

C0025 Molecular Insights into
Parkinson's Disease

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sp0020 Mutations in *SNCA*, *PINK1*, *parkin*, and *DJ-1* are associated with autosomal-dominant or autosomal-recessive forms of Parkinson's disease (PD), the second most common neurodegenerative disorder. Studies on the structural and functional properties of the corresponding gene products have provided significant insights into the molecular underpinnings of familial PD and the much more common sporadic forms of the disease. Here, we review recent advances in our understanding of four PD-related gene products: α -synuclein, parkin, PINK1, and DJ-1. In Part 1, we review new insights into the role of α -synuclein in PD. In Part 2, we summarize the latest developments in understanding the role of mitochondrial dysfunction in PD, emphasizing the role of the *PINK1/parkin* pathway in regulating mitochondrial dynamics and mitophagy. The role of *DJ-1* is also discussed. In Part 3, we point out converging pathways and future directions.

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I. Introduction

p0005 Parkinson's disease (PD) is a neurodegenerative disorder manifested by resting tremor, bradykinesia (slowness of movement), rigidity, and postural instability.¹⁻³ Some of these symptoms are attributed in large part to a loss of dopaminergic neurons in the substantia nigra of the midbrain. A defining neuropathological feature of PD brain is the presence in some surviving neurons of Lewy bodies, which are cytosolic inclusions enriched with fibrillar forms of the presynaptic protein α -synuclein (α Syn), encoded by the *SNCA* gene (also known as *PARK1*).^{4,5} Autosomal-dominant mutations in *SNCA* have been discovered in patients with early onset familial PD,⁶⁻¹⁰ and evidence suggests that these mutations promote α Syn aggregation. Thus, these neuropathological and genetic data suggest that α Syn aggregation is involved in PD pathogenesis.

p0010 A second characteristic feature of PD pathogenesis is an impairment of mitochondrial function. Biochemical studies have revealed a defect of mitochondrial complex I in the postmortem brains of PD patients.^{11,12} The decrease in complex I activity is predicted to cause an accumulation of reactive oxygen species (ROS) that damage proteins, lipids, and DNA.^{13,14} Dopaminergic neurons in the substantia nigra are hypothesized to be particularly susceptible to a buildup of ROS because they have high levels of oxidative stress (even under basal conditions) resulting from dopamine metabolism and auto-oxidation.^{13,15}

Additional evidence that mitochondrial deficits play a role in PD stems from the observation that three proteins mutated in autosomal-recessive early onset PD (DJ-1, Parkin, PINK1) regulate mitochondrial functions.^{16–19}

p0015 In Part 1 of this chapter, we provide an overview of current knowledge relating to the role of α Syn aggregation in PD. Questions that are addressed include (i) Which species are formed on the α Syn self-assembly pathway? (ii) Which of these species are responsible for neurotoxicity? (iii) How is α Syn aggregation modulated by cellular perturbations such as oxidative stress and membrane binding? (iv) How are α Syn aggregation and toxicity impacted by antioxidants and molecular chaperones? In Part 2, we review molecular mechanisms by which mitochondrial dysfunction elicits cellular defects in PD, with an emphasis on cellular pathways relating to *PINK1*, *parkin*, and *DJ-1*. Questions addressed include (i) What is the evidence that *PINK1* and *parkin* function in a common pathway to regulate mitochondrial integrity? (ii) What are cytoplasmic substrates for PINK1 and Parkin that mediate their neuroprotective functions? (iii) How do PINK1 and Parkin identify damaged mitochondria and mediate their removal? (iv) What is the relationship between *DJ-1* and the *PINK1/parkin* pathway? In Part 3, we highlight recent findings suggesting that α Syn aggregation and mitochondrial dysfunction act as “co-conspirators” to trigger dopaminergic cell death in PD. We conclude by raising key questions that need to be answered to better understand how α Syn aggregation and mitochondrial dysfunction contribute to PD pathogenesis, and we suggest potential strategies to target these two toxic phenomena in patients.

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II. Role of α Syn Aggregation in PD

s0015 A. Physiological Role of α Syn

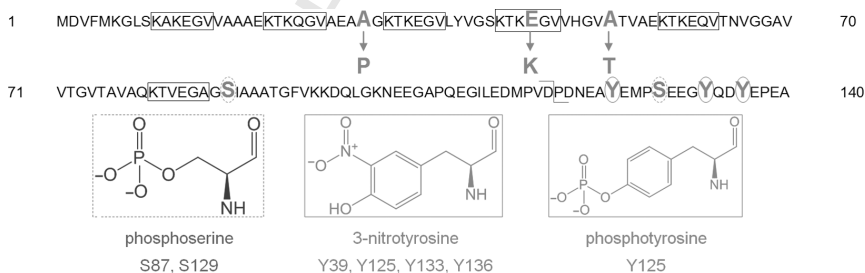
p0020 α Syn is a member of the “synuclein” family that also includes β -synuclein (β Syn) and γ -synuclein (γ Syn).²⁰ α Syn is expressed as multiple isoforms spanning 98-, 112-, 126-, and 140-amino acid residues as a result of alternative pre-mRNA splicing.²¹ The 140-residue (14-kDa) isoform has been characterized much more extensively than the other splice variants.

p0025 The sequence of the 14-kDa isoform of α Syn can be subdivided into three domains: (i) an N-terminal domain (residues 1–67), encompassing six repeats of the highly conserved hexamer sequence “KTK(E/Q)GV”; (ii) a central domain (residues 61–95; also referred to as the “non-A β component of AD amyloid” (NAC) domain), characterized by a high content of hydrophobic residues; and (iii) a C-terminal domain (residues 96–140), characterized by a high content of proline, aspartate, and glutamate residues. Analysis of aqueous

solutions of α Syn by circular dichroism (CD) or nuclear magnetic resonance (NMR) reveals a lack of stable secondary structure, and under these conditions the protein is referred to as “natively unfolded.”^{22,23} The N-terminal lysine-rich repeats are similar to lipid-binding motifs in amphipathic helical domains of exchangeable apolipoproteins, suggesting that the normal function of α Syn involves binding to phospholipid membranes.²⁴ Consistent with this idea, the N-terminal repeat region of α Syn binds anionic phospholipid vesicles and adopts an amphipathic α -helical structure as a result of its interaction with the membrane.^{24–31} In addition, α Syn is thought to play a role in regulating neurotransmission via interactions with synaptic vesicles^{32–38} or by regulating the SNARE complex assembly.³⁹ Data recently reported by Selkoe and colleagues⁴⁰ suggest that α Syn exists as an α -helical tetramer that remains intact when purified from mouse cortex or mammalian cell lines under non-denaturing conditions. A subsequent study showed that α Syn with an N-terminal 10-residue leader sequence derived from GST can be purified from a bacterial expression system as an apparent tetramer with some degree of helical structure.⁴¹ These intriguing findings (currently being validated by other groups) raise the possibility that the α -helical structure of α Syn can be stabilized by contacts between neighboring subunits in an α Syn oligomer, and not just by interactions with phospholipid membranes.

s0020 B. Effects of Familial Mutations on α Syn Self-assembly

p0030 Two types of α Syn gene mutations have been identified in patients with early onset familial PD: (i) substitution mutations encoding the α Syn variants A30P, E46K, and A53T (Fig. 1)^{6–8}; and (ii) mutations that increase the copy



f0005 FIG. 1. Amino acid sequence of α Syn in one-letter code. The lysine-rich repeats are enclosed in rectangular boxes. The diagram highlights the three substitutions associated with familial PD (A30P, E46K, and A53T) and posttranslational modifications identified in postmortem human brain: serine phosphorylation, tyrosine nitration, tyrosine phosphorylation, and C-terminal truncation (represented by a boundary line between residues 119 and 120, one of the several proteolytic cleavage sites in the C-terminal region⁴²). (See color plate.)

number of the wild-type gene, including a duplication and a triplication.^{9,10} The observation that Lewy bodies are enriched with fibrillar α Syn has led to the hypothesis that α Syn gene mutations cause early onset PD by promoting the formation of neurotoxic aggregates. In the case of the duplication and triplication mutants, the increased α Syn expression levels resulting from these mutations would be expected to favor aggregation of the protein via mass action. To address whether the three substitution mutations promote α Syn self-assembly, a common strategy has been to monitor the aggregation of α Syn variants in cell-free systems.

p0035 Upon prolonged incubation at 37 °C, recombinant wild-type and mutant α Syn form fibrils with characteristic features of classic amyloid deposits, similar to fibrillar α Syn isolated from Lewy bodies.^{43–47} α Syn fibrillization does not occur as a simple two-step process, but rather involves the formation of pre-fibrillar intermediates referred to as “protofibrils.”^{48,49} The results of far-UV CD, atomic force microscopy (AFM), and electron microscopy (EM) analyses indicate that protofibrils consist of spheres, chains, and rings enriched with β -sheet secondary structure.^{45,46,48–52} In addition, α Syn protofibrils are transient intermediates that accumulate to a maximum of ~15% of the total protein before being consumed by the formation of amyloid-like fibrils.^{45,46,52}

p0040 A study by Lansbury and colleagues⁴⁵ revealed that A53T formed fibrils more rapidly than wild-type α Syn, whereas A30P formed fibrils less rapidly than the wild-type protein. In contrast, A53T and A30P both formed protofibrils more rapidly than the wild-type protein.⁴⁵ Similar results have been published by other groups.^{53–55} These findings suggest that the neurotoxic effects of the A30P and A53T substitutions result from enhanced protofibril formation rather than accelerated fibrillization. Consistent with this idea, compounds that promote the conversion of α Syn from small aggregates to larger inclusions alleviate the protein's neurotoxicity in cellular and animal models relevant to PD.⁵⁶

p0045 α Syn protofibrils, but not the monomeric or fibrillar protein, bind phospholipid vesicles with high affinity^{50,57} and trigger membrane permeabilization.^{50,51,58,59} Other groups have reported that oligomeric α Syn increases the conductance across a phospholipid bilayer, although it is unclear whether this effect involves the formation of pore-like structures.^{60,61} Additional evidence suggests that oligomeric α Syn can perturb ion homeostasis by forming conducting membrane channels in cells.^{62–64} These observations provide a rationale for why α Syn protofibrils may be toxic (perhaps even more so than the fibrillar protein): namely, they may trigger dopaminergic cell death by permeabilizing lipid membranes, thus causing a disruption of ion gradients necessary for neuronal homeostasis.^{48,50,58} A30P and A53T have a greater membrane permeabilization activity per mole of protein than wild-type α Syn, and this property may contribute to the enhanced neurotoxicity of these two familial mutants (in addition to their increased propensity to form protofibrils).⁵⁸

p0050 Similar to A53T, E46K forms amyloid-like fibrils more rapidly than wild-type α Syn.^{54,65,66} However, in contrast to A30P and A53T, E46K does not have an enhanced propensity to form protofibrils, and protofibrillar E46K exhibits a decreased specific membrane permeabilization activity.⁶⁶ One way to interpret these findings is to infer that E46K elicits neurotoxicity via a mechanism that does not involve the formation of membrane-disrupting protofibrils. Alternatively, if we presume that E46K conforms to the “toxic protofibril hypothesis,” then cellular perturbations that are neglected in current cell-free systems (e.g., posttranslational modifications) must increase the ability of this variant to form protofibrils and/or permeabilize membranes *in vivo*.

