibits eIF4E, a translation initiation factor. Phosphorylation of 4E-BP releases eIF4E from inhibition, promoting translation. 4E-BP is also a target of mTOR. Therefore, it appears that LRRK2 and Akt/mTOR converge upon 4E-BP. Interestingly, both LRRK2 and mTOR appear to phosphorylate the same sites on 4E-BP (Thr37 and Thr46). These residues are considered priming sites, allowing other kinases to further phosphorylate 4E-BP and thereby inactivate it (174). It is interesting that Akt/mTOR activity is typically pro-survival, while LRRK2 activity favors cell death. Possible explanations for this conflict include the effect of other recruited pathways or cell-type specific effects. At any rate, this finding further supports the idea that translational regulation could play a role in the neurodegeneration seen in PD. The regulated transcript(s) that are important for 4EBP-dependent neuronal toxicity will be an important area of research going forward.

The role of neurite loss in the initiation of cell death

While neuronal loss is a defining feature of PD, loss of nigrostriatal projections is also a key feature of the disease. It is not surprising that cell death would be accompanied by loss of processes. However, the relationship between the two is unclear. It is notable that the degree of degeneration is greater in the striatal terminals than in the cell bodies of the SNpc, even when measured at earlier stages of disease (1). Likewise, in MPTP-treated primates, striatal terminals are lost before dopaminergic cell bodies. Finally, neurons with poorly myelinated, distant and extensive projections appear to be more susceptible to degeneration in PD (3). Therefore, axonal morphology appears to play a role in the selective vulnerability of affected neurons in PD. Together, these observations have led to the idea of a “dying back” process, in which damage to axons leads to subsequent death of the cell body. Several of the mechanisms mentioned in this review are likely to take place in processes. Indeed, axons and synapses may be more susceptible to these mechanisms of injury. One possible mechanism of cell death caused by axonal degeneration is loss of retrogradely transported trophic support from target tissues.

It certainly seems logical that survival of neuronal cell bodies and processes are tightly linked. However, many interventions that protect cell bodies against injury in PD models fail to save processes to a similar extent (104,175). Thus, survival of cell bodies and processes are separable. Moreover, degeneration of axons is an active process, not necessarily a passive result of cell body death (176). This is strikingly highlighted by the Wld(S) mouse strain, a spontaneously occurring mutant mouse line (177). In Wld(S) mice, transected axons can survive for weeks after being severed from their cell bodies. MPTP treated Wld(S) mice have less loss of nigrostriatal projections, despite similar levels of SNpc cell loss compared to wild type mice (178). In sum, there are distinct pathways that mediate maintenance of neuronal soma and processes. To date, the bulk of studies in PD models have focused on protection of cells bodies and the mechanisms of their degeneration. It is important to preserve both when evaluating neuroprotective intervention, and it will be important in future to explore the mechanisms by which neurites degenerate in PD.

Non-cell-autonomous mechanisms: Inflammation, glia

In the 1980s, clinical trials were performed studying striatal transplantation of fetal mesencephalic tissue. Recently, the first series of autopsies performed on participants from these trials after long-term follow-up have been reported. Two groups found LBs in the transplanted dopaminergic neurons in 3 patients (179,180); another group found no evidence of LBs in the grafts of 5 patients (181). The demonstration of Lewy bodies in transplanted neurons highlights the potential role of non-cell-autonomous factors in PD pathogenesis.

Inflammation is one potential contributor (reviewed in 110). Pathologically, there is significant microgliosis and astrocytosis in affected brain regions in PD. In addition, multiple cytokines, iNOS and COX2 are all increased in PD brain. Multiple epidemiological studies have found a reduced risk of PD in those who regularly use non-steroidal anti-inflammatory drugs (NSAIDs), implying that anti-inflammatory intervention may be protective. There is also extensive evidence from animal models of PD. Several anti-inflammatory interventions can ameliorate MPTP toxicity *in vivo*. Traditional immunosuppresants, such as dexamethasone, thalidomide, FK506, and cyclosporine A all attenuate MPTP toxicity. NADPH oxidase or iNOS null mice have less nigral cell loss and less oxidative damage in response to MPTP (153,182).

