Molecular Neurobiology

ISSN0893-7648/05/31(1–3): 273–282/$30.00

Environmental Toxins and α-Synuclein in Parkinson's Disease

Yanying Liu and Hui Yang*

Beijing Institute for Neuroscience, Capital University of Medical Science, Beijing Center of Neural Regeneration and Repairing, Beijing, China

Abstract

In recent years, environmental influences have been thought to play an important role in Parkinson's disease (PD). Evidence from epidemiological investigations suggests that environmental factors might take part in the disease process. Intriguingly, most of environmental toxins share the common mechanism of causing mitochondria dysfunction by inhibiting complex I and promoting α-synuclein aggregation, a key factor in PD. Therefore, understanding the mechanism of interactions between α-synuclein and environmental factors could lead to new therapeutic approaches to PD.

Index Entries: Environmental toxins; α-synuclein; Parkinson's disease; mitochondria; oxidative stress.

Introduction

Parkinson's disease (PD), a common neurodegenerative disorder, was first described by James Parkinson in 1817. The disease selectively affects dopaminergic neurons of the substantia nigra pars compacta. After 75 to 80% of striatal dopamine is lost, patients start to exhibit the classical symptoms of PD, including bradykinesia, resting tremor, and rigidity. The pathological hallmarks are the presence of round cytoplasmic inclusions in the forms of Lewy bodies and Lewy neuritis, which are composed of aggregated proteins, most notably α-synuclein and ubiquitin. In addition, α-synuclein gene has two missense mutations (A30P and A50T) in certain autosomal-dominant, early-onset, familial PD. α-Synuclein mutations are only responsible for a few familial PD, which further proves the specific role of α-synuclein in this disease. Most cases of PD appear to be sporadic. Evidence from epidemiological investigations supported the concept that environmental factors, including 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), rotenone, paraquat, and other

Received 6/18/04; Accepted 11/15/04.
* Author to whom all correspondence and reprint requests should be addressed. E-mail: huiyang@cpums.edu.cn

industrial chemicals could reduce the activity of complex I of mitochondria, which resulted in promoting α-synuclein upregulation and recapitulating most of the typical pathologic characteristics of PD. The real relationship between α-synuclein and environmental toxins needs to be further studied. It is likely that there is a complex interplay between α-synuclein and environmental factors in the causation of Parkinson's diseases *(1–3).*

Environmental Toxins

Despite many years of focused research, the causes of PD remain to be elucidated. The discovery that many environment toxins can cause parkinsonism in both human and nonhumans inspires the epidemiologist taking part in this research field. Presently, it is certain that farming, rural living, well-water drinking, and exposure to agriculture chemicals are all conditions that have been associated with an increased risk for PD *(4).* This been supported by many experiments. For example, herbicide use has been suggested to enhance PD risk *(5).* Dieldrin, an organochloride pesticide, was found to be present in a proportion of PD brains (6 of 20) and also to be a risk factor in one case-control study in Germany. Dithiocarbamates have been shown to enhance MPTP toxicity and to be associated with increased PD risk *(4,5).* The especially important observation that inadvertent exposure to MPTP could induce parkinsonism in human within 7 to 14 d further strengthened the hypothesis that PD has an environmental etiology *(3,4).*

MPTP is a powerful neurotoxin. It can penetrate the blood–brain barrier and is then converted primarily in glial cells into the active agent MPP^+ by monoamine oxidase B. MPP^+ is selectively taken up into dopamine neurons via high-affinity and energy-dependent dopamine transporters and is accumulated in mitochondria of dopaminergic neurons *(6,7).* In mitochondria, MPP^+ selectively inhibits the mitochondrial complex I of the electron transport chain and blocks oxidative phosphorylation, resulting in cellular energy failure, which indirectly causes neuronal depolarization, release of glutamate, and overexcitation of NMDA receptors and an increased production of reactive oxygen species (ROS) *(8–10).* In aged nonhuman primates, MPTP leads to intracellular inclusions that are filamentous and stain for α-synuclein *(2).* Paraquat is a complex I inhibitor with structural similarity to MPP^+. Its toxicity is known to involve the generation of free radicals via redox cycling reactions that transfer electrons to molecular oxygen. This oxidative stress can lead to upregulation and aggregation of α-synuclein *(5).* Rotenone, a high-affinity inhibitor of complex I (one of the five enzyme complexes of the inner mitochondrial membrane involved in oxidative phosphorylation), is usually used as a kind of insecticide and fish poison. Because it is a lipid compound, rotenone can easily penetrate the blood–brain barrier and enter the brain. In contrast to MPTP and paraquat, rotenone is not concentrated in dopaminergic neurons, but it induces selective dopamine (DA) cell death. The slow and chromic nature of rotenone toxicity leads to intraneuronal filamentous protein inclusions containing α-synuclein and ubiquitin that are remarkably similar to authentic Lewy bodies (LBs) *(2,11).*