s0025 C. Modulation of α Syn Aggregation by Long-Range Interactions

p0055 The solution structure of human wild-type α Syn has been characterized extensively using NMR methods that combine measurements of paramagnetic relaxation enhancement (PRE) and/or residual dipolar couplings (RDCs) with ensemble molecular dynamics simulations.^{67–69} The results indicate that the protein adopts an ensemble of conformations stabilized by long-range interactions between the C-terminal region and the N-terminal and NAC domains. In turn, the long-range interactions may result in inhibition of α Syn self-assembly via an auto-inhibitory mechanism involving the “shielding” of hydrophobic residues in the N-terminal and NAC regions by the C-terminal domain.^{55,67,68} In turn, one would predict that a loss of long-range interactions should lead to an increased rate of fibrillization. Consistent with this model, C-terminally truncated α Syn variants form fibrils more rapidly than the full-length protein.^{55,70–73} In addition, mouse α Syn, a variant with weaker long-range interactions compared to those of the human wild-type protein,⁷⁴ forms fibrils more rapidly than human wild-type α Syn or A53T.⁴⁶

p0060 On the basis of PRE and RDC data, Zweckstetter and colleagues⁷⁵ reported that the A30P and A53T substitutions destabilize long-range interactions between the C-terminal region and the hydrophobic NAC domain, and they inferred that this perturbation might account for the enhanced ability of the familial mutants to form oligomers compared to the wild-type protein. In contrast, Eliezer and colleagues⁷⁶ failed to observe a loss of long-range interaction in A30P and A53T, and in fact they found that C-to-N contacts were *stronger* in E46K compared to wild-type α Syn. Accordingly, these investigators concluded that parameters such as net charge or secondary structure propensity are more important than the strength of long-range interactions in determining relative rates of self-assembly of wild-type and mutant α Syn.

s0030 **D. Posttranslational Modifications of α Syn in Diseased Brains and Synucleinopathy Models**

p0065 Various posttranslational modifications are associated with aggregated α Syn in patients with synucleinopathy disorders. Examples of these modifications include tyrosine nitration,⁷⁷ phosphorylation of serine 129 (S129),^{42,78–80} ubiquitylation,^{42,81} and C-terminal truncations resulting from the removal of approximately 20–40 residues (Fig. 1).^{42,72,73,82} In contrast, phosphorylation of tyrosine 125 (Y125), was detected in the brains of aged, nondiseased individuals, but not in the brains of patients with dementia with Lewy bodies (DLB).⁸³

p0070 A number of posttranslational modifications of α Syn have been detected in cellular and animal models relevant to PD and other synucleinopathy disorders, including (i) oxidation of methionine residues 116 or 127 to methionine sulfoxide (MetSO) or methionine sulfone⁸⁴; (ii) nitration of tyrosine residues,^{85–88} including Y39^{89,90} in the N-terminal domain and Y125, Y133, and Y136 in the C-terminal domain (Strathearn *et al.*, unpublished observations)^{84,89}; (iii) phosphorylation of S129 (Strathearn *et al.*, unpublished observations)^{79,80,88,91–100}; (iv) phosphorylation of Y125^{83,84}; and (v) C-terminal truncation resulting from the cleavage of approximately 20–40 residues.^{72,73,101,102} One group has reported the presence of α Syn isoforms phosphorylated on serine 87 (S87) in the brains of synucleinopathy patients or transgenic mouse models of synucleinopathy disorders,¹⁰³ although another group failed to detect this modification in human patients or transgenic mice.¹⁰⁴

s0035 **E. Effects of Posttranslational Modifications on α Syn Self-assembly and Neurotoxicity**

p0075 A central question driving research in the field of synucleinopathy disorders is whether posttranslational modifications are a *cause* of enhanced α Syn aggregation and neurotoxicity. To address this question, a number of studies have been carried out in cell-free systems and cellular and animal models. The key findings from these studies are summarized below.

s0040 **1. α SYN OXIDATION**

p0080 Data obtained from studies in cell-free systems indicate that α Syn oligomer formation is promoted by H₂O₂ and Fe²⁺ or Cu²⁺^{105,106} and by dityrosine crosslink formation involving Y125 under conditions of oxidative stress.^{107–109} Modification of α Syn by metal-catalyzed oxidation¹¹⁰ or oxidation by the lipid peroxidation product 4-hydroxy-2-nonenal¹¹¹ results in inhibition of fibrillization and a buildup of soluble oligomers. Another lipid peroxidation product,

acrolein, also stimulates α Syn oligomer formation.¹¹² Oxidized cholesterol metabolites promote the self-assembly of recombinant α Syn to protofibrils and fibrils, apparently via a mechanism involving noncovalent interactions.¹¹³

p0085 Oxidation of all four methionine residues of α Syn (M1, M5, M116, and M127) to MetSO results in a nearly complete suppression of fibrillization at neutral pH,^{114,115} and the degree of inhibition increases with the number of oxidized methionine residues.¹¹⁶ The methionine-oxidized protein regains its ability to form fibrils under conditions that favor neutralization of C-terminal negative charges – notably, when incubated in the presence of various metal ions (e.g., Ti^{3+} , Zn^{2+} , Al^{3+} , Pb^{2+})¹¹⁷ or at low pH (pH = 3).¹¹⁵ These observations suggest that methionine oxidation interferes with α Syn fibrillization by favoring repulsive intermolecular interactions and/or auto-inhibitory long-range interactions involving the C-terminal domain.¹¹⁵ In contrast to the inhibitory effect of MetSO on α Syn fibrillization, soluble oligomers are found to accumulate in mixtures of methionine-oxidized and unoxidized α Syn^{114,116,118,119} or in pure solutions of the methionine-oxidized protein.¹¹⁵

s0045 2. α SYN–DA INTERACTIONS

p0090 A number of groups have shown that α Syn reacts with oxidized derivatives of DA, including indole-5,6-quinone, 5,6-dihydroxyindole, and dihydroxyphenylacetic acid (DOPAC), and α Syn loses the ability to form amyloid-like fibrils and instead accumulates as soluble oligomers.^{118,120–125} Data from other studies suggest that DA oxidation products stimulate α Syn oligomer formation and block fibril formation, and destabilize preformed fibrils by interacting with the protein noncovalently.^{122,123,126–128} DA may also promote α Syn oligomerization and inhibit α Syn fibrillization via oxidation of the protein's four methionine residues to MetSO (see above).¹²⁹ Some α Syn oligomers formed in the presence of DA appear similar to protofibrils on the basis of their size and morphology determined by AFM or EM^{120,127} or their elution behavior during gel filtration or SDS-PAGE,^{49,50,118,121,130} whereas others are smaller cross-linked multimers that lack a stable secondary structure.^{49,118,120,121,124,125,130,131} The results of molecular modeling studies involving docking of DA into α Syn conformations determined by solution NMR suggested that DA interacts with two sites on the protein: (i) the peptide sequence 125-YEMPS-129, via hydrophobic interactions; and (ii) residue E83, via electrostatic interactions.¹³² Consistent with these binding sites, the 125-YEMPS-129 segment was previously shown to play an important role in DA-mediated suppression of α Syn fibrillization via a noncovalent mechanism,^{123,127} and an α Syn mutant in which E83 was replaced with alanine (E83A) was found to be resistant to the inhibitory effects of DA on α Syn fibril formation.¹³²

p0095 In support of the above findings from studies in cell-free systems, Ischiropoulos and colleagues¹³³ showed that inclusion formation by α Syn A53T is suppressed in a neuroblastoma cell line engineered to produce high levels of intracellular DA via TH overexpression. The inhibitory effect of DA on α Syn aggregation in cell culture was dependent on the presence of the 125-YEMPS-129 segment.¹²³ Moreover, detergent-insoluble α Syn aggregates and soluble α Syn oligomers were found to be less abundant and more abundant (respectively) in nigral tissue compared to cortical tissue isolated from α Syn transgenic mice.¹²³ From these results, the authors inferred that (i) α Syn oligomers formed in the presence of DA are nontoxic, and (ii) a loss of DA in the substantia nigra may enhance α Syn neurotoxicity in this region by promoting the protein's conversion to amyloid-like fibrils. The degree to which this model relates to the pathogenesis of human PD is unclear, however, given that increased levels of α Syn expression are associated with increased dopaminergic cell death in the substantia nigra in the brains of patients^{9,10} but not in the brains of α Syn transgenic mice.¹³³ In addition, multiple lines of evidence suggest that interactions among cytosolic DA, α Syn, and Ca^{2+} ions contribute to preferential dopaminergic cell death in PD.¹³⁴⁻¹³⁶ Nevertheless, the apparent lack of dopaminergic cell death in response to a buildup of α Syn oligomers in the substantia nigra of transgenic mice is an important observation because it suggests that some α Syn assemblies formed in the presence of DA are not intrinsically toxic. Alternatively, a set of conditions in the substantia nigra of α Syn transgenic mice may prevent dopaminergic cell death which would normally be triggered by toxic α Syn oligomers (and thus identification of these conditions might reveal new strategies for treating PD).

s0050 3. α SYN NITRATION

p0100 In the presence of peroxyxynitrite, α Syn undergoes dimerization as a result of O,O' -dityrosine formation or nitration at one or more tyrosine residues.^{107,108,127} Nitrated α Syn has an increased propensity to form soluble oligomers but a decreased ability to form amyloid-like fibrils.^{137,138} Moreover, nitrated α Syn oligomers (but not nitrated monomers or dimers) suppress fibrillization of the unmodified protein.^{138,139} To address whether nitration plays a role in α Syn neurotoxicity, He and colleagues¹⁴⁰ generated a fully nitrated variant of α Syn112 which was fused to the TAT signal peptide to enable transport across cell membranes. Nitrated TAT- α Syn112 was found to elicit greater dopaminergic cell death and more pronounced motor deficits compared to the corresponding non-nitrated fusion protein after unilateral infusion into rat substantia nigra, suggesting that nitrated α Syn112 is more toxic than the unmodified protein. Although these findings revealed important new insights, they also raised questions about whether the toxicity of the nitrated fusion protein was affected by (i) the presence of the TAT peptide, which may perturb the subcellular distribution of its α Syn "cargo" compared to that of the unfused

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protein^{141,142}; and (ii) molecular properties of α Syn112 that are distinct from those of α Syn140. One way to address these issues would be to test the effects of ablating one or more nitration sites via site-directed mutagenesis on the neurotoxicity of α Syn140 expressed in a cell-culture model. Recently, we found that an α Syn140 variant in which Y39, Y125, and Y133 were replaced with phenylalanine (“3YF”) was substantially less toxic than the wild-type protein in a primary midbrain culture model, suggesting that one or more of the above tyrosine residues plays a role in dopaminergic cell death (Strathearn and Rochet, unpublished observations). A limitation of this approach is that one cannot be certain whether the reduced toxicity of the mutant protein results from the disruption of nitration, dityrosine formation (see above), or tyrosine phosphorylation (see below).

s0055 4. SERINE PHOSPHORYLATION

p0105 In an early study, Iwatsubo and colleagues⁷⁸ found that recombinant human α Syn phosphorylated by casein kinase 2 (CK2), an enzyme that phosphorylates α Syn at S129 and to a lesser degree S87,¹⁴³ formed amyloid-like fibrils more rapidly than the unphosphorylated protein. In contrast, a detailed biochemical analysis of α Syn phosphorylated uniquely at S129 (α Syn-pS129), formed by treating the S87A mutant with CK1, revealed that S129 phosphorylation disrupted long-range interactions in the natively unfolded monomer and interfered with the protein’s ability to undergo oligomerization or fibrillization.¹⁴⁴ It is unclear why serine phosphorylation was found to affect the rate of α Syn fibrillization differently in the two studies, although even small differences in the experimental conditions can have pronounced effects on the kinetics of α Syn self-assembly.¹⁴⁵ Importantly, α Syn mutants in which S129 was replaced with aspartate (S129D) or glutamate (S129E) differed substantially from α Syn-pS129 in terms of their conformations in solution and fibrillization rates (S129D and S129E had unperturbed long-range interactions and a similar ability to form fibrils as wild-type α Syn).¹⁴⁴ These findings suggest that the S129D and S129E substitutions are poor mimics of S129 phosphorylation, at least in the context of the purified recombinant protein.