Glial cells are another potential source of non-cell-autonomous influences on neurons. In PD SNpc homogenates, there are reductions of ~30-40% in several measures, including complex I and proteasome activity (110). However, dopaminergic neurons make up <5% of cells in the SNpc; therefore, to achieve reductions of such magnitude, non-neuronal cells have to be involved as well. Wild type α-Syn transgenic lines with expression restricted to neurons generally have a milder phenotype and less α-Syn deposition than lines with expression in both neurons and glia (183), suggesting that α-Syn expression in glial cells contributes to neuronal damage. Glial cultures from parkin-null mice show more baseline apoptosis, alterations in oxidative stress pathways, and conditioned medium from these glia is less trophic for neurons (184). Similarly, DJ-1 knockdown in astrocyte cultures results in a loss of neuroprotective activity from conditioned medium (185).

In conclusion, exclusively focusing on cell-autonomous factors in neuronal death might miss key elements in PD. Most cell culture approaches utilize homogenous cell populations, so non-cell-autonomous mechanisms could be overlooked. Neuron-glia co-cultures are an important approach in this regard. In addition, transgenic animals with cell-type-specific expression or deletion of target genes provide a valuable approach to studying non-cell-autonomous factors. Non-cell-autonomous mechanisms may be operational even when cell-autonomous mechanisms have been demonstrated. Thus, the roles of PD genes in non-neuronal cells, such as astrocytes, oligodendrocytes, and microglia, should be explicitly examined.

DISTAL PATHWAYS

Beyond the varied proximal events that initiate neurodegeneration in PD, a number of distal pathways have been identified that potentially lead directly to activation of the core cell death machinery in this disorder.

JNK signaling

c-jun N-terminal kinase, or JNK, is a member of the MAP kinase (MAPK) family and has been implicated in neuron cell death in a variety of circumstances including PD pathogenesis (reviewed in 165). Like all MAPKs, JNKs are activated by a cascade of upstream kinases. These include the MAPK kinase kinases (MAPKKKs) MLK1-3, DLK, ASK1 and ASK2, and the MAPK kinases (MAPKKs) MKK4 and MKK7, which in turn phosphorylate JNKs. JNK is the major activator of c-jun, a transcription factor that regulates expression of numerous cell

death genes. JNK also phosphorylates several Bcl-2 family members, causing inhibition of pro-survival members such as Bcl-2 and activation of pro-apoptotic members such as Bad and Bim.

JNK activation occurs in PD and contributes to cell death in numerous models of this disorder. In PD, phospho-JNK immunostaining is found in cytoplasmic granules adjacent to LBs in nigral neurons (186). In addition, in one study occasional dopaminergic neurons from PD but not control brains displayed nuclear c-jun staining, an indicator of c-jun activation (187). However, another study found no difference in c-jun expression or localization between PD and control SNpc (188). In *Drosophila*, parkin deletion results in activation of JNK in a small subgroup of dopaminergic neurons that undergo degeneration (189). This result suggests that parkin negatively regulates JNK signaling. This idea is consistent with cell culture studies showing that parkin overexpression reduces JNK phosphorylation and protects cells against 6OHDA-mediated cell death (190).

A great deal of experimental evidence links JNK activation with toxin-induced neuronal death. In both cell lines and primary cultures, MPP+ and 6OHDA cause JNK and c-jun activation (165). Cell death is reduced by blocking JNK activation via many different approaches—direct JNK inhibitors, MLK inhibitors, and expression of dominant negative forms of JNK. The JNK activator ASK1 is activated after MPTP treatment (191), and ASK1 siRNA can inhibit JNK activation and cell death in 6OHDA treated SH-SY5Y cells (192). In both the acute and subacute models of MPTP toxicity, inhibiting JNK activation protects dopaminergic cell loss and striatal terminals (175). Moreover, JNK2/3 double knockout mice are completely resistant to MPTP (187).

These models have also allowed further dissection of the JNK signaling pathway that may be relevant for PD. JIP is a scaffold for JNK signaling and interacts with multiple components of the JNK pathway. SNpc neurons are protected from MPTP by adenoviral transduction of a dominant negative form of JIP (193). Downstream of JNK activation, several Bcl-2 family members are upregulated and participate in cell death. In PC12 cells treated with MPP+, JNK activation leads to BimEL expression, with subsequent calpain activation, AIF release and caspase-independent cell death (194). In MPTP treated mice, JNK activation leads to upregulation of Bim, which in turn facilitates translocation of Bax to the mitochondria, cytochrome C release and apoptosis (195). Further definition of the regulators and effectors of JNK signaling relevant for PD should allow more targeted, specific approaches to JNK inhibition.