The fact that three complex I inhibitors cause cell death and induce the formation of LB-like filamentous inclusions as well as a systematic defect in complex I suggests that mitochondria dysfunction could be central to sporadic PD. Mitochondria not only are life essential organelles for the production of metabolic energy in the form of ATP but also are the primary site of ROS generation *(12,13).* During the course of normal metabolism, ROS are produced from within the respiratory chain of the mitochondria. Complex I and complex III in particular are associated with superoxide production. Inhibition of these complexes results in increased free-radical production. Environmental toxins impair mitochondrial function and thus lead to an increase in the concentration of free radicals and reduction in ATP formation in the neurons *(2,3).* Most free

radicals can extract an electron from neighboring molecules to complete their own orbital. Many biologic molecules, including DNA, proteins, and membrane lipids, are subject to oxidative damage *(9).* Oxidative stress from complex I inhibition would augment free-radical production, which, in turn, would further destroy complex I of the mitochondria *(14).* In addition, free radicals produced in the metabolism of DA could add to the oxidative stress in dopaminergic cells. Dopamine might be metabolized by mitochondrial monoamine oxidase and introduced into vesicles by the vesicular monoamine transporter, or it might spontaneously oxidize in a process that can generate toxic free radicals, giving semiquinone and quinone derivatives that result in the release of superoxide and hydrogen peroxide *(15,16).* In a word, mitochondria dysfunction resulting from environmental toxins leads to increased free-radical formation and reduction in ATP formation. Slow and chronic mitochondria deficiency results in the accumulation and aggregation of α-synuclein. In turn, α-synuclein promotes mitochondrial deficit and oxidative stress *(17,18,19).*

Environmental Toxins and α-Synuclein

Environmental toxins such as MPTP and paraquat can inhibit complex I and increase α-synuclein immunoreactivity in the brain. There is no direct evidence that α-synuclein is associated with complex I. Thus, the study of the interactions of α-synuclein with these environmental agents could provide a critical link between toxicant exposures and the pathogenesis of α-synuclein-containing inclusions in idiopathic PD.

Human α-synuclein is a 140-amino-acid presynaptic protein encoded by its gene on chromosome 4q21.3–q22 *(20,21).* Its main function has not been fully elucidated. It is known that α-synuclein can regulate synaptic plasticity and DA release *(22,23).* Also, it was hypothesized to function in the processes of lipid metabolism, signal transduction, axonal transport, and archoring cytoskeletal components to plasma membranes *(24,25,26).* Despite the lack of knowledge about the real function of this protein, LBs are strongly immunoreactive with anti-α-synuclein antibodies, denoting that filamentous α-synuclein might play an important role in the cause of PD *(27–29).* Native α-synuclein is a random-coil, unfolded protein with little ordered secondary structure. In LBs, α-synuclein assumes a fibrillary β-sheet conformation and binds other proteins *(30,31).* In a β-sheet conformation, α-synuclein might be very susceptible to self-aggregation *(32,33).* In recent years, it has been reported that mutant α-synuclein aggregates much more rapidly than the wild-type protein in vitro *(34–36).* Furthermore, the tendency of α-synuclein to aggregate into misfolded structures might confer toxic properties to the protein. For instance, in cultured cells, overexpression of human α-synuclein could produce neurotoxicity via different pathways *(33,37).* So far, experimental animal models of α-synucleinopathies have been produced by several laboratories (Table 1). They are essential for studying disease pathogenesis and for identifying ways to treat or prevent this disease. Several transgenic mouse models for α-synucleinopathy have now been developed. Models employing the platelet derived growth factor (PDGF) β, mThy-1, and mitochondria permeability transition pore (mPrP) promoters indicate that expression of α-synuclein lies in different regions of the brain *(38).* Models selectively targeting the nigral system have been developed using the tyrosine hydroxylase (TH) promoter or viral vectors. In transgenic animal models, oxidative stress accelerates α-synuclein accumulation and recapitulates many clinical features of the human PD, including loss of DA in the striatum, formation of α-synuclein and ubiquitin immunoreactive-positive inclusions, and motor defects. Different models might have different characteristics *(14,35).* For example, when lentiviral vectors expressing different human or rat forms of α-synuclein were injected into the substantia