p0110 Considerable research efforts have focused on elucidating how S129 phosphorylation affects α Syn aggregation and neurotoxicity in animal models of synucleinopathy disorders. One approach has been to investigate the impact of replacing S129 with (i) alanine, which cannot be phosphorylated; or (ii) aspartate, which may serve as an *in vivo* phosphoserine mimic. In one study, Chen and Feany⁹³ showed that the S129A mutant had a greater propensity to form inclusions but a reduced ability to trigger dopaminergic cell death than wild-type α Syn in a transgenic *Drosophila* model. In contrast, flies expressing S129D exhibited more pronounced neurodegeneration than flies expressing the wild-type protein. Contrary to these results, two groups reported that S129A and S129D exhibited increased and decreased neurotoxicity (respectively)

compared to wild-type α Syn when expressed from a recombinant adeno-associated virus (rAAV) vector injected in rat substantia nigra.^{146,147} In one of these studies, S129A was also shown to produce more abundant amyloid-like (thioflavin S-positive) nigral inclusions than wild-type α Syn or S129D.¹⁴⁷ A third group reported that wild-type α Syn, S129A, and S129D were essentially indistinguishable in terms of their neurotoxic effects in a rat rAAV model (the outcomes of this rAAV study may have differed from those of the other two outlined above because of differences in experimental conditions—e.g., rAAV serotype, rat strain, and duration of study).¹⁴⁸

p0115 Together, these findings indicate that S129 substitutions have opposite effects on α Syn neurotoxicity in transgenic *Drosophila* versus rAAV-infected rats: the rank order of toxicities is S129D>wild-type α Syn>S129A in flies, whereas it is S129A>wild-type α Syn>S129D in rats. Because the S129A mutant has an increased propensity to form amyloid-like fibrils compared to wild-type α Syn in cell-free systems,¹⁴⁴ it is not a reliable variant to determine the consequences of ablating S129 phosphorylation on α Syn neurotoxicity (in order to serve as a meaningful negative control, such a variant should have the same fibrillization propensity as the wild-type protein). It is unclear why the expression of S129D had opposite effects in rats versus flies. As one possibility, interactions between the human and rat proteins may modulate α Syn neurotoxicity in the rat rAAV model, whereas these interspecies effects are absent in *Drosophila* because flies do not express an endogenous α Syn homolog.^{46,147} It should also be noted that S129D (or S129E) may not faithfully reproduce the conformational properties or aggregation behavior of α Syn-pS129 *in vivo*, which is a limitation that has been demonstrated in cell-free systems (see above).¹⁴⁴

p0120 Kinases potentially involved in phosphorylating α Syn at S129 include casein kinase 2,^{95,97,98,104,143,149–151} G-protein coupled receptor kinases 2 and 5 (GRK2, GRK5),^{152,153} and polo-like kinases (PLKs),^{151,154,155} whereas α Syn-pS129 dephosphorylation is mediated by phosphoprotein phosphatase 2A (PP2A).¹⁵⁶ Experiments designed to modulate the activity or expression level of these enzymes may provide insight into the impact of S129 phosphorylation on α Syn aggregation or neurotoxicity. In one study, Chen and Feany⁹³ reported that the neurotoxicity of wild-type α Syn was enhanced upon coexpression of GRK2 in their transgenic fly model. In another study, expression of PLK2 was found to mitigate α Syn-mediated dopaminergic cell death in a *Caenorhabditis elegans* model and in rat primary midbrain cultures.¹⁵⁷ A third study revealed that activation of PP2A with eicosanoyl-5-hydroxytryptamide, an inhibitor of PP2A demethylation, interfered with S129 phosphorylation, α Syn aggregation, dendritic degeneration, glial activation, and motor dysfunction in α Syn transgenic mice.¹⁵⁶ A caveat in interpreting the results of these three studies is that modulation of kinase or phosphatase activity may affect α Syn aggregation or neurotoxicity via mechanisms independent of S129 phosphorylation.

p0125 Finally, α Syn-pS87 was recently shown to have a decreased ability to form oligomers or fibrils compared to wild-type α Syn.¹⁰³ The S87D and S87E variants had similar aggregation propensities as α Syn-pS87, suggesting that aspartate and glutamate are better phosphoserine mimics at position 87 than at position 129. Phosphorylation of S87 (but not S129) lowers the affinity of α Syn for phospholipid membranes and alters the conformation of the membrane-bound protein, suggesting that this modification may interfere with the protein's normal functions (e.g., modulation of neurotransmission).¹⁰³ Kinases implicated in S87 phosphorylation include CK1^{103,143} and the dual-specificity tyrosine-regulated kinase DYRK1A.¹⁵⁸

s0060 5. TYROSINE PHOSPHORYLATION

p0130 An early study revealed that the nonreceptor tyrosine kinase p72^{syk} suppressed α Syn aggregation in a cell-free system by phosphorylating residues Y125, Y133, and Y136 in the C-terminal tail.¹⁵⁹ In contrast, phosphorylation of Y125 alone by Lyn kinase had no effect on α Syn self-assembly. More recently, Feany and colleagues⁸³ reported that Y125F exhibited enhanced oligomerization and neurotoxicity compared to wild-type α Syn in transgenic flies, whereas coexpression of the tyrosine kinase shark (a *Drosophila* homolog of Syk) interfered with the ability of wild-type α Syn and S129D to form oligomers or elicit neurodegeneration in this model. The authors also showed that levels of α Syn-pY125 were lower in postmortem brains from old versus young individuals or from DLB patients versus age-matched controls. From these data, the authors inferred that (i) phosphorylation of Y125 antagonizes suppresses the formation of neurotoxic α Syn oligomers by antagonizing the pathologic effects of S129 phosphorylation; and (ii) a reduction in this protective effect of Y125 phosphorylation in older individuals may contribute to the increased risk of PD with aging. Our recent finding that the “3YF” mutant exhibits reduced neurotoxicity compared to wild-type α Syn in primary midbrain cultures (Strathearn and Rochet, unpublished observations) is inconsistent with the enhanced neurotoxicity of Y125F in transgenic *Drosophila*. The reasons for this discrepancy are unclear but may relate to obvious differences between the two experimental systems (e.g., presence of human and rat α Syn in midbrain cultures versus only human α Syn in fly brain; mutation of Y125/Y133/Y136 in cell-culture model versus mutation of just Y125 in *Drosophila* model).

s0065 6. C-TERMINAL TRUNCATION

p0135 Recombinant C-terminal truncation mutants spanning residues 1–110 or 1–120 of wild-type α Syn, similar to truncated α Syn variants identified in Lewy bodies,^{42,72,73,82} undergo fibrillization and/or oligomerization more rapidly than the full-length protein.^{47,55,70–73,160} In addition, substoichiometric levels of the truncation mutants accelerate the aggregation of full-length α Syn, and

this seeding effect is more pronounced in the case of truncated A53T than the truncated wild-type protein (paired with full-length A53T and wild-type α Syn, respectively).^{72,73} The stimulatory effect of C-terminal truncation on α Syn self-assembly is thought to involve the disruption of auto-inhibitory long-range interactions, a mechanism that may also account for the enhanced aggregation of full-length α Syn upon binding of metal ions or polyamines to the negatively charged C-terminal region of.^{55,67,68}

Au2

p0140 A question of central importance to the field is whether α Syn neurotoxicity is enhanced as a result of C-terminal cleavage. In one study, Feany and colleagues¹⁶¹ showed that transgenic *Drosophila* expressing a truncation mutant of human α Syn spanning residues 1–120 (α Syn_{1–120}) underwent a more pronounced accumulation of α Syn oligomers and inclusions and a more severe loss of dopaminergic neurons than flies expressing the full-length protein. In another study, Spillantini and colleagues¹⁶² showed that expression of α Syn_{1–120} in transgenic mice lacking the endogenous mouse protein triggered the formation of fibrillar α Syn inclusions, swelling of striatal neurites, loss of striatal dopamine, and motor deficits. Similarly, expression of α Syn_{1–119} in a conditional transgenic mouse model induced a loss of striatal dopamine and dopamine metabolites.¹⁶³ Transgenic mice expressing human α Syn_{1–130} exhibited more extensive dopaminergic cell death in the substantia nigra and more striking behavioral deficits than mice expressing the full-length protein.¹⁶⁴ Coexpression of full-length human α Syn and α Syn_{1–110} from AAV vectors stereotactically injected into rat substantia nigra (each at a multiplicity of infection below the toxic threshold determined for that virus alone) resulted in a pronounced neurodegenerative phenotype.¹⁶⁵ In contrast, stereotactic injection of an AAV vector encoding A53T_{1–93}, a product of matrix metalloproteinase-3 (MMP-3) cleavage, in rat substantia nigra resulted in a similar degree of neurodegeneration as injection of AAV encoding full-length A53T.¹⁰² Collectively, these results imply that C-terminally cleaved α Syn isoforms generally elicit greater neurotoxicity than full-length α Syn, and/or they may enhance the toxicity of the full-length protein. However, the effects of truncation on α Syn-mediated neurodegeneration are likely to vary with the site of cleavage (e.g., residue 110, 120, or 130 versus residue 93) and the α Syn sequence context (e.g., wild-type versus A53T sequence).

p0145 C-terminal truncation may enhance α Syn-mediated neurodegeneration by promoting fibrillization (e.g., perhaps by disfavoring interactions with DA via removal the 125-YEMPS-129 segment^{123,133}) and/or the formation of potentially toxic oligomers.¹⁶⁰ If C-terminally cleaved α Syn variants are indeed more toxic than the full-length protein, then one would infer that proteases responsible for cleaving α Syn may be reasonable therapeutic targets for PD and other synucleinopathy disorders. Several proteases are known to cleave α Syn in the C-terminal region, including calpain,^{166–168} the 20S proteasome,^{73,160} cathepsin D,¹⁶⁹ and MMP-3.¹⁰²

s0070 F. Effects of Phospholipids on α Syn Self-assembly

p0150 Because α Syn readily associates with cellular membranes, a high priority in the field of synucleinopathy diseases is to understand how the protein's interactions with phospholipids affect its self-assembly behavior. In an important study, Lee and colleagues¹⁷⁰ showed that α Syn formed SDS-resistant oligomers more rapidly in incubated membrane fractions versus cytosolic fractions of rat brain homogenates via a mechanism dependent on oxidative stress. The formation of membrane-bound α Syn oligomers was stimulated by the addition of cytosolic α Syn to membrane fractions, implying that the cytosolic protein was recruited into membrane-bound assemblies. α Syn was also shown to readily form oligomers in cells enriched with lipid droplets¹⁷¹ and to undergo accelerated oligomerization and fibrillization in the presence of long-chain polyunsaturated fatty acids.^{172–174} In addition, α Syn fibril formation was stimulated in the presence of anionic detergent micelles, phospholipid vesicles, or synaptosomal membranes.^{175–178} α Syn self-assembly occurred readily in these systems at a high protein–lipid ratio, whereas aggregation could be suppressed in the presence of excess phospholipids.^{176,179}

p0155 Upon interacting with phospholipid membranes, α Syn adopts a conformation in which the N-terminal repeat region is folded into an amphipathic α -helical domain (either bent or extended) that binds the membrane surface, whereas the C-terminal region is disordered and exposed to the aqueous environment.^{24–29,31,176,180–184} We and others have hypothesized that α Syn aggregation may be stimulated by interactions among α -helical molecules bound to the membrane surface.^{49,185–188} The underlying rationale is that neighboring α Syn conformers may interact more readily in a two-dimensional space at the membrane surface compared to the less geometrically constrained three-dimensional space of the bulk solution.^{189,190} Consistent with this hypothesis, we showed that α Syn formed clusters upon binding to a supported lipid bilayer consisting of a mixture of the anionic phospholipid phosphatidylglycerol (PG) and the zwitterionic phospholipid phosphatidylcholine (PC).^{191–193} The ability of α Syn to form membrane-bound clusters increased with increasing anionic lipid (PG) content and/or protein concentration, and regions on the bilayer where α Syn was clustered were also enriched in PG. From these data, we inferred that (i) α Syn induces separation of phospholipids into regions enriched in anionic and zwitterionic lipids in order to neutralize charges on helical α Syn and/or relieve unfavorable lipid–lipid interactions; and (ii) α Syn forms clusters associated with regions enriched with anionic lipids on the membrane surface in order to bury hydrophobic residues exposed when the protein adopts a helical structure.