A therapeutic aimed at JNK signaling has already been tried in the clinic. CEP-1347, a small molecule MLK inhibitor, inhibited JNK activation and provided significant neuroprotection in both *in vitro* and *in vivo* models of PD, including a primate MPTP model (165). However, a clinical trial testing CEP-1347 in early PD patients failed to show a neuroprotective benefit (196). While this result may suggest that MLK inhibition is not a valid target for neuroprotection in PD, there are several other potential explanations for the negative result in this trial. It is possible that other approaches to JNK inhibition, including direct JNK blockers or targeting other components of the pathway, such as ASK1, may still prove to have neuroprotective activity.

p53

Originally identified as a tumor suppressor gene, p53 also plays a role in neuronal cell death (reviewed in 197,198) The classical trigger for p53 activation is DNA damage. Oxidative stress also activates p53, although this also may occur via DNA damage. Activation of p53 occurs via multiple post-translational modifications with subsequent stabilization. Stabilized p53 enters the nucleus and regulates transcription of numerous genes whose products are involved

in cell death. In addition, p53 has transcription-independent activities that contribute to cell death, such as interacting with numerous Bcl-2 family members.

Three studies have looked at p53 expression in the SNpc of PD patients. Two saw no evidence for increased expression (188,199), while one reported increased levels of phospho-p53 in tissue homogenates (200). Activation of p53 may play a role in the increased sensitivity of DJ1-null cells to cell death triggers. Neurons in DJ1-null zebrafish have an increased level of p53 (201). DJ1 deletion causes increased dopaminergic cell death after oxidative stress or proteasomal inhibition, but pharmacologic inhibition of p53 is protective in these settings. Much work has been done with toxin-based models. In MPTP-treated mice, p53 inhibition causes reduced nigral cell loss (202). In cell culture and primary neurons, inhibition of p53, either with small molecules or dominant negative constructs, is protective against treatment with 6OHDA, MPP+, or proteasome inhibitors (203-206). Two downstream effectors of p53-mediated cell death have been studied more closely. Upregulation of Bax expression is necessary but not sufficient for MPTP-induced cell death *in vivo* (195). In multiple cell lines, upregulation of the BH3-only gene PUMA is necessary for cell death induced by 6OHDA (204,207), but not by proteasome inhibition (200). Bax and PUMA will be further considered below in a section on Bcl2 family members. In sum, p53 may play a role in neuronal death associated with PD, but the majority of the evidence comes from toxin-based models. Studying the role of p53 in some of the genetic models mentioned in this review, e.g. α-Syn transgenic animals, would help to clarify the importance of this protein in PD. However, given the role of p53 as a tumor suppressor, it would not appear that a systemic p53 inhibitor would be a useful therapeutic for PD.

Cell cycle regulators

In post-mitotic neurons, reactivation of cell cycle molecules has been associated with, and is often necessary for, cell death caused by diverse triggers (reviewed in 208,209). The cyclin dependent kinase (cdk) family of proteins, along with the partner cyclin proteins, regulates progression through specific phases of the cell cycle. For example, cyclin D-cdk4/6 and cyclin E-cdk2 operate during the G1-S transition. An important target of cyclinD-cdk4/6 is the retinoblastoma (Rb) protein family. Upon phosphorylation, Rb proteins dissociate from E2F transcription factors, allowing derepression and activation of E2F-responsive genes. In dividing cells, the net effect of cdk activation and Rb phosphorylation is DNA replication and cell cycle progression. However, in post-mitotic neurons, these lead to increases in expression of genes that induce apoptosis, including Bim, c-jun, and cdk1.

