Mechanistic Approaches to Parkinson's Disease Pathogenesis

Ranjita Betarbet[1]; Todd B. Sherer[1]; Donato A. Di Monte[2]; J. Timothy Greenamyre[1]

[1] Center for Neurodegenerative Disease and Department of Neurology, Emory University, Atlanta, Ga.
[2] Parkinson's Institute, Sunnyvale, Calif.

Parkinson's disease (PD) is a progressive neurological disorder marked by nigrostriatal dopaminergic degeneration and development of cytoplasmic proteinaceous aggregates known as Lewy bodies. Although the pathogenic mechanisms responsible for PD are not completely understood, many clues have come from biochemical, epidemiological, and genetic studies. Mutations in certain genes found in rare, familial cases of PD, such as α-synuclein and parkin, suggest a role for the ubiquitin-proteosome system and aberrant protein aggregation. Biochemical analyses have implicated mitochondrial dysfunction in PD. Epidemiological and animal model studies point to a role for environmental toxins, some of which are mitochondrial inhibitors. Mitochondrial dysfunction, resulting from either genetic defects, environmental exposures or an interaction between the two, may cause α-synuclein aggregation or neurodegeneration through oxidative stress or excitotoxicity. A better understanding of the mechanisms underlying PD should reveal novel therapeutic targets.

Brain Pathol 2002;12:499-510.

Introduction

Parkinson's disease (PD) is a late onset, progressive, neurodegenerative, movement disorder. Clinical symptoms of PD consist of tremor, rigidity and bradykinesia. The pathological hallmark of PD is the progressive and selective degeneration of the nigrostriatal pathway and dopaminergic cells of the substantia nigra. The subsequent dopamine deficiency in striatum is believed to underlie many of the clinical manifestations of PD (3, 21, 23, 33, 48).

An additional, important pathological feature of PD is the presence of cytoplasmic inclusions called Lewy bodies. Lewy bodies contain aggregates of many different proteins and are present in the dopaminergic neurons of substantia nigra and in brain regions such as the cerebral cortex and magnocellular basal forebrain nuclei (12). A major component of the Lewy body is a protein called α-synuclein (87). Mutations in the α-synuclein gene have been associated with rare familial cases of PD (74). However, Lewy bodies are positive for α-synuclein in the majority of idiopathic PD cases that lack α-synuclein mutations, suggesting a central role for α-synuclein protein in PD pathogenesis. Transgenic animal models have further implicated α-synuclein in the etiology of PD (26, 64). In addition to α-synuclein, mutations in 2 other genes, parkin and ubiquitin carboxy-terminal hydrolase L1, have been associated with familial PD and have suggested that dysfunctional protein degradation might be an important factor in the etiology of PD (67).

Mitochondrial dysfunction and subsequent oxidative stress have also been strongly implicated in the pathogenesis of PD. Several reports demonstrate modest but reproducible reductions in mitochondrial complex I function in a variety of tissues from PD patients, including brain, platelets, muscle and fibroblasts. This finding suggests a systemic complex I defect in PD (10, 15, 62, 69, 71, 79, 84). The role of mitochondria in PD has been further accentuated by the observation that MPP^+, the active metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and an inhibitor of complex I of the mitochondrial electron transport chain (ETC), causes an acute parkinsonian syndrome (41, 70, 94).

At several locations along the mitochondrial ETC, there are sites of "electron leaks." These electrons can combine with molecular oxygen and form reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2). The ROS can readily react with DNA, lipids and proteins and cause oxidative damage. Of particular relevance to PD is the site of electron leak within complex I of the ETC (36, 38, 95). Mounting evidence for oxidative stress and damage in brains of PD patients implicates oxidative damage as a mechanism of central importance in PD neurodegeneration.

Environmental toxins, including pesticides, have also been determined to be risk factors for PD based on epidemiological observations. Farming, living in rural areas, drinking well water and exposure to agricultural chemicals are associated with increased risk for PD (75, 76). Occupational exposure to certain metals, most

Corresponding author:
Dr R. Betarbet, Emory University, Center for Neurodegenerative diseases, Whitehead Biomedical Research Building, Room 505M, 615 Michael Street, Atlanta, GA 30322 (e-mail: rbetarb@emory.edu)

Benzimidazole	Hoe 110779
Bullactacin	Pyridaben
6-Chlorobenzothiadiazole	Pyrimidifen
Cyhalothrin	Sandoz 547A
Fenazaquin	Tebufenpyrad
Fenpyroximate	Thiangazole

From Degli Esposti (22) and Lummen (61)

Table 1. Pesticides known to inhibit Complex 1.

Compounds	**Source**
Rotenoids	Leguminosae plants
Piericidins	Streptomyces strains
Acetogenins	Annonacae plants (custard apple, paw-paw)
Antibiotics	Myxobacteria
Rhein	Rhubarb

From Degli Esposti (22)

Table 2. Natural compounds known to inhibit complex I.

notably manganese (32, 39, 57, 81), has been suggested as an additional environmental risk for PD.

Despite many years of research and numerous hypotheses, studies have failed to conclusively prove any specific cause for PD. It is commonly believed that a combination of genetic and environmental factors, converging on mitochondrial defects, oxidative stress and aberrant protein aggregation, account for most cases of PD. This review will therefore emphasize current hypotheses of how a combination of factors contribute to progressive neurodegeneration and parkinsonism.

PD and Environmental Toxins

Epidemiological studies indicate an association between numerous environmental factors and risk of developing Parkinson's disease. Some studies have linked geographical distribution of pesticide usage with prevalence of PD. For example, case-control surveys from several countries including the United States, Canada, Australia, Hong Kong, and Taiwan have shown statistically significant associations between pesticide exposures and PD (17, 29, 31, 39, 57, 68).

Experimental exposure of rodents to herbicides and pesticides further supports the involvement of environmental toxins in PD. Exposure of mice to the herbicides paraquat (13) and maneb (92) caused a dose-dependant decrease in dopaminergic nigral neurons and striatal dopaminergic innervation, followed by reduced ambulatory movement. The mechanism of action of paraquat is believed to involve oxidative stress and due to its structural similarity to MPP^+, its toxic effects may involve mitochondria. The observation that combined effects of parquat and maneb (93) have greater effects on the dopaminergic system than either of the chemicals alone suggests that exposure to a mixture of toxins may be relevant etiologically. The extent to which the mechanisms of action of environmental factors/toxins are related or independent is not clear.