Table 1
Comparison of Various α-Synuclein Transgenic Animal Models

Animal	Promoter/vector	Construct	Loss of DA neuron	Motor deficits	LB-like inclusion	Age at onset
Mouse	PDGF β	h α-synuclein (wt)	+ (↓ TH)	+	+	3–6 mo
	muThr-1	h α-synuclein (wt or A53T)	–	+	+	2 mo
	muPrP	h α-synuclein (wt/A53T/A30P)	–	+	+	10 mo
Rat	muTH	h α-synuclein (A53T/A30P)	+	+	+	9–12 mo
	ratTH	h α-synuclein (wt/A53T/A30P)	+	+	+	12 mo
	ratAAV	h α-synuclein (wt/A30P)	+	–	+	12 mo
	Lentivival vector	h α-synuclein (wt/A53T/A30P)	+	?	+	6 wk
Flies	GAL4-P UAST vector	h α-synuclein (wt/A53T/A30P)	+ (adult onset)	+	+	23–45 d

wt = wild type

nigra of rats, α-synuclein recapitulated the essential neuropathological features of PD and similarly led to protein aggregation but without cell loss *(27).* In contrast, Feany et al. reported that the transgenic flies showed adult-onset loss of dopaminergic neurons and the formation of LB-like inclusions *(40).* The level of human α-synuclein expression might explain the difference between the models. Therefore, transgenic models of α-synuclein overexpression will help to elucidate the role of α-synuclein in PD pathogenesis and provide a new method for treatment of this disease.

In the vast majority of patients with idiopathic PD, the lack of α-synuclein mutation indicates that additional mechanisms might lead to conformational change and consequent protein aggregation. One such mechanism could be the interaction of α-synuclein with other chemical species. Environmental toxins, including many pesticides and chemicals, markedly accelerated the in vitro rate of α-synuclein fibril formation in a dose-dependent manner *(41,42).* Animal models are commonly used for studying the pathogenic feature of PD in vivo. Systematic administration of MPTP in man, mice, and nonhuman primates induced α-synuclein aggregates and replicated the major features of PD *(6,43).* Lack of α-synuclein protected knockout mice against the neuronal loss trigged by the toxicant MPTP *(7).* When rats were systematically and chronically exposed to the pesticide and mitochondria toxin rotenone through jugular vein cannulation and implantation of subcutaneous osmotic pumps, selective degeneration of the nigrastriatal dopaminergic pathway, striatial oxidative damage, and formation of ubiquitin and α-synuclein-positive inclusions in nigra cells of rats were observed *(11–14).* When mice were exposed to the herbicide, brain levels of α-synuclein were significantly increased *(5,44).* These results suggest that regulation of α-synuclein as a consequence of toxicant insult and direct interactions between the protein and environmental agents are potential mechanisms leading to α-synuclein pathology in neurodegenerative disorders.

Mechanism

Many pesticides and herbicides share the common mechanism of causing mitochondria dysfunction by inhibiting complex I. The inhibition of complex I creates an environment of oxidative stress. Environmental toxins that cause α-synuclein to become insoluble might be related to oxidative damage, ultimately leading to aggregation of α-synuclein and the subsequent death of dopaminergic neurons *(2,45,46,47,48).* Based on evidence from various research fields, we propose that the interplay between α-synuclein and environmental toxins is a possible general mechanism for promoting cell death or cell survival.

The ubiquitin proteasome system (UPS) is the primary mechanism responsible for the elimination of mutant, damaged, misfolded, and other unwanted proteins. An effect of reduced mitochondrial complex I activity (e.g., MPTP, rotenone, paraquat) is to decrease cellular ATP levels, which might indirectly inhibit the highly ATP-dependent proteasome. Earlier evidence suggested that defects in the UPS could induce protein accumulation, formation of LB-like inclusions, and dopaminergic cell death. Thus, UPS dysfunction might contribute to α-synuclein aggregation *(16).* The propensity of α-synuclein to aggregate and fibrillize into structures that are resistant to cellular degradation might be central to its neurotoxicity. The proposed mechanisms of toxicity include inappropriate induction of apoptosis and inhibition of neuron-specific functions, which eventually would result in cell death. The presence of apoptosis has been reported in postmortem PD brains. It is clear that mitochondria are involved in the regulation of apoptosis *(2,43,44).* Opening of the mPTP and mitochondria depolarization seem to be part of the initiation of apoptosis. Multiple stimuli can trigger mitochondria to release caspase-activity proteins, among which are cytochrome-*c* and possibly other proteins such as apoptosis-inducing factor (AIF) and intramitochondrial caspases *(17,42,45).* For example, it is reported that overexpression of α-synuclein in GT1-7 cells leads to mitochondrial dysfunction *(46).* Hashimoto et al. *(47)* demonstrated that cytochrome-*c* interacted with α-synuclein in LBs. Thus, release of this mitochondrial molecule might not only trigger cell death but also promote α-synuclein aggregation *(53).* In addition to apoptosis as described earlier, dopaminergic neurons with autophagic vacuoles have been observed in the substantia nigra of PD patients, along with neurons displaying apoptotic features *(54,55).* Autophagy has been described from mammalian cells in response to nutrient starvation, physiological demands, or other insults. One study showed that A53T α-synuclein expression was autophagic and non-apopotic in PC12 cells *(56).* Despite the extensive morphological evidence of autophagy during neuronal cell death, the conclusive evidence for a prodeath role of autophagy during neuronal cell death is still missing. Therefore, the role of autophagy in neuronal cell death needs to be further studied.