There is expression of phospho-pRB, E2F-1 and proliferating cell nuclear antigen (PCNA), a marker of dividing cells, in a minority (3-6%) of SNpc neurons from PD brain, but not in controls (210). In addition, a similar percentage of neurons show aneuploidy of selected chromosomes (210), indicating activation of the DNA replication machinery. Immediately after MPTP treatment, 30% of dopaminergic SNpc neurons express PCNA and 10% are labeled with BrdU *in vivo* (210). Cultured midbrain neurons treated with MPP+ show immunostaining for phospho-pRB and E2F-1 (210). While many of these results are consistent with an attempt to re-enter the cell cycle, another interpretation is that oxidative DNA damage, which is seen after MPTP exposure and in PD autopsy material (211), has triggered DNA repair responses. Thus, components of the cell cycle machinery can be re-activated and participate in either repair or neuron death in PD.

Experimental support for a role of cell cycle molecules in PD comes in part from protection in several *in vitro* models conferred by inhibitors of cyclin-dependent kinases (212,213). In addition, the importance of E2F-1 for neuronal death was demonstrated both *in vitro* and *in vivo*. E2F-1 antisense protects cultured neurons from MPP+, and E2F-1-null mice show less nigral cell and striatal dopamine loss after MPTP treatment (210). In cultured neurons,

expression of multiple cyclins, especially cyclin D and E, goes up after MPP+ exposure, suggesting activation of cdk4/6 and cdk2, respectively (210). Blocking cdk2 *in vivo* (using adenoviral expression of a dominant negative cdk2 construct) leads to partial protection against MPTP-induced dopaminergic cell death (214). However, neither dominant negative cdk4 nor cdk6 had any effect on neuronal death (214), suggesting that the cdk2/cyclin E complex is more relevant for cell death in this model.

Taken together, these findings indicate that cell cycle molecules may participate as distal components of the cell death pathways in PD. However, this requires further confirmation, especially in genetically-based models, as well as a more refined definition of the relevant molecules involved in death. If such information is supportive, then examination of suitable cell cycle inhibitors for potential therapeutic intervention would be justified.

Cdk5-the black sheep of the cdk family

Despite its structural similarity to other cdk proteins, cdk5 is unusual in several regards (reviewed in 209). Cdk5 is normally expressed in both developing and post-mitotic neurons and has been linked with several aspects of neuronal biology. Cdk5 is not activated by a cyclin, but rather by a distinct protein, p35. Notably, p35 can be proteolytically cleaved, for example by activated calpains, to form p25, which appears to cause a more robust and long-lasting activation of cdk5. Excessive cdk5 activity contributes to neuronal death, and is even sufficient to produce cell death *in vitro*.

There is evidence for abnormal activation of cdk5 in neurodegenerative diseases, including PD. Lewy bodies show immunoreactivity for cdk5 (215,216). Another study examined homogenates from the cingulate gyrus, which is also affected in PD, and found increases in cdk5 protein, the ratio of p25:p35, and levels of a calpain cleavage product in PD vs. control tissue (217). One study has linked cdk5 with parkin (218). Parkin is phosphorylated by cdk5, causing reduced E3 ligase activity and decreased ubiquitylation of the parkin substrate synphilin. Several studies have delineated a signaling pathway involving cdk5 in MPTP-mediated neuronal death (214,219). MPTP promotes activation of calpains, which are Ca-dependent proteases, leading to p35 cleavage and formation of p25. This in turn causes stronger and more long-lasting cdk5 activation, resulting in neuronal apoptosis. Inhibiting calpains, either pharmacologically or via overexpression of an inhibitory peptide, limits dopaminergic cell death after MPTP exposure (219). In p35-null mice, MPTP treatment causes less nigral cell loss and striatal denervation (219). Finally, the cdk inhibitor flavopiridol (which blocks cdk5 along with other members of the cdk family) reduces MPTP-induced neuronal death in the SNpc (214). Two potential downstream targets of cdk5 phosphorylation have been identified: MEF2D and Prx2. MEF2D, a pro-survival transcription factor, is inactivated by cdk5 phosphorylation (219). Expression of an MEF2D mutant lacking the cdk5 phosphorylation site protects SNpc neurons from MPTP toxicity. Peroxiredoxin-2 (Prx2) is a neuronal antioxidant that reduces hydrogen peroxide. Phosphorylation of Prx2 by cdk5 leads to a reduction in its peroxidase activity (220). Expression of a Prx2 mutant lacking the cdk5 phosphorylation site protects dopaminergic SNpc neurons in the subacute MPTP model. Furthermore, there are higher levels of phospho-Prx2 in SNpc neurons in PD compared to controls, suggesting that this mechanism is operational in PD. Another intriguing potential target of cdk5 is pRb; in a neuronal cell line overexpressing p25, cdk5-p25 directly phosphorylates pRb, leading to the expression of multiple cyclin and cdk proteins (221). While this relationship needs to be confirmed in other systems where p25 is not overexpressed, it suggests a potential link between cdk5 and reactivation of cell cycle pathways during neuronal death.