p0160 An elegant series of NMR analyses by Bax and colleagues^{182,194} revealed that vesicle-bound α Syn adopts multiple conformations in which the N-terminal α -helical domain spans segments of different lengths along the polypeptide

chain. Two prominent long-lived conformations—referred to as the “SL1” and “SL2” states—had immobilized, membrane-bound helical domains spanning residues 1–25 and 1–97, respectively. Data reported by Beyer and colleagues¹⁹⁵ suggested that α Syn initially binds the membrane in the SL1 state, which then converts to the SL2 state via a mechanism in which the N-terminal helical domain spanning residues 1–25 nucleates the helical folding of the segment spanning residues 26–100. Consistent with these findings, residues 2–11 (and in particular, an aspartate residue at position 2) were found to play an important role in α Syn membrane binding and toxicity in a yeast model.¹⁹⁶ Because the SL1 state has a more extensive disordered region (spanning residues 26–140, including the hydrophobic NAC domain), this membrane-bound conformer should have a higher propensity to engage in intermolecular contacts compatible with the formation of β -sheet-rich, potentially toxic oligomers. The proportion of wild-type α Syn molecules existing in the SL1 state increased with increasing protein/lipid ratios,¹⁸² and all three familial PD mutants (A30P, E46K, and A53T) populated the SL1 conformation to a greater extent than the wild-type protein.¹⁹⁴

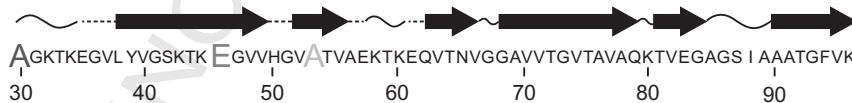
p0165 From these observations, we propose a model in which membranes enriched with anionic phospholipids promote the formation of neurotoxic α Syn oligomers. An initial step in this process involves the clustering of neighboring α Syn molecules via helix–helix contacts in membrane domains enriched with anionic phospholipids. Next, nonhelical segments of clustered α Syn molecules (particularly SL1 conformers) interact to form assemblies with increased β -sheet content and stability. Environmental perturbations may trigger α Syn self-assembly at the membrane surface by intervening at various stages of the pathway outlined above. For example, an increase in relative levels of anionic phospholipids in the brains of PD patients¹⁹⁷ may promote the clustering of membrane-bound helical conformers during the early stages of self-assembly.¹⁹¹ Nitration of the C-terminal tyrosine residues (Y125, Y133, and Y136), which was recently shown to lower the affinity of α Syn for phospholipid vesicles via an allosteric mechanism,¹⁹⁸ could potentially promote the formation of membrane-bound α Syn oligomers by increasing the proportion of α Syn molecules in the SL1 state,¹⁹⁴ or by increasing the lifetime of this conformation.¹⁹⁵ Interactions between nonhelical segments of membrane-bound α Syn may be favored by the presence of Ca^{2+} ions, which promote interactions of the C-terminal tail with the bilayer and induce an increase in overall β -sheet content.¹⁹⁹ The aggregation of membrane-associated α Syn may also be triggered by C-terminal truncation, based on evidence that the three familial mutants (and to a lesser extent the wild-type protein) undergo accelerated oligomerization when incubated in the presence of liposomes and the 20S proteasome, but not either agent alone.¹⁶⁰ Finally, strategies to interfere with the clustering of helical membrane-bound α Syn conformers or subsequent interactions between nonhelical segments may prove beneficial for the treatment of PD and other synucleinopathy disorders.

s0075 G. Characterization of α Syn Fibrillar Structure

p0170 The structural properties of α Syn amyloid-like fibrils were closely examined using various biophysical methods, including electron paramagnetic resonance spectroscopy,^{200,201} hydrogen exchange-mass spectrometry,²⁰² and solid-state NMR (SS-NMR).^{203–205} These studies revealed that fibrillar α Syn consists of a structured core domain encompassing residues ~30–110, flanked by an N-terminal, conformationally heterogeneous region and a C-terminal disordered region. The core region of fibrillar α Syn comprises five β -strands arranged as strand–loop–strand motifs that project along the fibril axis.^{200–204} This arrangement yields a five-layered structure in which each layer is composed of an extended β -sheet with parallel, in-register strands. The five-layered structure constitutes a protofilament, and two protofilaments are aligned in a straight or twisted fashion to form a fibril.²⁰⁴ Data obtained from a recent SS-NMR study suggested that the core region of fibrillar α Syn adopts a different structure consisting of two repeats of a motif encompassing a long β -strand followed by two shorter β -strands (Fig. 2).²⁰⁵ Together, these structural insights may have a profound impact on drug discovery efforts by stimulating the design of α Syn fibrillization inhibitors using structure-based methods.^{206–208}

s0080 H. Structure-Based Approaches to Assess Relative Toxicities of α Syn Oligomers and Fibrils

p0175 A central problem in the field of PD and other synucleinopathy disorders is whether α Syn neurotoxicity is mediated by prefibrillar intermediates (oligomers, protofibrils) or mature amyloid-like fibrils. To address this question, two groups have used the innovative approach of characterizing the neurotoxic properties of α Syn variants with a high propensity to form oligomers or protofibrils but not fibrils.^{209,210} In both cases, the α Syn variants were designed using a rational approach based on the structure of fibrillar α Syn described above. In one study, the engineered α Syn mutants A56P and A30P/A56P/A76P (“TP”) were found to undergo less rapid fibrillization than wild-type α Syn, and SS-NMR analysis of fibrils formed by the mutant proteins revealed a decrease in β -sheet



f0010 FIG. 2. Structural model of fibrillar α Syn determined from a recent solid-state NMR study. The core domain spans residues 38–96 and consists of two repeats of a β -sheet motif encompassing one long strand followed by two shorter strands. The three familial substitutions (A30P, E46K, and A53T) are shown in a larger font size. This figure (adapted from Ref. 205) was generously provided by Dr. Chad Rienstra (University of Illinois-Urbana/Champaign).

content.²⁰⁹ In addition, both proline variants (and especially the “TP” mutant) had an enhanced propensity to accumulate as oligomers in a cell-free system and exhibited increased neurotoxicity compared to wild-type α Syn when expressed in primary cell cultures, *C. elegans*, or *Drosophila*. In the second study, the α Syn variants E35K and E57K, designed on the basis of the rationale that these substitutions might disrupt salt bridges involved in stabilizing β -sheet structure in fibrillar α Syn, were found to have a decreased propensity to form amyloid-like fibrils but an increased ability to form oligomers, including ring-like structures.²¹⁰ Strikingly, E35K and E57K exhibited enhanced neurotoxicity compared to wild-type α Syn and A53T when expressed from a lentiviral construct in rat substantia nigra, and both variants formed SDS-resistant oligomers (particularly trimers) which were detected by immunoblot analysis of membrane fractions from rat midbrain homogenates. In contrast, an engineered α Syn mutant spanning residues 30–110 with an enhanced fibrillization propensity exhibited substantially reduced neurotoxicity in the rat lentiviral model.²¹⁰ Collectively, these findings support the hypothesis that α Syn oligomers, rather than amyloid-like fibrils, are the major α Syn species involved in neurodegeneration in PD.

s0085 I. A Role for Cell-to-Cell Transmission of α Syn in PD Pathogenesis

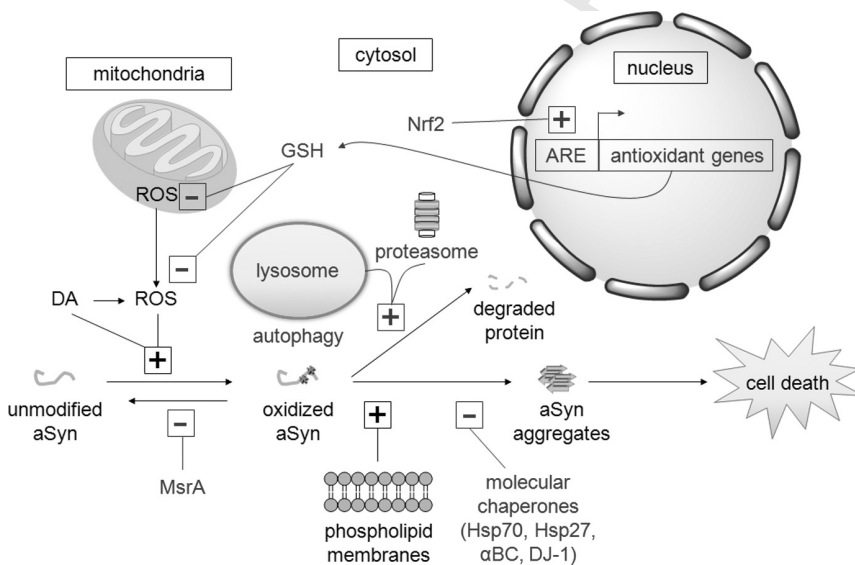
p0180 Emerging evidence over the past 5 years has led to the intriguing hypothesis that the spread of neuropathology in PD involves cell-to-cell transmission of α Syn.²¹¹ A number of groups have shown that monomeric and oligomeric forms of α Syn are secreted into the conditioned media of mammalian cell cultures, apparently via a nonclassical mechanism that depends on the presence of intracellular Ca^{2+} and involves the release of exosomes derived from multivesicular bodies.^{212–217} α Syn secretion is stimulated by various cellular stresses, including proteasomal inhibition and lysosomal impairment, and secreted α Syn has higher levels of oxidative damage than the intracellular protein.^{214,215} Oligomeric α Syn is internalized by mammalian cells via an endocytic mechanism and eliminated from recipient cells via lysosomal clearance pathways.²¹⁸ Additional studies have revealed that α Syn is transmitted between cells in culture or from host neurons to engrafted neuronal cells in α Syn transgenic mice, resulting in inclusion formation and activation of apoptosis in recipient cells.^{216,217,219} These findings are important because they suggest that cell-to-cell transmission of α Syn may contribute to the spread of neuropathology in PD described by Braak and colleagues.²²⁰ Moreover, the activation of astrocytes or microglia by extracellular α Syn (via mechanisms involving receptor binding and/or internalization) may contribute to neuroinflammatory pathways characteristic of the disease.^{221–225}

s0090 **J. Cellular Mechanisms to Suppress α Syn Aggregation and Neurotoxicity**

p0185 Various cellular mechanisms are in place to inhibit the accumulation of neurotoxic, aggregated forms of α Syn. Examples of proteins involved in these “surveillance” mechanisms include antioxidant proteins, molecular chaperones, and proteins involved in cellular clearance mechanisms (Fig. 3). Each of these types of proteins is reviewed in greater detail below.

s0095 **1. UPREGULATION OF ANTIOXIDANT RESPONSES**

p0190 Evidence that oxidative stress favors the formation of potentially toxic α Syn aggregates has prompted the hypothesis that α Syn-mediated neurodegeneration may be alleviated by proteins with antioxidant activity.^{226,227} In support of



f0015 **FIG. 3.** Model illustrating cellular phenomena that promote or inhibit α Syn self-assembly. A loss of mitochondrial function (e.g., impairment of complex I) or an increase in cytosolic dopamine levels triggers a buildup of ROS in dopaminergic neurons. In turn, ROS and/or dopamine oxidation products react with α Syn, converting the protein to oxidized forms with a high propensity to aggregate. α Syn self-assembly is promoted under some conditions by binding of the protein to phospholipid membranes. α Syn aggregation and neurotoxicity may be mitigated by (i) cellular clearance mechanisms, including the 26S proteasome and lysosomal autophagy; (ii) cellular antioxidant responses, including Nrf2-mediated transcription, resulting in increased glutathione (GSH) synthesis, and MsrA-dependent repair of oxidized α Syn; and (iii) molecular chaperones, including Hsp70, Hsp27, α B-crystallin, and DJ-1. (See color plate.)