α-Synuclein, however, is not intrinsically a cell death protein, as it exhibits neuroprotective activity in nondopaminergic human cortical neurons *(51).* Studies in various experimental models have failed to show a consistent relationship between α-synuclein expression and neuronal injury. One study showed that mice overexpressioning α-synuclein either the human wild type or the A53T mutant form of the protein displayed paraquat-induced protein aggregates, but they were completely protected against neurodegeneration *(52).* α-Synuclein overexpression in transgenic mice did not consistently induce neuronal damage nor did it exacerbate neurodegeneration caused by MPTP *(38,53).* The previous studies in our laboratory have proved that overexpression of α-synuclein in SH-SY5Y cells results in the formation of cytoplasmic inclusions, mitochondria dysfunction, and a large number of lysosomes and autophagosomes within the cells. Both the DCF loading analysis and the intracellular level of the reduced glutathione (GSH) measurement suggested that α-synuclein-transfected cells were under oxidative stress *(54).* When treated with rotenone, cells overexpressing α-synuclein cells underwent less insult than untransfected cells.

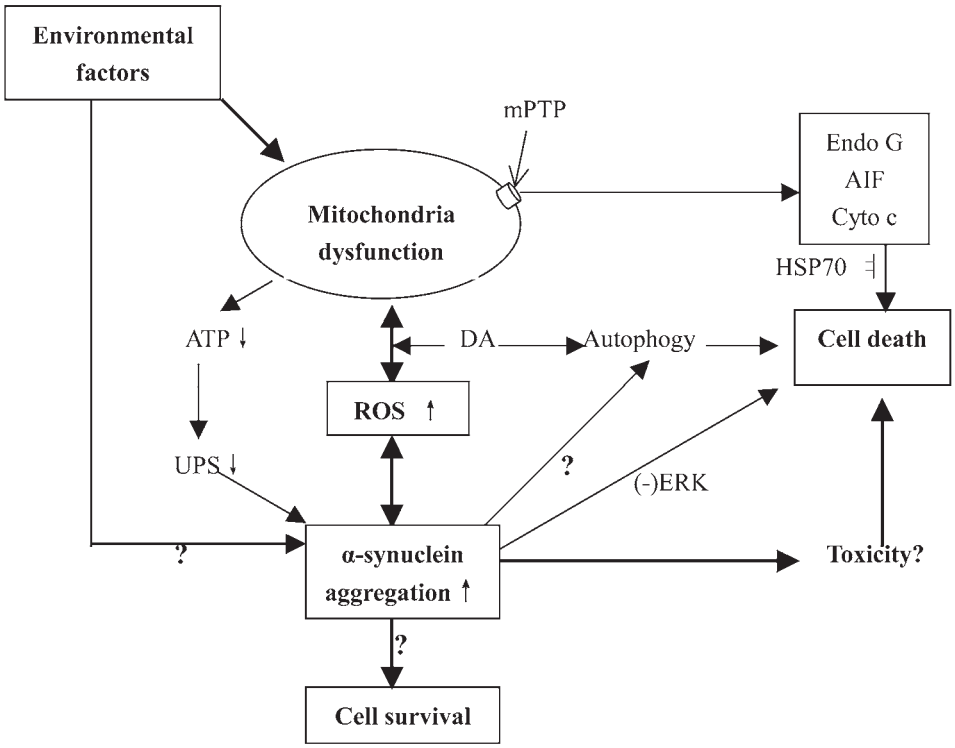

Fig. 1. Putative mechanism of cell survival or death in PD. Mitochondria is the primary site of ATP and ROS generation. α-Synuclein overexpression promotes mitochondrial deficit and oxidative stress. When cells are exposed to environmental factors, the activity of complex I is inhibited. Therefore, the production of free radicals with mitochondria and from DA metabolism can be enhanced and, eventually, cell death occurs. However, although α-synuclein overexpression cells were exposed to environmental factor, the aggregates of α-synuclein protects against those toxic agents and induces neurodegeneration. The end is cell survival. endo G, endonuclease G; UPS, ubiquitin–proteasomal system; ROS, reactive oxygen species; AIF, apoptosis-inducing factor; HSP70, heat shock protein 70; Cyto c, cytochrome-*c;* mPTP, mitochondria permeability transition pore.