Interestingly, many pesticides and herbicides share the common mechanism of causing mitochondrial dysfunction by inhibiting complex I (Table I). Many of these pesticides are used on a broad scale in commercial agriculture (22). In addition to synthetic compounds, there exist many natural substances that potently inhibit complex I (Table 2) and therefore have a potential role in PD pathogenesis (22). Thus various environmental factors converging on similar mechanisms of

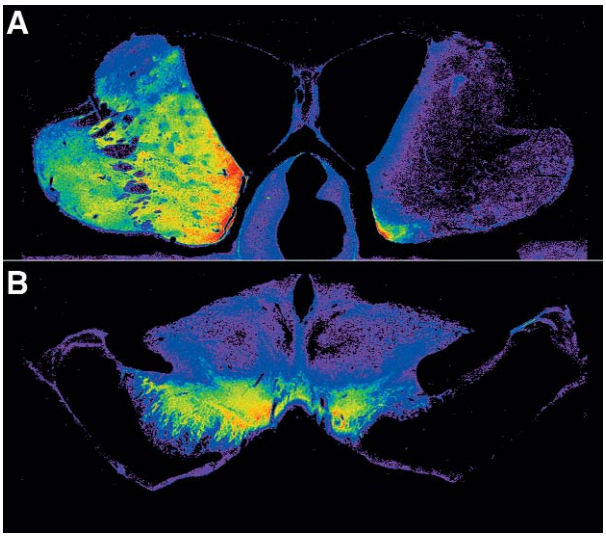

Figure 1. Photomicrographs of brain sections from a unilaterally MPTP-treated monkey. Sections through the striatum (**A**) and the substantia nigra (**B**) are stained for tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis. The gray levels of TH-immunoreactivity have been converted to a pseudocolor scale where in red depicts high and blue depicts low immunoreactivity. Note the markedly reduced TH-immunoreactivity in the lesioned striatum (top right) and lesioned substantia nigra (bottom right) versus the control striatum (top left) and control substantia nigra (bottom left).

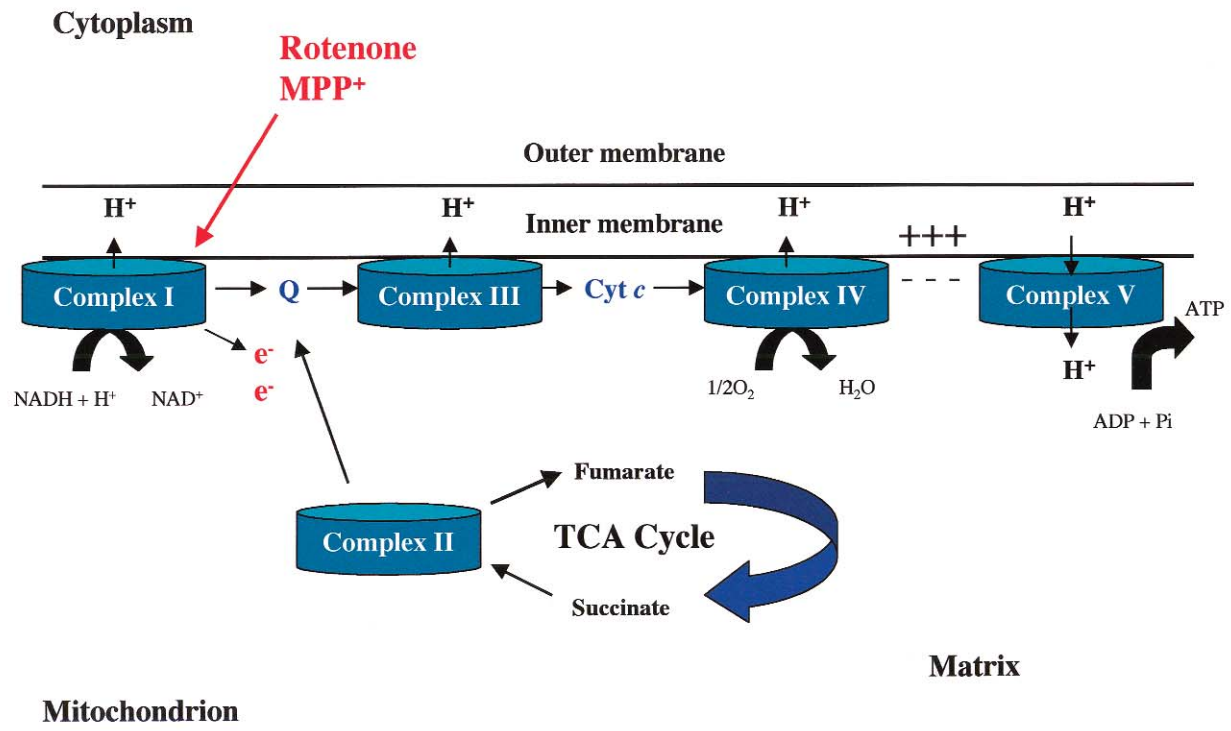

Figure 2. Schematic diagram of the mitochondrial ETC. Note the site of complex I inhibition by rotenone and MPP^+ and ROS production. Paraquat, due to its structural similarity to MPP^+, may also modify mitochondrial function at complex I.

action may eventually provide insights to PD pathogenesis.

To date, however, no environmental agent has been conclusively linked to PD pathogenesis. There are several explanations for this. Chronic low-grade exposure to toxins over many years may be essential for developing progressive neurodegeneration but might be very hard to detect. There may be long latent periods between toxin exposure and neurodegeneration such that clinical symptoms develop many years following exposure. Furthermore, genetic differences in the ability to metabolize causative environmental agents may explain the occurrence of PD in only some individuals exposed to a toxin (4, 68). As mentioned above, many potential toxins are naturally occurring plant or fungal products to which one may be exposed through water or food. It is therefore likely that an individual's cumulative lifetime exposure to a combination of environmental factors in association with genetic susceptibility or resistance, determines disease risk.

PD and Mitochondrial Dysfunction

MPTP and complex I defect. MPTP administration is one of the most common approaches to study parkinsonism in animal models. Exposure to MPTP results in selective, nigrostriatal, dopminergic degeneration (Figure 1). The experimental use of MPTP followed the inadvertent discovery that injections of an illicit meperidine analog resulted in acute, severe and permanent PD in several drug addicts. Langston and colleagues found MPTP to be responsible for these parkinsonian symptoms (53). Subsequent investigations showed that after administration, MPTP crosses the blood-brain barrier and is metabolized by monoamine oxidase B in astrocytes to its active metabolite 1-methyl-4-phenyl-2,3-dihydropyridinium ion (MPP^+). MPP^+ is selectively taken up into dopaminergic neurons via its affinity for the dopamine transporter and is thus selectively toxic to dopamine neurons (41). Furthermore, MPP^+ accumulates in millimolar concentrations in mitochondria and inhibits respiration at the level of complex I (Figure 2) (70, 94). Thus a selective, exogenous complex I toxin, specifically transported into dopaminergic neurons, could produce a parkinsonian syndrome in humans that was remarkably similar to "idiopathic PD." This finding suggests a role for mito-

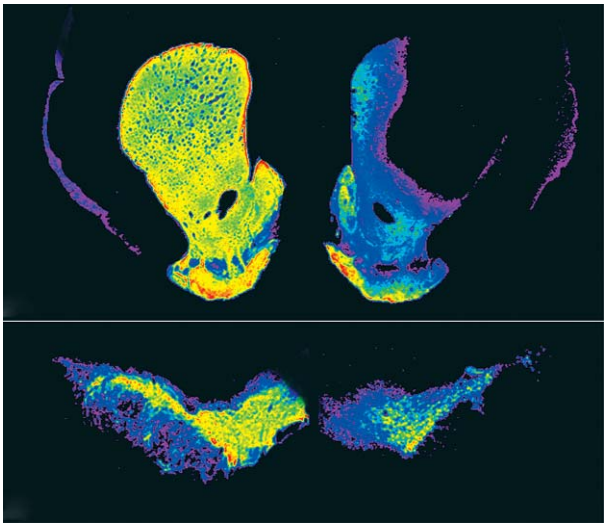

Figure 3. Photomicrographs of rat brain sections after rotenone administration. Sections through the striatum (**A**) and the substantia nigra (**B**) stained for tyrosine hydroxylase. The gray levels of TH-immunoreactivity have been converted to a pseudocolor scale where in red depicts high and blue depicts low immunoreactivity. Note the markedly reduced TH-immunoreactivity in the lesioned striatum (top right) and lesioned substantia nigra (bottom right) versus the control striatum (top left) and control substantia nigra (bottom left).

chondrial dysfunction and complex I defects in PD pathogenesis.