this hypothesis, expression of the antioxidant repair enzyme methionine sulfoxide reductase A (MsrA) was shown to inhibit α Syn aggregation and neurotoxicity in cellular and animal models relevant to PD (Fig. 3).^{228,229} MsrA reduces the *S*-stereoisomer of MetSO, including protein-bound MetSO, back to methionine. MsrA carries out its antioxidant function by (i) repairing oxidatively damaged proteins, thereby helping to preserve their activities; and (ii) depleting ROS by participating in cycles of methionine oxidation and reduction.^{230,231} In one study, overexpression of bovine MsrA was found to alleviate α Syn-mediated dopaminergic cell death and cause a decrease in the abundance of α Syn oligomers in primary midbrain cultures.²²⁹ In contrast, the ROS scavenger *N*-acetyl cysteine had a much less pronounced inhibitory effect, suggesting that MsrA interferes with α Syn aggregation and neurotoxicity by repairing oxidized α Syn rather than by depleting free radicals. Consistent with this interpretation, MsrA was shown to repair methionine-oxidized α Syn in a cell-free system.²²⁹ In another study, coexpression of bovine MsrA was found to rescue dopaminergic cell death and motor deficits induced by the expression of human α Syn in a transgenic *Drosophila* model.²²⁸ A similar protective effect was observed when the flies were fed *S*-methyl-L-cysteine (SMLC), a methionine analog that presumably triggered ROS depletion by undergoing cycles of oxidation and reduction catalyzed by endogenous MsrA in the fly brain.²²⁸

p0195 Collectively, these findings suggest that MsrA prevents a buildup of oxidized α Syn isoforms that readily form toxic oligomers in PD models.^{228,229} The neuroprotective effect of MsrA may involve direct repair of α Syn and/or ROS scavenging (the relative importance of each of these mechanisms may vary from one model to another). Cycles of oxidation and MsrA-catalyzed repair of membrane-bound α Syn are likely to play an important role in suppressing the oxidation of unsaturated membrane lipids.²³² Because MsrA is abundant throughout the brain including the substantia nigra,²³³ these observations imply that the enzyme may be involved in protecting nigral dopaminergic neurons against PD-related insults. If this is true, then the reported age-dependent decrease in MsrA activity²³⁴ may contribute to the increased risk of PD with aging.

p0200 The antioxidant enzyme Cu/Zn superoxide dismutase (SOD1) has also been shown to alleviate dopaminergic cell death and motor deficits when coexpressed in α Syn transgenic *Drosophila*.²³⁵ Moreover, activation of the Nrf2/Keap1 signaling pathway, resulting in increased expression of genes encoding enzymes involved in the cellular antioxidant response,^{236–238} rescues motor deficits and dopaminergic neuronal loss in α Syn-expressing flies (Fig. 3).²³⁹ These observations further substantiate the idea that α Syn neurotoxicity can be alleviated by the expression of proteins with antioxidant activity.

s0100 2. UPREGULATION OF CHAPERONE FUNCTION

p0205 Molecular chaperones contribute to protein quality control by facilitating the refolding of misfolded polypeptides, interfering directly with protein aggregation, or directing misfolded or aggregated polypeptides to cellular clearance pathways (e.g., UPP, lysosomal autophagy).^{227,240} Heat shock proteins (e.g., Hsp27, Hsp40, Hsp70, and Hsp90 in eukaryotes) are a major class of chaperones upregulated in response to elevated temperatures and other stresses that cause a buildup of misfolded polypeptides. Examples of molecular chaperones that have been characterized in terms of their ability to suppress α Syn aggregation or toxicity include Hsp70, Hsp27, α B-crystallin, and DJ-1 (Fig. 3).

s0105 3. HSP70

p0210 Hsp70 was found to inhibit α Syn fibril formation in cell-free systems via a mechanism involving binding of the chaperone to various species on the α Syn self-assembly pathway, including early oligomers and higher order protofibrils.^{241–243} The interaction of α Syn with Hsp70 led to the formation of soluble amorphous aggregates that were devoid of permeabilizing activity or cytotoxicity.^{241,242,244} Dobson and colleagues²⁴⁴ reported that Hsp70 and oligomeric α Syn formed a more compact complex in the presence of ATP or ADP, yielding toxic protofibrils that sequestered the chaperone via coaggregation. The stimulatory effects of nucleotides on α Syn protofibril formation and Hsp70 depletion were abrogated by co-incubation with Hip (ST13), a co-chaperone that is downregulated in PD patients,²⁴⁵ and siRNA-mediated knockdown of Hip induced an Hsp70-dependent increase in α Syn-YFP inclusions in a transgenic *C. elegans* model. In another study, stable β -sheet-rich α Syn oligomers were found to inhibit Hsp70/Hsp40-mediated unfolding/refolding of protein substrates, apparently via weak hydrophobic interactions with the Hsp40 co-chaperone.²⁴⁶ Together, these findings suggest that Hsp70-mediated suppression of α Syn neurotoxicity involves a balance between productive and nonproductive interactions among Hsp70, its co-chaperones, and various α Syn intermediates, resulting in the conversion of α Syn into nontoxic aggregates or in inhibition of the chaperone machinery, respectively.

p0215 Hsp70 has also been shown to modulate α Syn self-assembly and neurotoxicity in cellular and animal models. Groundbreaking studies by Bonini and colleagues^{247,248} revealed that Hsp70 overexpression or upregulation alleviated α Syn neurotoxicity without altering the degree of inclusion formation in α Syn transgenic flies, whereas inactivation of the constitutive Hsp70 isoform Hsc4 resulted in accelerated dopaminergic cell death in this model. These results suggested that Hsp70 facilitates the conversion of α Syn to nontoxic aggregates in the fly brain. Hsp70 expression was also shown to inhibit α Syn oligomerization

and toxicity in H4 human neuroglioma cells^{249,250} and primary midbrain cultures.²⁵¹ Hsp70 was also found to suppress α Syn aggregation in the brains of α Syn transgenic mice,²⁴⁹ although data from another study suggested that the chaperone had no impact on motor deficits or α Syn oligomerization in transgenic mice expressing A53T α Syn.²⁵² Finally, Hsp70 was recently shown to suppress the accumulation of extracellular α Syn oligomers in the H4 neuroglioma cell-culture model, potentially via a mechanism involving Hsp70 secretion.²¹⁶ Collectively, these data suggest that Hsp70 plays a major role in mitigating α Syn aggregation and toxicity in synucleinopathy disorders.

s0110 4. α B-CRYSTALLIN AND HSP27

p0220 The small heat shock proteins α B-crystallin and Hsp27 are composed of low molecular weight subunits assembled into large oligomeric complexes. α B-crystallin was found to inhibit the fibrillization of recombinant α Syn via a mechanism in which the chaperone binds prefibrillar forms of α Syn (e.g., partially folded monomeric species) and promotes their conversion to amorphous aggregates.^{253,254} A recent study revealed that α B-crystallin also interacts with amyloid-like α Syn fibrils, thereby blocking their elongation and promoting their dissociation to monomeric subunits.²⁵⁵ The inhibitory effect of α B-crystallin on α Syn fibrillization is mimicked by peptides derived from the conserved α -crystallin core domain²⁵⁶ and is augmented by phosphorylation of the chaperone at three serine residues (S19, S45, and S59).²⁵⁷ Finally, evidence suggests that α B-crystallin and Hsp27 attenuate α Syn aggregation and/or toxicity in various cell-culture models.^{258,259} These findings, together with neuropathological data showing that α B-crystallin and Hsp27 are present in synucleinopathy inclusions,^{259,260} suggest that both small heat shock proteins may play a role in mitigating α Syn aggregation and toxicity in the brain.

s0115 5. DJ-1

p0225 Mutations in the gene encoding DJ-1 (*PARK7*) cause very rare autosomal-recessive forms of PD.^{261–266} DJ-1 appears to function as a homodimer of ~20-kDa subunits, each of which has an α - β fold.^{267–271} The protein is a member of the large DJ-1/PfpI superfamily and has orthologs in most organisms.^{272,273} Many activities have been proposed for DJ-1 (outlined in greater detail in Part 2 of this chapter), and there is currently no consensus on what its functions are.^{274,275}

p0230 The crystal structure of human DJ-1 reveals the presence of a readily oxidized cysteine residue (cysteine 106) located at the subunit interface, and the protein exhibits a decrease in pI under conditions of oxidative stress due to the conversion of cysteine 106 to the sulfenic acid.^{271,276,277} DJ-1 has a conserved domain that is also shared by the heat shock protein Hsp31, and several groups have observed that DJ-1 exhibits molecular chaperone activity in cell-free systems.^{269,278–280} In one study, wild-type DJ-1, but not the familial mutant

L166P, was found to suppress the fibrillization and heat-induced oligomerization of α Syn.²⁷⁸ DJ-1 exhibited a markedly diminished chaperone activity with the substrate citrate synthase after preincubation with the reductant dithiothreitol (DTT) or as a result of replacing cysteine 53 with alanine, although the effects of DTT reduction and C53 substitution on the ability of DJ-1 to suppress α Syn self-assembly were not investigated. A study by Fink and Petsko²⁷⁹ revealed that human wild-type DJ-1 inhibits α Syn fibrillization via a mechanism driven by the “2O” form of DJ-1, in which cysteine 106 is oxidized to the sulfenic acid. DJ-1 isoforms oxidized to a greater or lesser extent than the 2O form had a substantially decreased ability to suppress α Syn fibril formation. Another group reported that two mutant forms of DJ-1 associated with early onset PD, namely, M26I and A104T, had a decreased ability to inhibit α Syn fibrillization than wild-type DJ-1, and the chaperone activity of the wild-type and mutant proteins was augmented by introducing a disulfide bond at the subunit interface.²⁸⁰

p0235 Collectively, these findings suggest that homodimeric DJ-1 in the “2O” state suppresses α Syn fibrillization, and this activity may be at least partly responsible for DJ-1-mediated neuroprotection. Presumably, DJ-1 must exist as a dimer to carry out this redox-sensitive chaperone function because (i) a hydrophobic patch that is predicted to interact with α Syn and other protein substrates is located at the dimer interface,²⁶⁹ and (ii) an “active site” pocket that appears to favor the oxidation of C106 to the sulfenic acid is lined with polar residues from both subunits.^{270,271,276,281} The DJ-1 chaperone activity is expected to be disrupted by primary structure alterations that destabilize the native dimeric structure, including mutations involved in early onset PD^{280,282–288} and oxidative modifications associated with sporadic PD and aging.^{286,289,290} Surprisingly, attempts to demonstrate a physical interaction between 2O DJ-1 and monomeric α Syn using a variety of biophysical approaches have been unsuccessful.²⁷⁹ However, DJ-1 may carry out its chaperone function by forming a complex with oligomeric or protofibrillar α Syn (Hulleman and Rochet, unpublished observations), similar to the mechanism proposed for Hsp70^{241,242} (see above).

p0240 Finally, we and others have reported that DJ-1 suppresses α Syn neurotoxicity and aggregation by inducing an increase in Hsp70 expression in cell-culture models.^{291–293} Recent evidence that DJ-1 promotes the folding of a chaperone-dependent fluorescent biosensor in a human neuroblastoma cell line²⁹⁴ is consistent with the idea that DJ-1 can enhance the function of other chaperones, in addition to carrying out its own chaperone function.