In aggregate, present findings indicate that cdk5 is an intriguing potential target for PD therapy either with respect to its potential cell cycle functions or other activities. However, cdk5

participates in a broad array of neuronal functions, making the risk of adverse effects relatively high. Selectively inhibiting the cdk5-p25 complex might target excessive activation while leaving more physiologic cdk5-p35 signaling intact.

Bcl2 family proteins and PD

The roles of Bcl2 family members in cell survival and death have been extensively reviewed, are well known to many, and will not be recounted here (reviewed in 222,223). Closely related to the unsettled issue of whether neuron death in PD is apoptotic in nature (see below) is the important question of whether such death involves members of the Bcl2 family. On one hand, if such death is apoptotic, then one might anticipate changes in the levels or distribution of Bcl2-related proteins. On the other hand, given the rapidity of cell death once the core apoptotic machinery is activated and the drawn-out progressive nature of PD, one might also find that changes in Bcl2-related protein levels or distribution, even if they occur, would be transient and therefore difficult to detect. Perhaps in reflection of this, there has been some disagreement in the literature regarding the disposition of Bcl2 family proteins (determined by immunohistochemistry) in post-mortem SN neurons. Several studies have indicated no change in Bcl2, Bax or Bcl-xL as well as of activated caspase 3 (188,224) while another observed increases in both Bax and caspase 3 (225). Two additional groups reported no overall change in Bax expression in PD, but did note changes in its distribution. In one report, there was an increase in Bax-rich inclusions (195,201,206,226). In the other, an antibody directed towards “activated” Bax inserted into the outer mitochondrial membrane revealed signal in a higher proportion of neuromelanin positive SN neurons with Lewy bodies than in neuromelanin positive neurons without these inclusions (227). Similar observations were made with an antibody recognizing activated caspase 3.

Given the difficulties inherent in assessing the roles of Bcl2 family members in neuron death associated with PD by examination of post-mortem tissues, researchers have turned to *in vitro* and animal models of the disease. Overall, these have supported the involvement of several such proteins, although specifics appear to depend on the particular model examined. In a variety of cell and animal models of PD, Bax has been reported to increase (195,201, 206), to undergo translocation to mitochondria (195,207) as well as to be required for death (73,195,206,228). In contrast, there are several reports of cell and animal models in which death was reported to be Bax-independent (200,229,230). In one instance, death was dependent rather on Bak (229). Given the widespread role of BH3-only proteins in apoptotic cell death, a number of investigators have examined these in the context of PD models. Several studies have observed induction of the BH3-only protein Bim (73,195,204,228). However, while Bim expression was required for death in the MPTP animal model (195), interference with its expression was not protective in several cell culture models (204,228). Another BH3-only protein, PUMA was induced and required for death in several *in vitro* models of PD (203, 204,207,228). PUMA is a direct target of p53; consistent with this connection, inhibition of p53 activity is protective in several PD culture and animal models (203,204,206,228). In other PD models, however, p53 appears to promote death by mechanisms independent of PUMA (195,200) and, in at least one case, PUMA induction was reportedly independent of p53 (207). Contrasting with the culture models, in the animal MPTP model, PUMA was not induced, nor was it required for neuron death (195). Several additional BH-3-only family members have also been found to be required for death in PD models including Noxa and Bnip3 (229) as well as Nix (231). In the latter case, Nix appeared to promote death by participating in activation of JNK signaling rather than by interaction with other Bcl2 family members.

Taken together, the present findings regarding Bcl2 proteins do support the notion that pro-apoptotic family members participate in neuron death in a variety of PD models. However, the identities and mechanisms of regulation of such proteins vary among, and are highly dependent

upon, the particular models employed. This situation in turn suggests both that further efforts should be directed towards examining the potential involvement of specific Bcl2 proteins in PD tissues and that a variety of family members in addition to Bax should be considered. In particular, expression of BH3-only family members seems worthy of more intensive study in PD tissues. It would also be useful to corroborate and extend present findings with “activated” Bax. If specific Bcl2 proteins can be linked to cell death in PD, then the advances that have been made to find small molecule regulators of such proteins may ultimately prove useful for treatment of this disorder. Understanding the potential roles of Bcl2 family proteins in PD may also provide useful biomarkers as well as insight about the intracellular pathways that drive neuron death in this disease.