Therefore, we supposed that α-synuclein expression in SH-SY5Y cells might partially protect mitochondria from rotenone (unpublished data). Moreover, earlier evidence also suggested that increased levels of α-synuclein did not necessarily lead to nerotoxicity. For instance, Dacosta et al. reported that neuronal cell lines transfected with α-synuclein were resistant to hydrogen peroxide-induced oxidative stress as well as to apoptotic stimuli *(61).* Presently, it has been proposed that under some circumstances, inclusions might serve a protective role. This function is supported by experimental models of polyglutamine disease *(62).* Consequently, it is conceivable that soluble oligomers of α-synuclein might be even more toxic than the insoluble fibrils found in LBs *(21,37, 38).* The aggregation of α-synuclein into insoluble structures, a concentration-dependent process, would reduce the concentration of the active α-synuclein molecule and might protect against α-synuclein-induced cell death. Thus, the inclusion formation could be a mechanism to assist in the clearance of misfolded noxious protein, but it is not without limit. In transgenic mouse models, neuroprotection of α-synuclein is associated more strictly with increased protein levels and is less dependent on the expression of different forms of α-synuclein. This is consistent with the notion that α-synuclein mutation only in the form of triplication causes PD because mutant α-synu-

clein behaves in a quantitative rather than qualitative manner *(63).* In addition, heat shock protein (Hsp) 70, a chaperone protein, could counteract oxidative damage to proteins and facilitate the degradation of oxidized proteins through the proteasomal system. Other studies suggested that Hsp70 could interact with AIF and cytochrome-*c* to prevent cell death *(64,65).* Transgenic flies carrying extra copies of Hsp70 have also been proved to be relatively resistant to paraquat toxicity *(66);* thus, Hsp70 is usually found during the process of neuroprotection.

In conclusion, environmental factors affect the activity of complex I of the mitochondria, which results in oxidative stress and α-synuclein aggregation. α-Synuclein aggregation further aggravates oxidative stress, which leads to activation of MAPK family members, lysosomal activation, accumulation of autophagic vesicular structures, increase in α-synuclein expression, and damage of mitochondria *(17,18,52).* The end is cell death (Fig. 1). Earlier evidence, however, also suggests that increased levels of α-synuclein do not necessarily lead to neurotoxicity. α-Synuclein might have a role of neuroprotection. Why are the results so different? The reasons might be as follows: (1) Different models. α-Synuclein expression and aggregation is likely to vary between different cell types, under different transfections with different promoters, and so forth and this might explain the apparent discrepancies. (2) Dual effects of α-synuclein. The first direct evidence that α-synuclein has neuron-specific dual effects depending on its concentration or level of expression was provided by Seo and colleagues. It has been demonstrated that low or physiological levels of α-synuclein can protect neurons against various toxic insults such as hypoxia or oxidative stress. In contrast, high concentrations or overexpression of α-synuclein exert a cytotoxic effect on neurons *(19).* (3) Complex interactions between environmental toxins and α-synuclein. The interactions between α-synuclein and environmental factors could change the toxicity of themselves. Alternatively, α-synuclein might possess properties that withstand toxic injury and its expression could affect different signaling pathways linked to neuronal survival or death. For example, one study reported that α-synuclein protected against oxidative stress via inactivation of the c-Jun N-terminal kinase stress-signaling pathway in neuronal cells *(67),* whereas another study suggested that α-synuclein regulated neuronal survival via the PI3/Akt pathway and promoted cell death through the Bcl-2 family (BAX)-related caspase pathway *(19).* (4) The particularity of DA neurons. Because of the specific metabolism of dopamine, dopaminergic neurons are uniquely sensitive to complex I impairments. Progressive oxidative damage induces release of cytochrome-*c* from mitochondria to the cytoplasm. This mechanism could explain the cytoplasmic inclusions found in neuronal cells *(53).* Moreover, α-synuclein–dopamine adducts might be an important mediator of toxicity in DA, consistent with the suggestion that α-synuclein is a direct requirement for MPTP-mediated neurotoxicity. Other causes such as poor UPS activity in DA neurons *(68)* and some unrecognized factors might regulate the interactions between α-synuclein and enveromental toxins. Therefore, it is likely that there is a complex interplay between α-synuclein and environmental agents in the cause of PD. Although the precise mechanism is unclear, the study of interactions between α-synuclein and environmental factors will provide important new insights into the pathogenesis of PD.

Acknowledgment

This work was supported by a grant from the National Science Foundation of Beijing (7022005).

References

1. Goedert M. (2001) Alpha-synuclein and neurodegenerative diseases. *Neuroscience* **2,** 492–501.
2. Dawson T.M. and Dawson V.L. (2003) Molecular pathways of neurodegeneration in Parkinson's disease. *Science* **302,** 819–822.
3. Collier K.S., Maries E., and Kordower J.H. (2002) Etiology of Parkinson's disease: genetics and environment revisited. *PNAS* **99,** 13,972–13,974.