Complex I and PD. The mitochondrial electron transport chain (ETC) produces ATP through oxidative phosphorylation. This process involves the activity of 5 complexes, namely, I, II, III, IV and V, located along the inner mitochondrial membrane (Figure 2). Protein subunits of these complexes are nuclear encoded or encoded by the mitochondrial genome. Schapira and colleagues first reported selective complex I defects (other complexes were not affected) in substantia nigra of PD patients (79). Later on, reports indicated that the complex I defect is systemic in PD, affecting tissues outside the brain such as platelets, lymphocytes and muscle (8, 10, 15, 62, 69, 71, 84). The nature of this complex I defect—whether genetic or acquired—remains uncertain. Investigations with cytoplasmic hybrids (or cybrids) have implied that PD patients may express mutations in subunits of complex I-encoding mitochondrial DNA. Cybrid cells express mitochondrial DNA from PD patients and age-matched controls on a common nuclear background. Since cells with mtDNA from PD patients maintained the decreased complex I activity, the reduced complex I activity may be due to mutations in the mitochondrial genome (35, 90). However, efforts to find causative mutations have been unsuccessful. Therefore it is possible that systemic complex I defects observed in sporadic cases of PD could be due to acquired abnormalities in protein subunits encoded in the nuclear or mitochondrial genome or could result from environmental toxins that inhibit complex I.

Rotenone and PD. To address the fundamental question of how a systemic defect in complex I could result in highly selective and progressive degeneration of the dopaminergic nigrostriatal pathway, a novel model of PD was developed based on chronic and systemic inhibition of complex I using rotenone (7). Rotenone is a naturally occurring, high affinity complex I inhibitor, and is commonly used as an "organic" insecticide, or to kill nuisance fish in lakes. Additionally, rotenone is a lipophilic compound that easily crosses the blood brain barrier and gets into the brain rapidly. By systemically and chronically exposing rats to low doses of rotenone, a uniform inhibition of complex I was produced throughout brain. In this way, rotenone exposure differs from that of MPTP, which selectively inhibits complex I in dopaminergic neurons due to its dependence on the dopamine transporter. Despite uniform complex I inhibition, rotenone caused selective degeneration of the nigrostriatal dopaminergic pathway (Figure 3), selective striatal oxidative damage, and formation of ubiquitin and α-synuclein positive inclusions in nigral cells, which were similar to the Lewy bodies of PD. Behaviorally, rotenone-exposed rats were hypokinetic with flexed posture, similar to the stooped posture of PD patients. Some developed severe rigidity and a few had spontaneously shaking paws that were reminiscent of resting tremor in PD (7).

The rotenone model shows that features of PD can be produced by systemic complex I inhibition. This indicates that the nigrostriatal pathway is intrinsically and selectively sensitive to complex I dysfunction. In addition, the occurrence of complex I dysfunction in PD may further link environmental toxins like rotenone to the pathogenesis of PD. Many other environmental agents as mentioned before, affect mitochondrial function at complex I (Tables 1, 2). Furthermore, complex I impairment may predispose neurons to excitotoxicity and oxidative damage, both of which have been implicated in the pathogenesis of PD (2, 34, 82).

The rotenone model appears to be an accurate model in that systemic complex I inhibition results in specific, progressive and chronic degeneration of the nigrostriatal pathway similar to that observed in human PD. It also reproduces the neuronal inclusions and oxidative damage seen in PD. Thus, the rotenone model recapitulates

most of the mechanisms thought to be important in PD pathogenesis.

Excitotoxicity in PD. Complex I impairment associated with PD may predispose neurons to excitotoxic death. Depletion of cellular ATP levels, due to complex I dysfunction, can alter cellular homeostasis. Loss of ATP would reduce Na^+/K^+ ATPase function, resulting in partial neuronal depolarization and decreasing the voltage-dependent Mg^{2+} blockade of the *N*-methyl-D-aspartate (NMDA) glutamate receptor. Under these conditions, even normal levels of glutamate stimulation may cause excitotoxic activation of NMDA receptors and cause large intracellular calcium transients. These calcium transients may be lengthened or elevated by inadequate mitochondrial calcium buffering and by decreased calcium ATPase activity resulting from energy impairment. In summary, complex I dysfunction may result in excitotoxic activation of NMDA receptors rendering neurons vulnerable to this insult (34).

This situation is particularly dangerous in PD due to the neurochemistry of the basal ganglia circuitry. Decreased nigrostriatal dopaminergic input leads to overactivity of the subthalamic nucleus, which sends excitatory glutamatergic projections to the already damaged nigrostriatal neurons. This altered circuit may render nigrostriatal neurons vulnerable to excitotoxic insults. In fact, both pharmacological and surgical manipulations of the subthalamic nucleus protect substantia nigra in rodent models (9, 73).

Inappropriate activation of NMDA receptors may also influence mitochondrial function leading to mitochondrial calcium overload, mitochondrial depolarization, and production of reactive oxygen species (77, 89). These effects may result in a feed-forward cycle of mitochondrial impairment and may exacerbate the oxidative damage that results from complex I inhibition alone.

Oxidative damage and PD. During ATP production through oxidative phosphorylation, molecular oxygen is reduced to water at complex IV of the ETC (Figure 2). A small portion of this oxygen gets reduced non-enzymatically, to superoxide (O_2^-) and hydrogen peroxide (H_2O_2) by electrons that leak at sites in the ETC. One such site is complex I, upstream of the rotenone binding site. Partial inhibition of complex I, as produced by rotenone and as observed in PD, can enhance ROS production (36, 38, 95). PD cybrid cells also produce elevated ROS (16). Local ROS production can further damage complex I, resulting in a feed-forward cycle of complex I damage and ROS generation. Free radicals produce damage by reacting with DNA, lipids and proteins.

In addition to mitochondrial production, H_2O_2 is also produced as a by-product of dopamine metabolism and dopamine auto-oxidation (59), which can participate in a positive feedback loop responsible for progressive oxidative damage (42). Thus dopaminergic neurons and their nerve terminals, the primary targets in PD, are believed to exist in a constant state of oxidative stress. Cellular free radical scavenging systems, including glutathione (GSH) and superoxide dismutase (SOD), can, to a large extent, prevent ROS from damaging cellular and mitochondrial structures. Partial inhibition of complex I greatly increases ROS production (38), which may overwhelm such protective mechanisms. Conversely, depletion of GSH can cause oxidative damage to complex I and reduction in its activity (44).

Evidence for the involvement of oxidative stress in PD has also been obtained from PD patients. Increased lipid peroxidation and oxidative damage to DNA and proteins have been observed in substantia nigra of PD patients (24, 27, 42, 100). Decreased levels of glutathione have also been found in the substantia nigra of PD brains, further implicating oxidative stress (85). Oxidative damage in PD may not be selective to substantia nigra as elevated oxidative protein damage has also been reported throughout PD brain (1).