s0120 6. UPREGULATION OF PROTEIN DEGRADATION

p0245 Cellular clearance systems play a key role in protein quality control by ensuring the removal of misfolded or damaged polypeptides.^{227,240} Two clearance systems have been extensively characterized in terms of their ability to modulate intracellular α Syn levels: proteasomal degradation and lysosomal

autophagy, including chaperone-mediated autophagy (CMA) and macroautophagy. In general, α Syn overexpression results in inhibition of each of these catabolic pathways. Conversely, upregulation of these pathways results in enhanced α Syn degradation and thus suppression of α Syn aggregation (Fig. 3).

s0125 7. PROTEASOMAL DEGRADATION

p0250 A number of studies have revealed that α Syn accumulates in cells exposed to proteasome inhibitors,^{295–298} suggesting that the proteasome plays an important role in α Syn clearance. More recently, the proteasome was shown to preferentially degrade α Syn-pS129.^{98,299} Because polyubiquitylated α Syn species were not detected in cells subjected to proteasome impairment, it was inferred that α Syn degradation occurred via a ubiquitin-independent mechanism in these models.^{296,299} Other groups have reported that α Syn levels are not affected by interfering with proteasome function.^{300–302} A potential explanation for these discrepancies is that soluble α Syn may be eliminated by the proteasome, whereas aggregated α Syn may be degraded by macroautophagy, and the distribution of the protein between soluble and aggregated forms may vary in different cell-culture models.²⁹⁸ α Syn overexpression results in disruption of the UPP in various cell lines,^{301,303,304} and oligomeric α Syn interferes with the enzymatic activity of the 26S proteasome.^{305–308} α Syn neurotoxicity was found to be attenuated by overexpression of components of the UPP, including (i) parkin, an E3 ubiquitin ligase (described in greater detail in Part 2 of this chapter) in mouse primary midbrain cultures³⁰¹ and (ii) ubiquitin variants compatible with the formation of lysine-48 linkages in a *Drosophila* model.³⁰⁹

s0130 8. AUTOPHAGY

p0255 In a landmark paper, Cuervo and colleagues³¹⁰ reported that α Syn is a substrate for CMA in primary neurons and in a cell-free system containing purified lysosomes. Consistent with these data, a later study revealed that α Syn turnover was disrupted in mammalian cell lines and primary neuronal cultures by introducing two amino acid substitutions (D98A and Q99A) that disrupt the α Syn CMA recognition motif (₉₅VKKDQ₉₉) or via RNAi-mediated knockdown of the lysosomal LAMP2A receptor, a central player in the CMA pathway.³⁰² CMA has also been implicated in the removal of α Syn from the brains of transgenic mice.³¹¹ The familial mutants A30P and A53T and dopamine-modified wild-type α Syn have been shown to elicit CMA impairment, apparently via interactions with the LAMP2A receptor that interfere with lysosomal uptake and degradation of α Syn and other substrates.^{310,312,313} The inhibitory effects of the mutant and dopamine-modified proteins on CMA may be related to the high propensity of these variants to form prefibrillar oligomers.^{45,118}

p0260 Several lines of evidence suggest that α Syn is also a substrate for macroautophagy. The macroautophagy inhibitor 3-methyladenine induces α Syn accumulation in dopaminergic PC12 cells and in cultured neurons.^{298,302} A recent *in vivo* study showed that macroautophagy plays an important role in α Syn clearance when the protein is expressed at high levels in transgenic mouse brain, whereas at lower expression levels (i.e., in nontransgenic animals) the protein is eliminated primarily via proteasomal degradation.³¹⁴ Data obtained by the Rubinsztein group³¹⁵ suggest that α Syn overexpression inhibits macroautophagy by disrupting the formation of AP precursors termed “omegasomes.” Another group reported that α Syn overexpression results in a buildup of the AP marker protein LC3-II,³¹⁶ suggesting that α Syn may also disrupt macroautophagy downstream of AP formation (e.g., perhaps at the AP-lysosome fusion step). Importantly, α Syn clearance is stimulated in cells and transgenic mice exposed to pharmacological inducers of macroautophagy, including rapamycin,^{298,317–321} suggesting that stimulation of this clearance mechanism may be a reasonable strategy to alleviate α Syn aggregation and neurotoxicity in PD.

s0135

III. Role of Mitochondrial Dysfunction in PD

s0140 A. Parkin, A Multifunctional E3 Ubiquitin Ligase

p0265 Mutations in *PARKIN*, the product of the *PARK2* locus, were first discovered in Japan in multiple families with a syndrome known as “autosomal-recessive juvenile parkinsonism” (AR-JP).^{322,323} Mutations in *PARKIN* may also play an important role in some sporadic cases of PD.^{324,325} Parkin is primarily localized to the cytoplasm, but as discussed further below, it also translocates to the mitochondria in response to various signals, where it plays important roles in mitochondrial homeostasis. Parkin has an N-terminal ubiquitin-like domain (Ubl), followed by a recently described zinc-coordinating motif termed RING0, and two RING-finger motifs separated from each other by a cysteine-rich in-between-RING (IBR) motif.

p0270 Ubiquitin conjugation to proteins is usually effected by a series of enzymes: an E1 that activates ubiquitin, an E2 that accepts the activated ubiquitin and works with a third enzyme, and the E3-ubiquitin ligase that provides specificity in determining which substrates will be ubiquitinated.³²⁶ Parkin is a RING family E3 ligase.^{327–329} It undergoes autoubiquitination and can ubiquitinate a number of different substrates. Pathogenic mutations occur throughout the protein-coding region and many have effects on ligase activity (reviewed in Refs. 330–332). Parkin ligase activity can also be inactivated by oxidative stress, and following nitrosylation or covalent modification by dopamine.^{333–337}

Inactivation may occur through modification of residues (such as cysteines) essential for Parkin's ligase activity. Alternatively, and/or in addition, modification may result in Parkin becoming insoluble, leading indirectly to a loss of ligase activity on some or all substrates.^{333,335,338} α -Syn overexpression has also been shown to promote Parkin insolubility.³³⁹ Much of the time, Parkin inactivation through these mechanisms may be pathological; but it is also possible that these modifications serve under some circumstances to modulate Parkin activity for normal physiological ends, an area that remains relatively unexplored. Since there is evidence that each of the above insults can play a role in PD pathogenesis in sporadic cases, it is likely that disruption of Parkin ligase activity plays a role in at least some cases of sporadic PD (reviewed in Ref. 331). Needless to say, an important goal in the field has been to identify substrates of this activity.

s0145 B. Cytoplasmic Parkin Substrates

p0275 Many potential Parkin substrates have been identified. In the section below, we discuss substrates in the cytoplasm. In a later section, we discuss roles for Parkin ligase activity at the mitochondrial membrane. RING domain ligases such as Parkin can catalyze K48-mediated polyubiquitination,^{327–329} which targets substrates for proteasome-dependent degradation (reviewed in Ref. 326). Thus, one important hypothesis in the field has been that loss of *parkin* results in the aberrant accumulation of toxic proteins. Important predictions of this model are that the substrate should accumulate in *parkin* knockout mice and in patients carrying *parkin* mutations, and perhaps in other contexts in which *parkin* has been inactivated, such as following MPTP intoxication and/or in sporadic PD cases. Additional predictions of this model are that decreasing expression levels of the substrate in a *parkin* mutant background should prevent cell death, while overexpression should result in toxicity. A number of Parkin binding partners and substrates have been identified, but most have not been characterized in sufficient detail to know whether they meet these criteria (reviewed in Refs. 330–332). Several intriguing exceptions that meet at least several criteria are aminoacyl-tRNA synthetase (AIMP2)^{340,341} and the far upstream element binding protein 1 (FBP-1).³⁴² AIMP2 is present in the Lewy bodies, and Parkin is able to promote its degradation, presumably via polyubiquitination and proteasome-dependent degradation. AIMP2 also accumulates in the brains of *parkin*-null mice, patients with *PARKIN* mutations, and in some sporadic PD patients, and is toxic to dopaminergic neurons when overexpressed. FBP-1 also accumulates in *parkin* knockout mice, patients with mutations in *PARKIN* sporadic cases, and animal models of MPTP intoxication. How these proteins mediate their toxic functions is unknown and warrants further investigation.

p0280 Paris, a third protein recently identified as a Parkin substrate, is interesting because a number of lines of evidence suggest that its expression is both necessary and sufficient to mediate loss of dopaminergic neurons in cells lacking *parkin*.³⁴³ Paris and Parkin can co-immunoprecipitate from cells and brains, and Paris is ubiquitinated through a K48 polyubiquitin linkage in a Parkin-dependent manner. Paris levels are also increased in the brains of *PARKIN* patients and patients with sporadic PD, consistent with the hypothesis that Parkin-dependent ubiquitination of Paris results in its degradation. Finally, silencing of *paris* expression prevents the loss of dopaminergic neurons observed in a conditional *parkin* knockout, while overexpression of *paris* results in loss of DN neurons, which can be suppressed through coexpression of *parkin*. These latter observations are particularly important because they suggest that PD pathology critically depends on the levels of Paris.

p0285 How does upregulation of Paris lead to pathology? Paris binds the *PGC-1-alpha* promoter and represses its transcription, as well as the transcription of a *PGC-1-alpha* target, *NRF-1*. These interactions are likely to be significant because overexpression of *PGC-1-alpha* prevents the death associated with upregulation of Paris, as well as death induced in other PD models.³⁴⁴ Downregulation of *PGC-1-alpha* target gene expression has also been observed in *PARKIN* patients³⁴⁵ and dopaminergic neurons from sporadic cases.³⁴⁴ How might downregulation of *PGC-1-alpha* and its target gene *NRF-1* (and perhaps other genes as well) cause pathology? A major effect of *PGC-1-alpha* expression is to promote mitochondrial biogenesis and regulate the metabolism of ROS.^{346–349} As discussed below, Parkin also participates in a process that removes damaged mitochondria. Therefore, it is tempting to propose that stress-dependent stimulation of Parkin's ligase activity promotes the removal of damaged mitochondria and, through downregulation of *paris*, promotes a compensatory increase in mitochondrial biogenesis. In such a model, inactivation of *parkin* would result in a particularly toxic situation in which damaged mitochondria (and their damaged genomes) are retained and compensatory biogenesis fails to occur. Conversely, silencing of *paris* and/or activation of *PGC-1-alpha* through other means may provide a therapeutic route to maintaining mitochondrial function to some extent, even if the Parkin-dependent removal of damaged mitochondria is unable to occur or occurs less efficiently.

s0150 C. Parkin's Cytoprotective Ability Involves Multiple Forms of Ubiquitination

p0290 Parkin expression has also been shown to be neuroprotective in response to a variety of stresses (reviewed in Refs. 330,332). This activity is generally thought to require Parkin's ligase activity—as implied by the fact that ligase-

dead forms of the protein show little or no activity—but the exact mechanism of action is unknown. K48-dependent polyubiquitination may be important in some contexts, as discussed above. However, Parkin can also mediate mono-ubiquitination, K63-linked polyubiquitination (reviewed in Ref. 330) as well as a very recently described K27-linked ubiquitination,³⁵⁰ and perhaps even E2-independent ubiquitination.³⁵¹ These modifications can influence cellular processes such as signal transduction, transcriptional regulation, and protein and membrane trafficking, without promoting substrate degradation.^{352,353} Finally, Parkin has also been suggested to bind directly to the 19S regulatory domain of the proteasome,^{354,355} activating the proteasome in an E3 ligase activity-independent manner by increasing the affinity of 19S subunits for each other.³⁵⁵ The fact that Parkin expression can provide protection from death associated with proteasome inhibition^{334,356} suggests that at least some component of Parkin's neuroprotective activity when overexpressed—which may or may not be the same activity lost in the absence of *parkin*—involves processes other than K48-linked ubiquitin and proteasome-dependent protein degradation. We return to some of these points in the sections below.