4. Warner T.T. and Schapira A.H.V. (2003) Genetic and environmental factors in the cause of Parkinson's disease. *Ann. Neurol.* **53 (Suppl. 3),** s16–s25.
5. Manning Bog A.B., McCormack A.L., Li J., Uversky B.N., Fink A.L., and Di Monte D.A. (2002) The herbicide paraquat causes up-regulation and aggregation of α-synuclein in mice. *J. Biol. Chem.* **277,** 1641–1644.
6. Isacson O. (2002) Models of repair mechanisms for future treatment modalities of Parkinson's disease. *Brain Res. Bull.* **57,** 839–846.
7. Dauer W., Kholodilov N., Vila M., et al. (2002) Resistance of α-synuclein null mice to the Parkinsonian neurotoxin MPTP. *Proc. Natl. Acad. Sci. USA* **99,** 14,524–14,529.
8. Wang H.M., Shimoji M., Yu S.W., Dawson T.M., and Dawson V. (2003) Apoptosis inducing factor and PARP-mediated injury in the MPTP mouse model of Parkinson's disease. *Ann. NY Acad. Sci.* **991,** 132–139.
9. Beal M.F. (2003) Mitochondria, Oxidative Damage, and Inflammation in Parkinson's disease. *Ann. NY Acad. Sci.* **991,** 120–131.
10. Collier T.J., Collier K.S., Mcguire S., and Sortwell C.E. (2003) Cellular models to study dopaminergic injury responses. *Ann. NY Acad. Sci.* **991,** 140–151.
11. Sherer T.B., Kim J.H., Betarbet R., and Greennmyre J.T. (2003) Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and α-synuclein aggregation. *Exp. Neurol.* **179,** 9–16.
12. Brunk U.T. and Terman A. (2002) The mitochondrial–lysosomal axis theory of aging accumulation of damaged mitochondria as a result of imperfect autophagocytosis. *Eur. J. Biochem.* **269,** 1996–2002.
13. Orth M., Tabrizi S.J., Schapira A.H.V., and Cooper J.M. (2003) α-Synuclein expression in HEK293 cells enhances the mitochondrial sensitive to rotenone. *Neurosci. Lett.* **351,** 29–32.
14. Betarbet R., Sherer T.B., Di Monte D.A., and Greenamyre J.T. (2002) Mechanistic approaches to Parkinson's disease pathogenesis. *Brain Pathol.* **12,** 499–510.
15. Kathleen A., Grire-Zeiss M.A., and Federoff H.J. (2003) Convergent pathobiologic model of Parkinson's disease. *Ann. NY Acad. Sci.* **991,** 152–166.
16. Gómez-Santos C., Ferrer I., Reiriz J., Vinals F., Barrachina M., and Ambrosio S. (2002) MPP^+ increases α-synuclein expression and ERK/MAP-kinase phosphorylation in human neuroblastoma SH-SY5Y cells. *Brain Res.* **935,** 32–39.
17. Mc Naught K.S.P. and Olanow C. (2003) Proteolytic stress: a unifying concept for the etiopathogenesis of Parkinson's disease. *Ann. Neurol.* **53,(Suppl. 3),** s73–s86.
18. Cookson M.R. (2003) Pathways to parkinsonism. *Neuron* **37,** 7–10.
19. Seo J.H., Rah J.C., Choi S.H., et al. (2002) α-Synuclein regulates neuronal survival via Bcl-2 family expression and PI3/Akt kinase pathway. *FASEB J.* **16,** 1826–1829.
20. George J.M. (2001) The synucleins. *Genome Biol.* **3,** 3002.1–3002.6.
21. Ma Q.L., Chan P., Yoshii M., and Uéda K. (2003) α-Synuclein aggregation and neurodegenerative disease. *J. Alzheimer's Dis.* **5,** 139–148.
22. Yu X., Tsunao S., Uéda K., et al. (2001) Characterization of the human α-synuclein gene: genomic structure, transcription start site, promoter region and polymorphisms. *J. Alzheimer's Dis.* **3,** 485–494.
23. Lücking C.B. and Brice A. (2000) Alpha-synuclein and Parkinson's disease. *Cell. Mol. Life Sci.* **57,** 1894–1908.
24. Paxinou E., Chen Q., Giasson Bi W.M., et al. (2001) Induction of alpha-synuclein aggregation by intracellular nitrative insult. *J. Neurosci.* **21,** 8053–8061.
25. Iseki E., Marui W., Sawada H., Uéda K., and Kosaka K. (2000) Accumulation of human α-synuclein in different cytoskeletons in Lewy bodies in brain of dementia with lewy bodies. *Neurosci. Lett.* **290,** 41–44.
26. Saha A.R., Hill J., Utton M.A., et al. (2004) Parkinson's disease (alpha-synuclein) mutations exhibit defective axonal transport in cultured neurons. *J. Cell Sci.* **117,** 1017–1024.
27. Bianco C.L., Ridet J.L., Schneider B.L. Déglon N., and Aebischer P. (2002) α-Synucleinopathy and selective dopaminergic neuron loss in a rat lentiviral-based model of Parkinson's disease. *PNAS* **99,** 10,813–10,818.
28. Abeliovich A., Schmitz Y., Farinas I., et al. (2000) Mice Lacking α-synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron* **25,** 239–252.
29. Goerts J., Manning-Bog A.B., Cormack A.L.M., et al. (2003) Nuclear localization of α-synuclein and its interaction with histones. *Biochemistry* **42,** 8465–8471.
30. Alim M.A., Hossain M.S., Arima K., et al. (2002) Tubulin seeds α-synuclein fibril formation. *J. Biol. Chem.* **277,** 2112–2117.