Animal models of PD have also suggested the involvement of oxidative stress. MPTP-treated mice demonstrate elevated levels of ROS, lipid peroxidation (36, 88) and 3-nitrotyrosine (65). Antioxidants and spin trap agents attenuated MPTP-induced toxicity (65, 80, 101). There is ample evidence for the involvement of oxidative stress in 6-hydroxydopamine (6-OHDA)-induced degeneration, another animal model of PD. Studies have demonstrated that the neurotoxic effects of 6-OHDA involves generation of hydrogen peroxide and hydroxyl radicals (78), reduction in GSH and SOD activity (72) and an increase in malondialdehyde levels in the striatum (52). In addition, it has been shown that 6-OHDA is toxic to mitochondrial complex I, and leads to production of superoxide free radicals (18, 36). The partial or even complete prevention of neurotoxic effects of 6-OHDA by prior administration of vitamin E (14) and the MAO-B inhibitor, selegiline (49), may also be regarded as indirect evidence for the formation of free radicals and involvement of oxidative stress. Furthermore, the in vitro rotenone model of PD has also suggested the involvement of oxidative stress in neurodegeneration. Neuroblastoma cells, chronically exposed to rotenone

have demonstrated reduced GSH and elevated oxidative damage to DNA and proteins prior to cell death (82).

As mentioned previously, peroxynitrite (ONOO-) formation has also been implicated in PD pathogenesis. Peroxynitrite is a highly reactive oxidant formed by the reaction of nitric oxide with superoxide anion. Reaction of proteins with peroxynitrite results in modification of proteins at tyrosine residues (3-nitrotyrosines) and brains from PD patients have shown elevated levels of 3-nitrotyrosine suggesting that protein nitration may play a role in the neurodegeneration in PD (5, 30).

The oxidative stress hypothesis for PD provides a potential explanation for the selective toxicity observed in dopaminergic neurons. From the numerous lines of evidence mentioned above it appears that the dopaminergic cells in PD are under tremendous oxidative stress: oxidative stress as a consequence of systemic mitochondrial complex I inhibition, in addition to oxidative stress from feed- forward cycles of dopamine metabolism and dopamine auto-oxidation. It is therefore hypothesized that dopaminergic cells are selectively vulnerable to complex I toxins. However, why the nigrostriatal dopamine system is selectively affected when other dopamine systems are resistant is as yet unknown.

PD and Aberrant Protein Aggregation

Lewy bodies, prominent in substantia nigra of PD patients, are proteinaceous accumulations of neurofilaments, α-synuclein fibrils, ubiquitin, ubiquitin carboxy-terminal hydrolase L1 (UCHL1), parkin, proteasomal elements, nitrated proteins (including α-synuclein) and numerous, probably as yet unidentified, proteins (11). Various components of Lewy bodies have been associated with genetic mutations in familial PD (19, 43, 56, 83). Therefore, it has been suggested that Lewy bodies, (whether causative or secondary to PD pathogenesis) may be crucial in providing clues about pathogenesis. In addition, genetic mutations linked to familial PD also point to the possibility that altered protein conformation and/or degradation could be a key and a common factor in the degenerative process in sporadic PD. In fact, dysfunctional protein degradation has recently been proposed as being an important player in the degenerative process that occurs in PD (67).

The ubiquitin-proteasome system (UPS) is essential for non-lysosomal degradation and clearance of abnormal (ie, mutated or oxidatively damaged) proteins. Through a series of enzyme-mediated reactions, proteins are first identified and then linked with multiple ubiquitin molecules as a signal for degradation. Activated ubiquitin is generated by a ubiquitin-activating enzyme (E1) through an ATP-dependent mechanism. It is then transferred to ubiquitin-conjugating enzymes (E2) and ligated to lysine residues of protein substrates in a reaction catalysed by many different ubiquitin protein ligases (E3), such as *parkin*, that, together with specific E2s, ensure specific protein targeting. Ubiquitin-protein conjugates are subsequently recognized and degraded by 26S proteasomes, which are multi-subunit proteases found in eukaryotic cells. The degraded products are short peptide fragments and amino acids that can be recycled to produce new proteins. At the same time, the polyubiquitin chains are disassembled by ubiquitin carboxy-terminal hydrolases (such as UCHL1) to produce monomeric ubiquitin molecules that re-enter the UPS pathway.

Mutation in 3 different genes associated with familial PD link impaired protein degradation to PD pathogenesis. Deletion or point mutations in the *parkin* gene cause autosomal recessive, juvenile parkinsonism (AR-JP). AR-JP is characterized by juvenile onset, prolonged survival, L-DOPA responsiveness and loss of pars compacta neurons. Cytoplamic inclusions such as Lewy bodies or neurofibrillary tangles however, are rare in these patients (25, 37, 47, 60). Parkin is a ubiquitin-protein ligase whose activity is lost with the disease-causing mutation (83). Thus, in AR-JP patients, the ability of the UPS to clear abnormal proteins is impaired. Toxicity, due to accumulation of improperly degraded proteins, could be responsible for dopaminergic cell death in substantia nigra. A second mutation associated with the UPS was identified in members of another kindred with early-onset, autosomal dominant, typical PD. These patients showed a mutation in the gene for UCH-L1. Beneficial responses to L-DOPA treatment support the diagnosis of PD in these cases but it is unknown if these individuals have Lewy body pathology (56). Loss of UCHL1 activity could lead to reduced labeling with ubiquitin and impaired clearance of abnormal proteins and subsequently to neurodegeneration. Association of PD with *parkin* and *UCH-L1* mutations indicate that altered UPS function may have a role in PD pathogenesis.

Defective UPS or increased production of abnormal proteins that fail to be degraded by the UPS would result in abnormal protein accumulation. Mutated, misfolded, and oxidatively damaged proteins that accumulate have a tendency to aggregate and form insoluble inclusions (45, 51, 99) such as Lewy bodies. Evidence in support of impaired UPS and protein aggregation, comes from the occurrence of elevated levels of oxidatively damaged proteins (1), increased protein aggrega-

tion (58) and impaired proteolysis (66) in substantia nigra of patients with sporadic PD. In addition, formation of α-synuclein positive cytoplasmic inclusions in dopamine neurons in animal models of PD (7, 26, 64) and from the research done on α-synuclein (6) suggests the involvement of impaired protein degradation in PD. However, the exact role of the UPS remains unclear.

The neuronal protein α-synuclein has been directly linked to PD pathogenesis. Two different point mutations in α-synuclein gene, A53T (19) and A30P (43), have been identified in separate families with dominantly transmitted PD. α-Synuclein is the major component of Lewy bodies, even in the more common forms of PD that are not associated with α-synuclein mutations. α-Synuclein mutations cause dominantly inherited, early onset PD with L-DOPA responsiveness and pathology that includes Lewy bodies and severe loss of neurons in the substantia nigra pars compacta. Evidence for the role of α-synuclein in PD pathogenesis comes from transgenic animal models for PD that express the wild type or the mutated α-synuclein (26, 64). Transgenic mice overexpressing human α-synuclein demonstrate a number of features of PD, including loss of nigrostriatal dopaminergic terminals in the striatum, development of α-synuclein and ubiquitin-positive cytoplasmic inclusions, and motor impairments (64). Dopaminergic and behavioral defects were only observed in the high expressing line of transgenic mice, suggesting that a critical threshold of α-synuclein expression may be required for the dopaminergic and behavioral abnormalities. Other transgenic mice had α-synuclein-positive inclusions and motor deficits, but there was no evidence of nigrostriatal dopaminergic degeneration. In fact, in these mice, neurons of the brainstem and motor neurons were most vulnerable (97). The conflicting results observed in α-synuclein transgenic mice suggest that other factors, such as background strain, in addition to α-synuclein pathology may be involved in PD pathogenesis. Furthermore, it should be kept in mind that one of the mutations associated with the human PD is normally expressed in mice. How mutations in α-synuclein can induce dopamine cell death is not known; however, it has been speculated that altered protein degradation may have a role in the underlying mechanisms leading to neurodegeneration.