s0155 D. PINK1, A Serine/Threonine Kinase with Multiple Forms and Localizations

p0295 In parallel with the above work, a number of observations demonstrate that Parkin, together with the kinase PINK1, plays an important role at the mitochondrial membrane in the regulation of mitochondrial homeostasis and quality control. In the following section we outline this pathway and its significance for PD. Mutations in *PINK1* (the *PARK6* locus) were first described in Spanish and Italian families with a syndrome of AR-JP.³⁵⁷ As with *PARKIN*, a variety of mutations have been reported in patients (though patients with *PINK1* mutations are less common), and the one family characterized showed Lewy body pathology,³⁵⁸ as observed in some, but not all patients homozygous or trans-heterozygous for mutations in *PARKIN*.^{322,359,360} *PINK1* encodes a putative serine/threonine kinase. The N-terminus contains a mitochondrial targeting sequence. This is followed by a hydrophobic transmembrane domain, the kinase domain, and a putative C-terminal regulatory domain. Expression of PINK1 is cytoprotective in some contexts, and this activity often appears to require kinase activity (reviewed in Ref. 361). The majority of mutations associated with familial PD disrupt kinase activity, while others affect other aspects of PINK1 function (see below). Multiple forms of PINK1 protein are generated, and some, but not all PINK1, localize to mitochondria with the kinase domain facing the cytoplasm. These features of PINK1 biology are discussed further below.

s0160 E. The PINK1/Parkin Pathway of Mitochondrial Homeostasis and Quality Control in *Drosophila* and Beyond

p0300 Links between *parkin* and *PINK1* were first identified in *Drosophila* (reviewed in Refs. 17,362,363). Flies lacking *parkin* exhibit dramatic mitochondrial defects—swollen mitochondria that have severely fragmented cristae—in several energy-intensive tissues, including the male germline and adult flight muscle.^{364,365} The flight muscles ultimately die, and their death shows features of apoptosis.³⁶⁴ Flies lacking *parkin* also display a small but significant degeneration of a subset of dopaminergic neurons.³⁶⁶ Although severe defects in mitochondrial morphology are not observed in *parkin* knockout mice, these animals do display mitochondrial functional defects including reduced mitochondrial respiratory activity.³⁶⁷

p0305 Flies lacking the *Drosophila* homolog of *PINK1* show phenotypes very similar to those of flies lacking *parkin*: mutants are viable but exhibit increased stress sensitivity and mitochondrial morphological defects in testes and muscle.^{368–370} *PINK1* mutants also show reduced ATP levels and mitochondrial DNA (mtDNA) content. Flies lacking endogenous *PINK1* function but expressing PD-associated mutant forms of *PINK1*^{370,371} show phenotypes similar to those of the *PINK1* null mutant, consistent with the *PINK1*-associated disease being the result of a loss of *PINK1* function.^{370,371} As in *parkin* mutant flies, mitochondria in *PINK1* mutant flight muscle are swollen with fragmented cristae, and these cells ultimately undergo apoptotic death.^{368–370} Mitochondria within dopaminergic neurons in *PINK1* mutants also display aberrant morphology, and there is a small but statistically significant loss of a subset of these neurons with age.^{369,370}

p0310 What is the relationship between *PINK1* and *parkin*? Stringent genetic studies in *Drosophila* allow one to construct a genetic pathway. *parkin* overexpression suppresses all *PINK1* mutant phenotypes tested, while *PINK1* overexpression does not compensate for loss of *parkin* function.^{368–370} In addition, double mutants lacking both *PINK1* and *parkin* have phenotypes identical to, rather than stronger than, either single mutant.^{368,369} Together, these observations indicate that *PINK1* and *parkin* act in a linear pathway to regulate mitochondrial integrity, with *parkin* functioning downstream of *PINK1*.

p0315 Observations on *PINK1* and *parkin* function in flies are relevant to humans for several reasons. First, expression of human *PINK1*^{368,370} or *PARKIN* in *Drosophila* suppresses phenotypes caused by loss of function of *PINK1* or *parkin*, respectively, indicating that the human and fly proteins are functionally conserved. Second, as noted above, PD patients who harbor mutations in *PINK1* or *PARKIN* are clinically indistinguishable,³⁷² and mice lacking both *PINK1* and *parkin* show phenotypes no worse than those of the single

mutants,³⁷³ consistent with the hypothesis that these genes function in a common genetic pathway. Third, cells from patients and/or mouse knockout models of *PINK1* or *parkin* also show defects in mitochondrial morphology and/or mitochondrial respiration, particularly in complex I activity in a variety of cell types.^{364,367,374–388}

p0320 Important clues as to the mechanism by which *PINK1* and *parkin* regulate mitochondrial function came from the study of mitochondrial morphology in *Drosophila* mutants. Mitochondria are continually undergoing cycles of fission and fusion. This allows them to change shape and share components. It also plays an important role in facilitating recruitment to specific cellular compartments such as synapses where ATP or Ca²⁺ buffering demands are high. Not surprisingly, dysfunction of mitochondrial fission/fusion has been linked to the pathogenesis of a number of neurodegenerative diseases (reviewed in Ref. 389). Fusion is promoted by *mitofusin* (*mfn*), which is required for outer membrane fusion, and *opa-1*, which is required for inner membrane fusion, while fission is promoted by Drp1, a predominantly cytoplasmic protein recruited to mitochondria during fission. Recruitment and/or function of Drp-1 depends on mitochondrial outer membrane proteins such as Fis1 and Mff through mechanisms still being explored.^{389–391} During *Drosophila* spermatogenesis, mitochondria undergo significant morphological changes. Early spermatids undergo mitochondrial aggregation and fusion, creating a spherical structure known as the nebenkern, which is composed of two intertwined mitochondria.³⁹² During subsequent spermatid elongation, the nebenkern unfurls, yielding two mitochondrial derivatives that are maintained throughout subsequent stages of spermatogenesis. In both *PINK1*³⁹³ and *parkin*^{393,394} mutants, only one leaf blade is seen, suggesting a defect in mitochondrial fission or an overabundance of fusion.³⁹³ Several other pieces of evidence support this hypothesis. First, when mitochondria in *PINK1* or *parkin* mutants are visualized with mitochondrially targeted GFP, they are clumped in large aggregates in both dopaminergic neurons and flight muscle. These phenotypes, as well as others, such as the degeneration of flight muscle and a decrease in the levels of dopamine in fly heads, can be suppressed by increasing the levels of *Drp1* or *Fis1*, and/or decreasing levels of *mfn* or *Opa1*.^{380,393,395,396} Finally, heterozygosity for *drp1* is lethal in a *PINK1* mutant background, consistent with the idea that *PINK1* and *drp1* work in the same direction to promote fission.^{380,393}

p0325 *PINK1* and *parkin* also regulate mitochondrial morphology in mammalian systems. However, in contrast to the story in *Drosophila*, which is consistent across cell types and labs, in mammalian systems various effects have been observed. Observations consistent with the *Drosophila* work have been obtained in some systems. Thus, enlarged mitochondria have been observed in *PINK1* striatal neurons³⁷⁸ and COS7 cells in which *PINK1* was silenced

using RNAi. In the latter system, this phenotype was suppressed by overexpressing *Fis1* or *Drp1*, as in *Drosophila*.³⁹⁵ Cultured fibroblasts from *PARKIN* patients also contained longer and more branched mitochondria than wild-type controls.³⁹⁷ Most recently, Yu and colleagues found that expression of *PINK1* or *parkin* in hippocampal neurons resulted in an increase in mitochondria number and a decrease in size, while silencing of *PINK1* resulted in elongated mitochondria.³⁹⁸ As expected, expression of *Drp1* or silencing of *Opa1* suppressed these phenotypes. Midbrain dopaminergic neurons responded similar to manipulations of *PINK1* and *parkin*. In contrast, others have observed that loss of *PINK1* results in fission, with decreased levels of *Drp1* resulting in suppression of this phenotype.^{377,382,383,399} The reasons for these differences are unknown, but likely to be interesting. One possibility is that loss of *PINK1* or *Parkin* results in an increase in the amount of mitochondrial damage, and that this leads, through other pathways, to mitochondrial fragmentation. Screens in yeast^{400,401} and *C. elegans*⁴⁰² have shown that disruption of many genes leads to changes in mitochondrial morphology, including fragmentation or elongation. Thus, it is likely that the final mitochondrial morphology phenotype observed in any particular cell type with respect to the presence or absence of *PINK1/parkin* will depend on multiple variables. In any case, what is most important is not the specific morphology observed, but the functional state of the mitochondrial population and the ways in which this is influenced by *PINK1* and *parkin*, discussed below.

p0330 How could defective mitochondrial fission resulting from mutation of *PINK1* or *parkin* lead to defects in cell physiology? A major function of mitochondria is to oxidize dietary reducing equivalents to generate ATP and heat through oxidative phosphorylation, a process in which electron transport through the respiratory chain creates a potential difference across the inner mitochondrial membrane, which is used to produce ATP. Unfortunately, inefficiencies in electron transport result in the production of ROS, making mitochondria the major source of free-radical production in the cell. These radicals damage mitochondrial proteins, lipids, and DNA, which impair mitochondrial function and can lead to further increases in free-radical production, ultimately compromising cellular function and/or leading to cell death. Mitochondrial cycles of fusion and fission allow content mixing, which presumably functions to make the cellular population of mitochondria relatively homogeneous in protein content, stabilizing overall mitochondrial function over the short term. However, because mitochondrial genomes undergo mutation at high frequency, nonselective cycles of fusion and fission do nothing to prevent (and may promote) the accumulation of defective genomes (there is no selective pressure to remove them and genomes with deletions often accumulate), resulting in a time-dependent decrease in overall cellular mitochondrial function. Therefore, mechanisms must exist to specifically eliminate defective mitochondria and,

presumably, any associated defective mitochondrial genomes. A number of observations suggest that this removal occurs through the process of mitophagy, a specialized form of autophagy in which cellular components are degraded following engulfment by autophagosomes (reviewed in Ref. 403). Importantly for the purposes of this review, the process of mitophagy is intimately linked with changes in mitochondrial size and shape brought about through fission and fusion (reviewed in Refs. 404,405). As we will see, loss of pink1 and/or parkin inhibits the removal of damaged mitochondria.