31. Neystat M., Rzhetskaya M., Holodilov N., and Burke R.E. (2002) Analysis of synphilin-1 and synuclein interactions by yeast two-hybrid β-galactosidase liquid assay. *Neurosci. Lett.* **325,** 119–123.
32. Duda J.E., Lee V.M.Y., and Trojanowski J. (2000) Neuropathology of α-synuclein aggregates new insights into mechanisms of neurodegenerative disease. *J. Neurosci. Res.* **61,** 121–127.
33. Saha A.R., Ninkin N.N., Hanger D.P., and Bham D.A. (2000) Induction of neuronal death by alpha-synuclein. *Eur. J. Neurosci.* **12,** 3073–3077.
34. Conway K.A., Lee S.J., Rochet J.C., et al. (2000) Acceleration of oligomerization not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. *Proc. Natl. Acad. Sci. USA* **97,** 571–576.
35. Spira P.J., Sharpe D.M., Halliday G., Cavanagh J., and Nicholson G.A (2001) Clinical and pathological features of a Parkinsonian syndrome in a family with an Ala53Thr alpha-synuclein mutation. *Ann. Neurol.* **l49,** 313–319.
36. Giasson B.I., Duda J.E, Quinn A.M., Zhang B., Trojanowski J.Q., and Lee V.M. (2002) Neuronal alpha-synucleinopathy with severe movement disorder in mice expressing A53T human alpha-synuclein *Neuron* **34,** 521–523.
37. Cristina G.S., Ferrer I., Santidrian A.F., and Barrachina M. (2003) Dopamine induces autophagic cell death and α-synuclein increase in human neuroblastoma SH-SY5Y cells. *J. Neurosci. Res.* **73,** 341–350.
38. Hashimoto M., Rockenstein E., and Masliaii E. (2003) Transgenic models of α-synuclein Pathology past present, and future. *Ann. NY Acad. Sci.* **991,** 171–188.
39. Dawson T.M., Mandir A.S., and Lee M.K. (2002) Animal models of PD: pieces of the same puzzle? *Neuron* **35,** 219–212.
40. Feany M.B. and Bendera W.W. (2000) *Drosophila* model of Parkinson's disease. *Nature* **404,** 394–398.
41. Sherer T.B., Betarbet R., Stout A.K., et al. (2002) An in vitro model of Parkinson's disease: linking mitochondrial impairment to altered α-synuclein metabolism and oxidative damage. *J. Neurosci.* **22,** 7006–7015.
42. Uversky V.N., Li J., and Fink A.L. (2001) Pesticides directly accelerate the rate of α-synuclein fibril formation: a possible factor in Parkinson's disease. *FEBS Lett.* **500,** 105–108.
43. Masliah E., Rockenstein E., Veinbergs I., et al. (2000) Dopaminergic loss and inclusion body formation in α-synuclein mice: implications for neurodegenerative disorders. *Science* **287,** 1265–1269.
44. Kostrzewa R.M. and Juan S.A. (2003) Novel mechanisms and approaches in the study of neurodegeneration and neuroprotection. *Neurotox. Res.* **5,** 375–384.
45. Betarbet R., Sherer T.B., Mackenzie G., Caucica-Osuna M., Panov A.V., and Greenamgre J.T. (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.* **3,** 1301–1306.
46. Orth M. and Schapira A.H.V. (2002) Mitochondrial involvement in Parkinson's disease. *Neurochem. Int.* **40,** 533–541.
47. Vila M., Vukosavic S., Jackson L.V., Neystat M., Jakowec M., and Przedborski S. (2000) α-Synuclein up-regulation in substantia nigra of dopaminergic neurons following administration of the Parkinsonian toxin MPTP. *J. Neurochem.* **74,** 721–729.
48. Lim K.L., Dawson V., and Dawson T.M. (2003) The cast of molecular characters in Parkinson's disease. *Ann. NY Acad. Sci.* **991,** 80–92.
49. Loo G.V., Saelens X., Gurp M.V., Farlane M.M., Martin S.J., and Vandenabeele P. (2002) The role of mitochondrial factors in apoptosis: a Russian roulette with more than one bullet. *Cell Death Differ.* **9,** 1031–1042.
50. Ameisen J.C. (2004) Looking for death at the core of life in the light of evolution. *Cell Death Differ.* **11,** 4–10.
51. Tatton W.G.., Ruth C.R., Brown D., and Tatton N. (2003) Apoptosis in Parkinson's disease: signals for neuronal degradation. *Ann. Neurol.* **53(Suppl. 3),** s61–s72.
52. Hsu L.J., Sagara Y., Arroyo A., et al. (2000) α-Synuclein promotes mitochondrial deficit and oxidative stress. *Am. J. Pathol.* **2,** 401–410.
53. Hashimoto M., Takeda A., Hsu L.J., Takenouchi T., and Masliah E. (1999) Role of cytochrome *c* as a stimulator of α-synuclein aggregation in lewy body disease. *J. Biol. Chem.* **274,** 28,849–28,852.
54. Yuan J.Y., Lipinski M., and Degterev A. (2003) Diversity in the mechanisms of neuronal cell death. *Neuron* **40,** 401–413.
55. Webb J.L., Ravikumar B., Atkins J., Skepper J.N., and Rubinsztein D.C. (2003) α-Synuclein is degraded by both autophagy and the proteasome. *J. Biol. Chem.* **278,** 25,009–25,013.
56. Stefanis L., Larsen K.E., Rideout H.J., Sulzer D., and Greene L.A. (2001) Expression of A53T