Chemical analysis suggests that wild-type α-synuclein has a tendency to form insoluble aggregates. Changes in α-synuclein, whether following mutations (19, 43) or oxidation (28, 86), cause the protein to become less soluble and further promote its aggregation. The A30P mutation appears to disrupt binding of α-synuclein to vesicles, thus

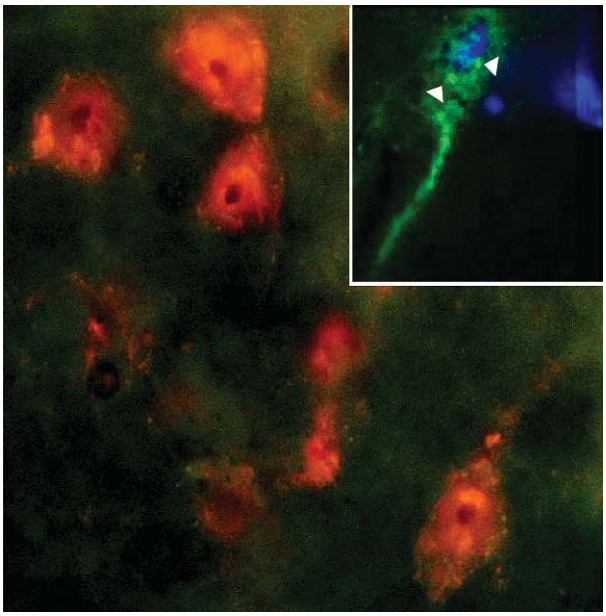

Figure 4. Paraquat-induced protein aggregation. Coronal sections from the mouse midbrain were either stained with hematoxylin and thioflavine S or bisbenzimide (inset) and an antibody against α-synuclein. Treatment of animals with a regimen of 3 consecutive weekly injections of 10 mg/kg paraquat caused the formation of thioflavine S-positive and (inset) α-synuclein-imunoreactive aggregates within neurons of the substantia nigra pars compacta. Arrowheads (inset) indicate two perinuclear deposits. In control animals injected with saline, no thioflavine S staining was detected above background and α-synuclein immunoreactivity was diffuse throughout the substantia nigra and stained mostly neuronal fibers (not shown).

making excess protein available for aggregates (43). The A53T mutation produces a protein that is more prone to spontaneous aggregation than wild-type protein (19). Furthermore, impaired degradation of altered α-synuclein protein can exacerbate its accumulation in neurons. Also, unusual folding patterns may not permit the modified α-synuclein to undergo degradation. Studies have also shown that oxidatively modified α-synuclein is also more prone to aggregate than the native protein (86). Dopamine, itself, may also influence α-synuclein aggregation. Dopamine-α-synuclein adducts may stabilize α-synuclein and cause it to selectively accumulate in dopaminergic cells (20). In these cell-free systems, pesticide mitochondrial inhibitors such as rotenone and paraquat also induced α-synuclein aggregation (96).

α-Synuclein appears to be selectively nitrated in PD (28). Furthermore, complex I toxins such as MPTP (98) and rotenone (82) also cause an apparent upregulation in α-synuclein levels in specific brain regions. Exposure to the herbicide paraquat in mice was accompanied by α-

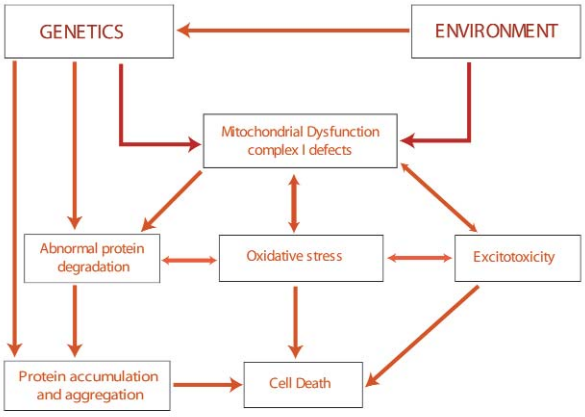

Figure 5. Suggested mechanisms involved in PD pathogenesis. Genetic mutations (ie, α-synuclein, mitochondrial DNA), whether inherited or acquired through environmental exposures, may result in altered mitochondrial function, abnormal protein degradation and/or protein aggregation. Many environmental factors including rotenone and paraquat can also cause mitochondrial impairment. Mitochondrial dysfunction can cause abnormal protein degradation, oxidative stress or render cells vulnerable to excitotoxicity. Finally, oxidative stress, excitotoxicity, or protein aggregation can lead directly or indirectly to cell death.

synuclein aggregation in neurons of substantia nigra. These aggregates were also positive for thioflavine S (Figure 4) suggesting the formation of α-synuclein positive inclusions similar to amyloid fibrils (63). These observations further strengthen the role of environmental agents in PD pathogenesis and indicate abnormal protein degradation as a potential mechanism for PD pathogenesis (6).

Evidence from in vitro studies using neuronal cell lines over-expressing α-synuclein (54, 55), have further strengthened the role of α-synuclein or rather its mutated/altered, aggregated form, in PD pathogenesis. Exposure to complex I inhibitors such as rotenone caused acute aggregation of over-expressed α-synuclein (54, 55), and chronic accumulation of endogenous α-synuclein (82). These results suggest an interaction between mitochondrial function and α-synuclein pathology. Additionally, α-synuclein can itself inhibit mitochondrial function and induce oxidative stress (40). Elevated α-synuclein levels sensitized cells to exogenous oxidative challenges and dopamine toxicity (46, 50, 91).

Conclusions

There are numerous lines of evidence implicating various factors in the pathogenesis of PD, but to date there is no "smoking gun" pointing to a specific cause. The absence of a specific cause strengthens the common belief that a combination of environmental factors and genetic susceptibilities account for most sporadic cases of PD. Epidemiological and biochemical studies on PD patients have suggested the involvement of a low-grade systemic complex I defect as one of the underlying mechanisms in PD pathogenesis, due to chronic exposure to environmental toxins, or from genetic mutations of nuclear or mitochondrial DNA, or from a combination of genetic and environmental factors. The range of vulnerabilities would depend on genetic differences in the ability to metabolize or activate environmental toxins. Mitochondrial defects would further result in oxidative damage and/or excitotoxicity (Figure 5). Oxidative stress from complex I inhibition would augment the free radical production that results from dopamine metabolism and dopamine auto-oxidation, in dopaminergic cells. The cumulative effects of oxidative stress would result in a feed-forward cycle that would further damage complex I of the mitochondrial ETC (Figure 5), and dopamine metabolism in dopaminergic cells.

Oxidation of α-synuclein and its subsequent aggregation in cells is one example of an oxidative stress-related alteration. In addition, α-synuclein accumulation suggests a defective protein degradation and clearance pathway, possibly involving the UPS, and again resulting in a feed-forward cycle wherein increased α-synuclein levels would lead to protein accumulation and aggregation (Figure 5).