THE FINAL STEP: Is cell death in PD apoptotic?

Thus far, we have considered both proximal and distal events in neuron death associated with PD. Clearly, there remain many issues to be resolved, and this is no less so for the question of the final mechanism of neuronal death in PD. The answer is important in part because it will inform our interpretation of data relating to distal and proximal mechanisms and because it will consequently aid in the design of potential therapeutic strategies.

Apoptosis is typically identified by characteristic cell morphology, e.g. nuclear condensation and cell and nuclear pyknosis. These characteristics are not consistently seen in PD (232). Another characteristic apoptotic feature, DNA fragmentation, can be detected *in vivo* with TUNEL staining, which labels free ends of DNA. Studies that performed TUNEL labeling on brain samples have generated wildly variable results, ranging from no TUNEL-positive neurons observed in PD brain to a high percentage of TUNEL-positive neurons in both PD and control cases (232). Clearly, this technique is difficult to use reliably, and it is difficult to draw conclusions from these disparate results.

If neuron death does occur by an apoptotic mechanism in PD, detection of apoptotic neurons in PD is likely to be very difficult, given the relatively slow rate of SNpc dopaminergic cell loss and the rapid clearance of apoptotic cells. Therefore, several groups have looked for the expression of death-related gene products in PD that are associated with either the extrinsic or intrinsic apoptotic pathways. Again, there are conflicting results, with one study finding no difference in the immunostaining patterns of multiple Bcl2 family members, activated caspase-3, or Fas in PD vs. control neurons (188). However, other studies have demonstrated activation of caspase-3, caspase-8, as well as changes in Bcl2 family members (see prior section on Bcl2 proteins) in SNpc dopaminergic neurons in PD (225,227,232).

While the evidence in PD autopsy material is conflicting, the overwhelming majority of animal and *in vitro* models of PD demonstrate morphological apoptosis and activation of caspases. Neuronal cell death (typically *in vitro*) associated with several PD genes, including α-Syn, parkin, PINK1, DJ-1, and LRRK2, involves caspase activation (for example, refs 27,103, 117,233-235). Similarly, the PD-mimetic toxins MPTP, rotenone, and 6OHDA produce caspase activation and mitochondrial release of cytochrome c (for example, refs 73,101,236, 237). In many of these paradigms, though not all (194,238), cell death can be blocked with caspase inhibitors. The involvement of the JNK and cell cycle pathways in death induced by such treatments also favors an apoptotic mechanism.

Is there evidence for another pattern of cell death in PD? Necrotic cell death has not been observed in PD, nor would it be expected to play a large role in a slowly progressive neurodegenerative disorder. Interestingly, many of the toxins used to model PD, e.g. MPTP, will produce necrosis when given in high doses and/or over a relatively short period of time. Thus, similar insults may produce apoptosis and/or necrosis, depending on experimental conditions. Autophagic cell death, discussed previously, is another potential form of cell

demise. As mentioned previously, there is morphological evidence of autophagy in degenerating neurons in PD. However, given that autophagy can either suppress or promote to cell death, such findings are difficult to interpret.

Even if neuron death in PD were apoptotic, direct anti-apoptotic therapies would appear to face several challenges. Systemic application of global anti-apoptotics could interfere with physiologic cell death as well as suppression of tumors. Experimental evidence also suggests that in many cases, despite suppression of apoptotic pathways, cells exposed to stresses eventually die by alternative mechanisms (227). Finally, blockade of only the distal components of an apoptotic pathway may prevent death, but not preserve neuronal function. Such considerations argue for therapeutic strategies that address the more proximal causes of neuron degeneration in PD.