mutant but not wild-type alpha-synuclein in PC 12 cells induces alterations of the ubiquitin-dependent degradation system loss of dopamine release and autophagic cell death. *J. Neurosci.* **21,** 9549–9560.
57. Xu J., Kao S.Y., Lee F.J.S., Song W.H., Jin L.W., and Yonkner B.A. (2002) Dopamine dependent neurotoxicity of α-synuclein: a mechanism for selective neurodegeneration in Parkinson's disease. *Nat. Med.* **8,** 600–606.
58. Manning-Bog A.B., McCormack A.L., Purisai M.G.., Bolin L.M., and Di Monte D.A. (2003) α-Synuclein overexpression protects against paraquat-induced neurodegeneration. *J. Neurosci.* **23,** 3095–3099.
59. Rathke-Hartlieb S., Hahle P.J., Neumann M., et al. (2001) Sensitivity to MPTP is not increased in Parkinson's disease-associated mutant α-synuclein transgenic mice. *J. Neurochem.* **77,** 1181–1184.
60. Zhang Y.X., Yang H., Cai Q., et al. (2003) The influence of overexpression α-synuclein EGFP on formation of inclusion and mitochondrial ultrastructure in SH-SY5Y cells in vitro. *Chin. J. Neuroanal.* **19,** 251–256.
61. Dacosta C.A., Ancolio K., and Checler F. (2000) Wild type but not Parkinson's disease-related ala-53-Thr mutant α-synuclein protects neuronal cells from apoptotic stimuli. *J. Biol. Chem.* **275,** 24,065–24,069.
62. Taylor J.P., Hardy J., and Fischbeck K.H. (2002) Toxic proteins in neurodegenerative disease. *Science* **14,** 1991–1995.
63. Singleton A.B., Farrer M., Johnson J., et al. (2003) α-synuclein locus triplication causes Parkinson's disease. *Science* **302,** 841.
64. Saleh A., Srinivasula S.M., Balkir L., Robbins P.D., and Alnemri E.S. (2000) Negative regulation of the Apaf-1 apoptosis by Hsp 70. *Nat. Cell Biol.* **2,** 476–483.
65. Ravagnan L., Gurbuxani S., Susin S.A., et al. (2001) Heat-shock protein 70 antagonizes apoptosis-inducing factor. *Nat. Cell Biol.* **3,** 839–843.
66. Auluck P.K., Edwin Chan H.Y., Trojanowski J.Q., Lee V.M.-Y., and Bonini N.M. (2002) Chaperonne suppression of α-synuclein toxicity in a drosophila model for Parkinson's disease. *Science* **295,** 865–868.
67. Hashimoto M., Hsu L.J., Rockenstain E., Takenouchi T., Mallory M., and Masliah E. (2002) α-synuclein protects against oxidative stress via inactivation of the c-Jun N-terminal kinase stress-signaling pathway in neuronal cells. *J. Biol. Chem.* **277,** 11,465–11,472.
68. Tanaka Y.I., Engelender S., Igarashi S., et al. (2001) Inducible expression of mutant α-synuclein decreases proteasome activity and increases sensitivity to mitochondria-dependent apoptosis. *Hum. Mol. Genet.* **10,** 919–926.