A more complete understanding of PD pathogenesis will uncover novel targets for rational drug therapy. However, even with our incomplete understanding of PD etiology there are a number of potential avenues for treatment. These include, but are not limited to, brain-permeable antioxidants and spin trap agents, glutamate receptor antagonists, and compounds that prevent protein aggregation. Testing of these compounds in accurate animal models of PD could uncover important clues to the mechanisms that underlie PD neurodegeneration.

References

1. Alam ZI, Daniel SE, Lees AJ, Marsden DC, Jenner P, Halliwell B (1997) A generalised increase in protein carbonyls in the brain in Parkinson's but not incidental Lewy body disease. *J Neurochem* 69:1326-1329.
2. Albin RL, Greenamyre JT (1992) Alternative excitotoxic hypotheses. *Neurology* 42:733-738.
3. Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci* 12:366-375.

4. Barbeau A, Cloutier T, Roy M, Plasse L, Paris S, Poirier J (1985) Ecogenetics of Parkinson's disease: 4-hydroxylation of debrisoquine. *Lancet* 2:1213-1216.
5. Beckman JS, Koppenol WH (1996) Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol* 271:C1424-1237.
6. Bennett MC, Bishop JF, Leng Y, Chock PB, Chase TN, Mouradian MM (1999) Degradation of alpha-synuclein by proteasome. *J Biol Chem* 274:33855-33858.
7. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* 3:1301-1306.
8. Bindoff LA, Birch-Machin M, Cartlidge NE, Parker WD, Jr, Turnbull DM (1989) Mitochondrial function in Parkinson's disease [letter; comment]. *Lancet* 2:49.
9. Blandini F, Nappi G, Greenamyre JT (2001) Subthalamic infusion of an NMDA antagonist prevents basal ganglia metabolic changes and nigral degeneration in a rodent model of Parkinson's disease. *Ann Neurol* 49:525-529.
10. Blin O, Desnuelle C, Rascol O, Borg M, Peyro Saint Paul H, Azulay JP, Bille F, Figarella D, Coulom F, Pellissier JF et al (1994) Mitochondrial respiratory failure in skeletal muscle from patients with Parkinson's disease and multiple system atrophy. *J Neurol Sci* 125:95-101.
11. Braak H, Braak E (2000) Pathoanatomy of Parkinson's disease. *J Neurol* 247 Suppl 2:II3-10.
12. Braak H, Braak E, Yilmazer D, Schultz C, de Vos RA, Jansen EN (1995) Nigral and extranigral pathology in Parkinson's disease. *J Neural Transm* Suppl 46:15-31.
13. Brooks AI, Chadwick CA, Gelbard HA, Cory-Slechta DA, Federoff HJ (1999) Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. *Brain Res* 823:1-10.
14. Cadet JL, Katz M, Jackson-Lewis V, Fahn S (1989) Vitamin E attenuates the toxic effects of intrastriatal injection of 6- hydroxydopamine (6-OHDA) in rats: behavioral and biochemical evidence. *Brain Res* 476:10-15.
15. Cardellach F, Marti MJ, Fernandez-Sola J, Marin C, Hoek JB, Tolosa E, Urbano-Marquez A (1993) Mitochondrial respiratory chain activity in skeletal muscle from patients with Parkinson's disease. *Neurology* 43:2258-2262.
16. Cassarino DS, Fall CP, Swerdlow RH, Smith TS, Halvorsen EM, Miller SW, Parks JP, Parker WD, Jr, Bennett JP, Jr. (1997) Elevated reactive oxygen species and antioxidant enzyme activities in animal and cellular models of Parkinson's disease. *Biochim Biophys Acta* 1362:77-86.
17. Chan DK, Woo J, Ho SC, Pang CP, Law LK, Ng PW, Hung WT, Kwok T, Hui E, Orr K, Leung MF, Kay R (1998) Genetic and environmental risk factors for Parkinson's disease in a Chinese population. *J Neurol Neurosurg Psychiatry* 65:781-784.
18. Cleeter MW, Cooper JM, Schapira AH (1992) Irreversible inhibition of mitochondrial complex I by 1-methyl-4-phenylpyridinium: evidence for free radical involvement. *J Neurochem* 58:786-789.
19. Conway KA, Harper JD, Lansbury PT (1998) Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease. *Nat Med* 4:1318-1320.
20. Conway KA, Rochet JC, Bieganski RM Lansbury PT, Jr, (2001) Kinetic stabilization of the alpha-synuclein protofibril by a dopamine- alpha-synuclein adduct. *Science* 294:1346-1349.
21. Crossman AR (1989) Neural mechanisms in disorders of movement. *Comp Biochem Physiol* A 93:141-149.
22. Degli Esposti M (1998) Inhibitors of NADH-ubiquinone reductase: an overview. *Biochim Biophys Acta* 1364:222-235.
23. DeLong MR (1990) Primate models of movement disorders of basal ganglia origin. *Trends Neurosci* 13:281-285.
24. Dexter DT, Carter CJ, Wells FR, Javoy-Agid F, Agid Y, Lees A, Jenner P, Marsden CD (1989) Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *J Neurochem* 52:381-389.
25. Farrer M, Chan P, Chen R, Tan L, Lincoln S, Hernandez D, Forno L, Gwinn-Hardy K, Petrucelli L, Hussey J, Singleton A, Tanner C, Hardy J, Langston JW (2001) Lewy bodies and parkinsonism in families with parkin mutations. *Ann Neurol* 50:293-300.
26. Feany MB, Bender WW (2000) A Drosophila model of Parkinson's disease. *Nature* 404:394-398.
27. Floor E, Wetzel MG (1998) Increased protein oxidation in human substantia nigra pars compacta in comparison with basal ganglia and prefrontal cortex measured with an improved dinitrophenylhydrazine assay. *Journal of Neurochemistry* 70:268-275.
28. Giasson BI, Duda JE, Murray IV, Chen Q, Souza JM, Hurtig HI, Ischiropoulos H, Trojanowski JQ, Lee VM (2000) Oxidative damage linked to neurodegeneration by selective alpha- synuclein nitration in synucleinopathy lesions. *Science* 290:985-989.
29. Golbe LI, Farrell TM, Davis PH (1990) Follow-up study of early-life protective and risk factors in Parkinson's disease. *Mov Disord* 5:66-70.
30. Good PF, Hsu A, Werner P, Perl DP, Olanow CW (1998) Protein nitration in Parkinson's disease. *J Neuropathol Exp Neurol* 57:338-342.
31. Gorell JM, Johnson CC, Rybicki BA, Peterson EL, Richardson RJ (1998) The risk of Parkinson's disease with exposure to pesticides, farming, well water, and rural living. *Neurology* 50:1346-1350.
32. Gorell JM, Rybicki BA, Cole Johnson C, Peterson EL (1999) Occupational metal exposures and the risk of Parkinson's disease. *Neuroepidemiology* 18:303-308.
33. Greenamyre JT (1993) Glutamate-dopamine interactions in the basal ganglia: relationship to Parkinson's disease. *J Neural Transm Gen Sect* 91:255-269.
34. Greene JG, Greenamyre JT (1996) Bioenergetics and glutamate excitotoxicity. *Prog Neurobiol* 48:613-634.
35. Gu M, Cooper JM, Taanman JW, Schapira AH (1998) Mitochondrial DNA transmission of the mitochondrial defect in Parkinson's disease. *Ann Neurol* 44:177-186.
36. Hasegawa E, Takeshige K, Oishi T, Murai Y, Minakami S (1990) 1-Methyl-4-phenylpyridinium (MPP+) induces NADH-dependent superoxide formation and enhances NADH-dependent lipid peroxidation in bovine heart submitochondrial particles. *Biochem Biophys Res Commun* 170:1049-1055.