CONCLUDING REMARKS: Sorting facts from artifacts in PD-related neuron death

In summary, a number of diverse mechanisms have been implicated in the pathogenesis of PD. The key issue remains determining how relevant each one is to the disease itself. This is crucial for the development of therapeutic strategies. We believe that a few broad approaches could help in this regard. First, in post-mortem studies, areas outside the SNpc should be more routinely examined for expression of cell death-related markers. The neuroanatomical distribution of cell loss in PD could serve as a map for validating disease-related mechanisms. For example, the locus ceruleus is equally affected compared to the SNpc in PD, yet is examined in relatively few studies of the disease. Similar patterns of expression of a marker of interest in the locus ceruleus and SNpc would help substantiate the pathogenic relevance of that marker. In addition, according to the Braak staging scheme, PD affects progressively more rostral brain regions (3). Studying areas, such as the limbic system or even neocortex, with less extensive cell loss may reveal pathogenic mechanisms that occur at earlier stages of degeneration. Furthermore, neurodegeneration in non-nigral regions contributes to disabling non-motor symptoms in PD, such as dementia and depression. These non-motor symptoms have more limited treatment options compared to the motor symptoms, which can be treated successfully with dopaminergic therapy. Therefore, understanding neuronal loss outside the dopaminergic SNpc would contribute to the understanding of underlying mechanisms in PD, as well as lead to potential targets for addressing non-motor symptoms.

Second, further effort is needed to integrate the various proximal pathophysiological mechanisms that have been implicated in PD. For instance, does α-Syn aggregation contribute to oxidative stress and vice versa? Do these stresses reinforce each other in a loop? What about the relationships between these and all the other potential proximal events discussed here? Due to their inter-connectedness, it may be that irrespective of the initiating causes of neurodegeneration in PD, the same sets of proximal cellular responses will be ultimately activated.

Third, more work is needed to clarify the disease-relevant links between the more proximal insults and distal cell death pathways. Most of the work in this area has utilized toxin-based models. However, numerous *in vitro* and *in vivo* systems are now available that model PD based on more relevant gene abnormalities. Testing the importance of the distal pathways discussed in this review, such as JNK, p53, and specific Bcl2 family members, in these gene-based models would add important information regarding the relevance of these pathways to PD.

Translating insights about cell death mechanisms in PD into neuroprotective therapies has been difficult thus far. Many of these difficulties are likely related to our incomplete understanding

of the mechanisms of neuron degeneration in this disorder. However, there are a few practical points to consider. First, given the number of linked proximal and distal events that appear to contribute to neurodegeneration in PD, it seems likely that combination therapy will be necessary to achieve a meaningful slowing of disease progression. Second, a general issue is whether to block more proximally or distally in the cell death cascade. Intervening at a proximal point would be more likely to rescue neurons when there is the least amount of damage already done. However, there is a greater potential for parallel or redundant pathways to get around the blockade of an upstream event. Thus, efforts are needed to identify potential "bottlenecks" where inputs from different proximal pathways are integrated and for which there are limited alternative mechanisms to trigger cell death. Lastly, PD-related stresses on neurons not only activate responses that are destructive, but also those that are potentially neuroprotective. The ultimate fate of a given neuron in PD therefore appears to reflect the balance between these two types of events (239). Thus, although much effort has gone into identifying and suppressing destructive responses in PD, it may be equally fruitful to more fully recognize and exploit those that are protective.

Acknowledgments

Support for this review was provided in part by grants from the NIH-NNDS, Parkinson's Disease Foundation, and American Parkinson Disease Association.

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Table 1

Parkinson disease associated genes

Gene	Locus	Inheritance pattern	Clinical phenotype
α-Syn (alpha-synuclein)	PARK1, PARK4	Dominant, or increased copy number	Duplication, A30P: typical. Triplication, A53T: atypical.
Parkin	PARK2	Recessive (partial penetrance in heterozygotes?)	Young onset, typical
UCH-L1 (ubiquitin C-terminal hydrolase L1)	PARK5	Susceptibility (dominant?)	Typical
PINK1 (PTEN-induced kinase 1)	PARK6	Recessive	Young onset, typical
DJ-1	PARK7	Recessive	Young onset, typical
LRRK2 (leucine rich rpeat kinase 2)	PARK8	Dominant, incomplete penetrance	Typical
ATP13A2	PARK9	Recessive	Atypical
HtrA2/Omi	PARK13	Susceptibility	Typical
Glucocerebrosidase/ß-glucosidase		Susceptibility	Typical