37. Hattori N, Matsumine H, Asakawa S, Kitada T, Yoshino H, Elibol B, Brookes AJ, Yamamura Y, Kobayashi T, Wang M, Yoritaka A, Minoshima S, Shimizu N, Mizuno Y (1998) Point mutations (Thr240Arg and Gln311Stop) [correction of Thr240Arg and Ala311Stop] in the Parkin gene [published erratum appears in Biochem Biophys Res Commun 1998 Oct 20;251(2):666]. *Biochem Biophys Res Commun* 249:754-758.

38. Hensley K, Pye QN, Maidt ML, Stewart CA, Robinson KA, Jaffrey F, Floyd RA (1998) Interaction of alpha-phenyl-N-tert-butyl nitrone and alternative electron acceptors with complex I indicates a substrate reduction site upstream from the rotenone binding site. *J Neurochem* 71:2549-2557.

39. Hertzman C, Wiens M, Bowering D, Snow B, Calne D (1990) Parkinson's disease: a case-control study of occupational and environmental risk factors. *Am J Ind Med* 17:349-355.

40. Hsu LJ, Sagara Y, Arroyo A, Rockenstein E, Sisk A, Mallory M, Wong J, Takenouchi T, Hashimoto M, Masliah E (2000) alpha-synuclein promotes mitochondrial deficit and oxidative stress. *American Journal of Pathology* 157:401-410.

41. Javitch JA, D'Amato RJ, Strittmatter SM, Snyder SH (1985) Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc Natl Acad Sci U S A* 82:2173-2177.

42. Jenner P (1998) Oxidative mechanisms in nigral cell death in Parkinson's disease. Mov Disord 13:24-34.

43. Jensen PH, Nielsen MS, Jakes R, Dotti CG, Goedert M (1998) Binding of alpha-synuclein to brain vesicles is abolished by familial Parkinson's disease mutation. *J Biol Chem* 273:26292-26294.

44. Jha N, Jurma O, Lalli G, Liu Y, Pettus EH, Greenamyre JT, Liu RM, Forman HJ, Andersen JK (2000) Glutathione depletion in PC12 results in selective inhibition of mitochondrial complex I activity. Implications for Parkinson's disease. *J Biol Chem* 275:26096-260101.

45. Johnston JA, Ward CL, Kopito RR (1998) Aggresomes: a cellular response to misfolded proteins. *J Cell Biol* 143:1883-1898.

46. Kanda S, Bishop JF, Eglitis MA, Yang Y, Mouradian MM (2000) Enhanced vulnerability to oxidative stress by alpha-synuclein mutations and C-terminal truncation. *Neuroscience* 97:279-284.

47. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392:605-608.

48. Klockgether T, Turski L (1989) Excitatory amino acids and the basal ganglia: implications for the therapy of Parkinson's disease. *Trends Neurosci* 12:285-286.

49. Knoll J (1986) The pharmacology of (-)deprenyl. *J Neural Transm* Suppl 22:75-89.

50. Ko L, Mehta ND, Farrer M, Easson C, Hussey J, Yen S, Hardy J, Yen SH (2000) Sensitization of neuronal cells to oxidative stress with mutated human alpha-synuclein [In Process Citation]. *J Neurochem* 75:2546-2554.

51. Kopito RR (2000) Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol* 10:524-530.

52. Kumar R, Agarwal AK, Seth PK (1995) Free radical-generated neurotoxicity of 6-hydroxydopamine. *J Neurochem* 64:1703-1707.

53. Langston JW, Ballard P, Tetrud JW, Irwin I (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 219:979-980.

54. Lee HJ, Choi C, Lee SJ (2002(a)) Membrane-bound alpha-synuclein has a high aggregation propensity and the ability to seed the aggregation of the cytosolic form. *J Biol Chem* 277:671-676.

55. Lee HJ, Shin SY, Choi C, Lee YH, Lee SJ (2002(b)) Formation and removal of alpha-synuclein aggregates in cells exposed to mitochondrial inhibitors. *J Biol Chem* 277:5411-5417.

56. Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, Harta G, Brownstein MJ, Jonnalagada S, Chernova T, Dehejia A, Lavedan C, Gasser T, Steinbach PJ, Wilkinson KD, Polymeropoulos MH (1998) The ubiquitin pathway in Parkinson's disease [letter]. *Nature* 395:451-452.

57. Liou HH, Tsai MC, Chen CJ, Jeng JS, Chang YC, Chen SY, Chen RC (1997) Environmental risk factors and Parkinson's disease: a case-control study in Taiwan. *Neurology* 48:1583-1588.

58. Lopiano L, Fasano M, Giraudo S, Digilio G, Koenig SH, Torre E, Bergamasco B, Aime S (2000) Nuclear magnetic relaxation dispersion profiles of substantia nigra pars compacta in Parkinson's disease patients are consistent with protein aggregation. *Neurochem Int* 37:331-336.

59. Lotharius J, O'Malley KL (2000) The parkinsonism-inducing drug 1-methyl-4-phenylpyridinium triggers intracellular dopamine oxidation. A novel mechanism of toxicity. *J Biol Chem* 275:38581-38588.

60. Lucking CB, Abbas N, Durr A, Bonifati V, Bonnet AM, de Broucker T, De Michele G, Wood NW, Agid Y, Brice A (1998) Homozygous deletions in parkin gene in European and North African families with autosomal recessive juvenile parkinsonism. The European Consortium on Genetic Susceptibility in Parkinson's Disease and the French Parkinson's Disease Genetics Study Group [letter]. *Lancet* 352:1355-1356.

61. Lummen P (1998) Complex I inhibitors as insecticides and acaricides. *Biochim Biophys Acta* 1364:287-296.

62. Mann VM, Cooper JM, Krige D, Daniel SE, Schapira AH, Marsden CD (1992) Brain, skeletal muscle and platelet homogenate mitochondrial function in Parkinson's disease. *Brain* 115:333-342.

63. Manning-Bog AB, McCormack AL, Li J, Uversky VN, Fink AL, Di Monte DA (2002) The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and alpha-synuclein. *J Biol Chem* 277:1641-1644.

64. Masliah E, Rockenstein E, Veinbergs I, Mallory M, Hashimoto M, Takeda A, Sagara Y, Sisk A, Mucke L (2000) Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. *Science* 287:1265-1269.

65. Matthews RT, Klivenyi P, Mueller G, Yang L, Wermer M, Thomas CE, Beal MF (1999) Novel free radical spin traps protect against malonate and MPTP neurotoxicity. *Exp Neurol* 157:120-126.

66. McNaught KS, Jenner P (2001) Proteasomal function is impaired in substantia nigra in Parkinson's disease. *Neurosci Lett* 297:191-194.

67. McNaught KS, Olanow CW, Halliwell B, Isacson O, Jenner P (2001) Failure of the ubiquitin-proteasome system in Parkinson's disease. *Nat Rev Neurosci* 2:589-594.

68. Menegon A, Board PG, Blackburn AC, Mellick GD, Le Couteur DG (1998) Parkinson's disease, pesticides, and glutathione transferase polymorphisms [see comments]. *Lancet* 352:1344-1346.

69. Mizuno Y, Yoshino H, Ikebe S, Hattori N, Kobayashi T, Shimoda-Matsubayashi S, Matsumine H, Kondo T (1998) Mitochondrial dysfunction in Parkinson's disease. *Ann Neurol* 44:S99-109.

70. Nicklas WJ, Vyas I, Heikkila RE (1985) Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4- phenyl-pyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl- 1,2,5,6-tetrahydropyridine. *Life Sci* 36:2503-2508.

71. Parker WD, Jr., Boyson SJ, Parks JK (1989) Abnormalities of the electron transport chain in idiopathic Parkinson's disease. *Ann Neurol* 26:719-723.

72. Perumal AS, Gopal VB, Tordzro WK, Cooper TB, Cadet JL (1992) Vitamin E attenuates the toxic effects of 6-hydroxydopamine on free radical scavenging systems in rat brain. *Brain Res Bull* 29:699-701.

73. Piallat B, Benazzouz A, Benabid AL (1996) Subthalamic nucleus lesion in rats prevents dopaminergic nigral neuron degeneration after striatal 6-OHDA injection: behavioural and immunohistochemical studies. *Eur J Neurosci* 8:1408-1414.

74. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease [see comments]. *Science* 276:2045-2047.

75. Priyadarshi A, Khuder SA, Schaub EA, Priyadarshi SS (2001) Environmental risk factors and Parkinson's disease: a metaanalysis. *Environ Res* 86:122-127.

76. Priyadarshi A, Khuder SA, Schaub EA, Shrivastava S (2000) A meta-analysis of Parkinson's disease and exposure to pesticides. *Neurotoxicology* 21:435-440.

77. Reynolds IJ, Hastings TG (1995) Glutamate induces the production of reactive oxygen species in cultured forebrain neurons following NMDA receptor activation. *J Neurosci* 15:3318-27.

78. Sachs C, Jonsson G (1975) Mechanisms of action of 6-hydroxydopamine. *Biochem Pharmacol* 24:1-8.

79. Schapira AH, Cooper JM, Dexter D, Jenner P, Clark JB, Marsden CD (1989) Mitochondrial complex I deficiency in Parkinson's disease [letter] [see comments]. *Lancet* 1:1269.

80. Schulz JB, Henshaw DR, Matthews RT, Beal MF (1995) Coenzyme Q10 and nicotinamide and a free radical spin trap protect against MPTP neurotoxicity. *Exp Neurol* 132:279-283.

81. Semchuk KM, Love EJ, Lee RG (1993) Parkinson's disease: a test of the multifactorial etiologic hypothesis. *Neurology* 43:1173-1180.

82. Sherer TB, Betarbet R, Stout AK, Lund S, Baptista M, Panov AV, Cookson MR, Greenamyre JT (2002) An in vitro model of Parkinson's Disease: Linking mitochondrial impairment to altered alpha-synuclein metabolism and oxidative damage. *J Neurosci* 22:7006-7015.

83. Shimura H, Hattori N, Kubo S, Mizuno Y, Asakawa S, Minoshima S, Shimizu N, Iwai K, Chiba T, Tanaka K, Suzuki T (2000) Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nat Genet* 25:302-305.

84. Shoffner JM, Watts RL, Juncos JL, Torroni A, Wallace DC (1991) Mitochondrial oxidative phosphorylation defects in Parkinson's disease [see comments]. *Ann Neurol* 30:332-339.

85. Sian J, Dexter DT, Lees AJ, Daniel S, Agid Y, Javoy-Agid F, Jenner P, Marsden CD (1994) Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. *Ann Neurol* 36:348-355.

86. Souza JM, Giasson BI, Chen Q, Lee VM, Ischiropoulos H (2000) Dityrosine cross-linking promotes formation of stable alpha -synuclein polymers. Implication of nitrative and oxidative stress in the pathogenesis of neurodegenerative synucleinopathies. *J Biol Chem* 275:18344-18349.

87. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M (1997) Alpha-synuclein in Lewy bodies [letter]. *Nature* 388:839-840.

88. Sriram K, Pai KS, Boyd MR, Ravindranath V (1997) Evidence for generation of oxidative stress in brain by MPTP: in vitro and in vivo studies in mice. *Brain Res* 749:44-52.

89. Stout AK, Raphael HM, Kanterewicz BI, Klann E, Reynolds IJ (1998) Glutamate-induced neuron death requires mitochondrial calcium uptake. *Nat Neurosci* 1:366-373.

90. Swerdlow RH, Parks JK, Miller SW, Tuttle JB, Trimmer PA, Sheehan JP, Bennett JP, Jr, Davis RE, Parker WD, Jr (1996) Origin and functional consequences of the complex I defect in Parkinson's disease. *Ann Neurol* 40:663-671.

91. Tabrizi SJ, Orth M, Wilkinson JM, Taanman JW, Warner TT, Cooper JM, Schapira AH (2000) Expression of mutant alpha-synuclein causes increased susceptibility to dopamine toxicity. *Hum Mol Genet* 9:2683-2689.

92. Takahashi RN, Rogerio R, Zanin M (1989) Maneb enhances MPTP neurotoxicity in mice. *Res Commun Chem Pathol Pharmacol* 66:167-170.

93. Thiruchelvam M, Richfield EK, Baggs RB, Tank AW, Cory-Slechta DA (2000) The nigrostriatal dopaminergic system as a preferential target of repeated exposures to combined paraquat and maneb: implications for Parkinson's disease. *J Neurosci* 20:9207-9214.

94. Tipton KF, Singer TP (1993) Advances in our understanding of the mechanisms of the neurotoxicity of MPTP and related compounds. *J Neurochem* 61:1191-1206.

95. Turrens JF, Boveris A (1980) Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. *Biochem J* 191:421-427.

96. Uversky VN, Li J, Fink AL (2001) Pesticides directly accelerate the rate of alpha-synuclein fibril formation: a possible factor in Parkinson's disease. *FEBS Lett* 500:105-108.

97. van der Putten H, Wiederhold KH, Probst A, Barbieri S, Mistl C, Danner S, Kauffmann S, Hofele K, Spooren WP, Ruegg MA, Lin S, Caroni P, Sommer B, Tolnay M, Bilbe G (2000) Neuropathology in mice expressing human alpha-synuclein. *J Neurosci* 20:6021-6029.

98. Vila M, Vukosavic S, Jackson-Lewis V, Neystat M, Jakowec M, Przedborski S (2000) Alpha-synuclein up-regulation in substantia nigra dopaminergic neurons following administration of the parkinsonian toxin MPTP. *J Neurochem* 74:721-729.

99. Wigley WC, Fabunmi RP, Lee MG, Marino CR, Muallem S, DeMartino GN, Thomas PJ (1999) Dynamic association of proteasomal machinery with the centrosome. *J Cell Biol* 145:481-490.

100. Yoritaka A, Hattori N, Uchida K, Tanaka M, Stadtman ER, Mizuno Y (1996) Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. *Proc Natl Acad Sci U S A* 93:2696-2701.

101. Zou L, Xu J, Jankovic J, He Y, Appel SH, Le W (2000) Pramipexole inhibits lipid peroxidation and reduces injury in the substantia nigra induced by the dopaminergic neurotoxin 1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine in C57BL/6 mice. *Neurosci Lett* 281:167-170.