p0335 How are defective mitochondria selected for removal? Live cell imaging of mitochondrial dynamics in mammalian cells shows that, while many products of mitochondrial fission rapidly fuse again with the mitochondrial network, others, which also exhibited a depolarized membrane potential, showed a decreased probability of fusion and were often targeted for degradation through mitophagy.^{406,407} These and other observations (reviewed in Refs. 404,408) suggest a general model of mitochondrial quality control in which fission allows the segregation of mitochondrial components, including proteins, lipids, and genomes, into separate mitochondrial units. These units are then "tested" for their ability to acquire a hyperpolarized membrane potential, an honest signal of organelle and genome health since many normal mitochondrial activities ultimately function to make the inner mitochondrial compartment hyperpolarized so as to generate ATP. Those mitochondria that fail the test are kept segregated (through mechanisms to be discussed shortly) and are ultimately targeted for degradation through mitophagy. Importantly, decreased mitophagy results in the accumulation of oxidized proteins and decreased cellular respiration, strongly suggesting that the end result of this process is the selective removal of damaged mitochondria.⁴⁰⁷

p0340 How are dysfunctional mitochondria identified and targeted for mitophagy? Youle and colleagues showed that Parkin is selectively recruited to mitochondria whose membrane is depolarized, resulting in their elimination through mitophagy. Parkin recruitment to mitochondria is independent of *drp1*, while mitophagy requires *drp1*, indicating that Parkin-dependent mitophagy requires fission. Work from a number of labs has gone on to show that recruitment of Parkin to mitochondria is PINK1 dependent,^{350,409–415} including in neurons differentiated from *PINK1* mutant patient cells.⁴¹⁶ PINK1 is a membrane protein with its C-terminus facing the cytoplasm.⁴¹⁷ PINK1 protein levels are specifically upregulated on damaged mitochondria.^{410,411,413} In healthy mitochondria, PINK1 is constitutively cleaved, releasing its C-terminal kinase domain into the intermembrane space and/or cytoplasm where it is degraded in a proteasome-dependent manner. In damaged mitochondria that have lost their membrane potential, full-length PINK1 remains anchored to the membrane. Outer mitochondrial anchorage of PINK1 is all that is required to recruit Parkin, because tethering of PINK1 to the mitochondrial membrane through other methods is sufficient to recruit Parkin.⁴¹⁰

p0345 How is PINK1 cleavage regulated? Early work in *Drosophila* showed that PINK1 cleavage required the inner mitochondrial membrane protease rhomboid 7.⁴¹⁸ This, coupled with an earlier observation that the *rhomboid-7* mutant phenotype includes a loss of mitochondrial fusion,⁴¹⁹ suggested a model in which Pink1's profission activity is negatively regulated through cleavage by rhomboid-7. Early experiments in mammalian systems excluded the rhomboid protease PARL as the protease cleaving PINK1, on the basis of the results of RNAi knockdown.⁴¹⁰ However, more recent observation by the same group⁴²⁰ and others^{421–423} clearly demonstrate that PINK1 is cleaved by PARL and that this activity is regulated by mitochondrial membrane potential. In healthy mitochondria, PINK1 is guided to the inner mitochondrial membrane using the general mitochondrial import machinery. Here it is cleaved by PARL, resulting in release of a cytoplasmic form that is degraded by an MG132-sensitive protease. In contrast, when the mitochondrial membrane potential is depolarized, newly synthesized full-length PINK1 is somehow shunted onto the outer mitochondrial membrane, where it is able to recruit Parkin. An unrelated yeast protein shows a similar mitochondrial membrane potential-dependent shift in localization, suggesting that alternations in the mitochondrial membrane potential may be more generally used to signal energy or health status to the rest of the cell through differential protein localization.⁴²⁴

p0350 How PINK1 stabilization on the outer mitochondrial membrane recruits Parkin is unknown. There is also evidence that recruitment of Parkin results in an activation of its ligase activity, also through an unknown mechanism.⁴⁰⁹ What is clear is that, once recruited and activated, Parkin has a number of effects on the mitochondria-associated proteome. Many mitochondrial proteins are ubiquitinated and/or degraded in a Parkin-dependent manner. These include Mfns,^{413,425–430} Milton and Miro,⁴²⁸ Bcl-2,⁴³¹ which are components of the TOM mitochondrial protein import complex,^{428,432} VDAC,^{350,433} and Drp-1.⁴³⁴ Most recently, Chan and colleagues carried out a comprehensive analysis in HeLa cells of proteins associated with depolarized mitochondria to which Parkin had been recruited.⁴²⁸ They confirmed many previous findings and discovered much that is new. In particular, they found that Parkin recruitment to the mitochondrial membrane results in the recruitment of the proteasome and the K-48 and K63-linked ubiquitination, and in some cases degradation, of a number of mitochondrial proteins. Components of the autophagy pathway were also recruited. Chan *et al.*,⁴²⁸ Tanaka *et al.*,⁴²⁷ and to a lesser extent Yoshii *et al.*⁴³² also found that inhibition of the proteasome, or specifically K48-linked ubiquitination,⁴²⁸ suppressed mitophagy, but not recruitment of Parkin, arguing that protein degradation downstream of Parkin recruitment is essential for mitophagy, at least in these contexts.

p0355 What are the key substrates of Parkin in this context? As noted above, a number of groups have shown that Parkin recruitment results in the degradation of Mfns. However, mitophagy still occurs in *mfn* null cells.⁴²⁸ Therefore, the degradation of the *mfn*s presumably serves primarily to segregate dysfunctional mitochondria by preventing fusion. Voltage-dependent cleavage and inactivation of Opa1 by the mitochondrial protease Oma1 probably serves a similar purpose with respect to the inner mitochondrial membrane.⁴³⁵ Recruitment of Parkin to mitochondria also results in the mitochondrial accumulation of p97, a AAA-ATPase⁴²⁷ that participates in a number of processes including endoplasmic reticulum-associated degradation in which p97 provides the driving force required to extrude ubiquitinated membrane proteins from the ER so that they can be degraded in the cytosol.⁴³⁶ Mfn degradation and mitophagy require p97, suggesting a model in which p97 may be similarly required to promote the extrusion into the cytoplasm and degradation of critical substrates. VDAC, an abundant mitochondrial outer membrane protein, is ubiquitinated through a K63 linkage by Parkin and has been suggested to be important for mitophagy.³⁵⁰ However, VDAC null cells still undergo mitophagy, indicating that its ubiquitination is not absolutely required.⁴³³ It has also been suggested that K63-linked ubiquitination of mitochondrial proteins such as VDAC promotes mitophagy through recruitment of ubiquitin-binding adaptors such as HDAC6 and the autophagy adaptor p62.^{350,437,438} However, as with VDAC, *p62* null cells still undergo Parkin-dependent mitophagy, indicating that *p62* ubiquitination may play other roles, perhaps involving altering mitochondria location within the cell to facilitate access to the autophagy machinery.^{433,439}

p0360 To summarize, recruitment of Parkin to the depolarized mitochondria results in the ubiquitination, and in some cases degradation, of a number of proteins. In the case of the Mfns, Milton and Miro, proteins involved in mitochondrial trafficking along microtubules, degradation probably serves to sequester damaged organelles away from the mitochondrial network and promote their localization to sites of mitophagy, respectively. Recruitment of the autophagy p62 may also function to facilitate localization of damaged mitochondria to sites of mitophagy. The proteasome and p97 are required for Parkin-dependent mitophagy, but exactly why they are required is unknown. They may be needed to remove inhibitors of mitophagy. If this is the case, it may be possible to identify these substrates as genes whose silencing allows mitophagy to occur in *mfn* null cells exposed to proteasome inhibitors. Alternatively, or in addition, p97 and the proteasome may have effects on mitophagy through action on targets in other cellular compartments. With respect to PD, at least for those with *PINK1* or *PARKIN* mutations, PD may result from a failure of mitophagy—an important aspect of mitochondrial quality control.

p0365 As attractive as the above model of *PINK1/parkin*-dependent clearance of damaged mitochondria is, it is important to note that thus far mitophagy has only been demonstrated in cultured cells, not *in vivo*. Thus, an important goal in the field is to provide evidence supporting the role of *PINK1/parkin* in mediating mitophagy to clear damaged mitochondria *in vivo*. Also, it has been reported that Parkin translocation to damaged mitochondria occurs only in nonneuronal cells, not in neurons.⁴¹⁵ Whether mitophagy occurs in some tissues but not others awaits future studies.

s0165 F. Other Roles for PINK1

p0370 Loss of *PINK1* results in defects in mitochondrial calcium handling. The mitochondrial matrix accumulates Ca^{2+} to higher basal levels, as a result of a decreased efflux capacity. Mitochondria from cell lacking *PINK1* are therefore less able to buffer cytoplasmic Ca^{2+} increases in response to stimuli.^{440–442} This can lead to increased ROS, decreased ATP production, and opening of the mitochondrial permeability transition pore, which can lead to death. Loss of *PINK1* is also associated with increased expression of cytokines in response to LPS but decreased expression of cytokine-induced NF- κ B, which promotes cell survival.⁴⁴¹ It is possible that some of these effects occur downstream of the accumulation of defective mitochondria because of loss of mitophagy. However, it is also possible that *PINK1* has other important targets in mitochondria and other cellular compartments that are important for maintaining mitochondrial homeostasis. For example, at the mitochondria, *PINK1* has been reported to phosphorylate the TNF receptor-associated protein 1 (TRAP1), a molecular chaperone, and phosphorylation of TRAP1 by *PINK1* is important for preventing oxidative-stress-induced release of cytochrome *c*, which promotes apoptosis.⁴⁴³

p0375 A number of groups have found some fraction of *PINK1* localized to the cytoplasm.^{418,444–448} Importantly, expression of an N-terminally deleted version of *PINK1* lacking its mitochondrial targeting sequence protects neurons from death induced by MPTP, and this activity requires putative *PINK1* kinase activity. This raises the possibility that, at least when overexpressed, *PINK1* can have pro survival functions outside the mitochondria.⁴⁴⁷ Recent work suggests that one pathway through which this may work involves phosphorylation of the mammalian Target of Rapamycin Complex 2 (TORC2) component Rictor by *PINK1*, which promotes TORC2-dependent phosphorylation of the prosurvival kinase AKT.⁴⁴⁹ Interestingly, it was recently reported that expression of DJ-1 also promotes the oxidative-stress-induced phosphorylation of AKT and that DJ-1 promotes AKT's ability to protect cells from MPTP-induced stress.⁴⁵⁰ It will be interesting to determine whether DJ-1's activity in this assay requires *PINK1* kinase activity or physical interaction. Interactions between *PINK1*/

Parkin and DJ-1 in the cytoplasm have been identified by one group,⁴⁵¹ but not by a second group,⁴⁵² leaving the relationships between these proteins in the cytoplasm unclear.

s0170 G. Links Between the PINK1/Parkin Pathway and Other PARK Loci

p0380 Mutations in *DJ-1* cause very rare autosomal-recessive forms of PD.²⁶² DJ-1 has also been shown to have oncogenic activity.⁴⁵³ The DJ-1 protein is small (~20kDa) and probably functions as a homodimer. DJ-1 is a member of the large DJ-1/PfpI superfamily and has members in almost all organisms. Many activities have been proposed for DJ-1, and there is currently no consensus on what its functions are. Suggested functions include a redox-regulated chaperone, a cysteine protease, a transcription coactivator, an RNA-binding protein, and a regulator of survival signaling through interactions with Daxx or the kinase ASK1 (reviewed in Refs. 275,454). What is clear is that DJ-1 is cytoprotective in a number of different contexts; this activity requires a conserved cysteine (Cys106 in mammals). Oxidation of this cysteine to cysteine sulfonate and cysteine sulfinic acid occurs under oxidative stress conditions, and oxidation of this residue is critical for cytoprotection in a number of systems. Mutants that mimic the oxidized form of DJ-1 but that lack Cys106 are still cytoprotective, indicating that DJ-1 is unlikely to be cytoprotective simply because oxidation of Cys106 consumes ROS.^{455,456} Importantly for the purposes of this review, while DJ-1 is predominantly cytoplasmic,⁴⁵⁷ oxidative stress enhances its association with mitochondria.^{276,455,458–460} In addition, versions of DJ-1 targeted specifically to mitochondria by fusing a mitochondrial localization sequence to its N-terminus show enhanced cytoprotective functions, suggesting that the mitochondria is one (of perhaps many) important site of DJ-1 action.⁴⁶¹

p0385 Is there a relationship between *DJ-1* and the *PINK1/parkin* pathway? Lymphoblastoid cells from *DJ-1* PD patients have an increased percentage of fragmented mitochondria. *DJ-1* mutant mice show defects in muscle mitochondrial function,⁴⁶² and the loss of *DJ-1* in a mouse neuroblastoma cell line, cortical neurons, or embryonic fibroblasts from *DJ-1* null embryos leads to mitochondrial membrane depolarization, accumulation of ROS, fragmentation of mitochondria, accumulation of markers of autophagy around mitochondria,^{452,463,464} and, in the case of the *DJ-1* null cells, increased autophagic flux. In both studies, antioxidants reversed these effects and, in the neuroblastoma cells, expression of *DJ-1* blocked mitochondrial fragmentation in response to rotenone, which are results consistent with the idea that *DJ-1* functions to maintain mitochondrial health in the face of oxidative stress. Expression of *PINK1* or *parkin* could restore mitochondrial connectivity in cells lacking *DJ-1*,^{452,464} and expression of *DJ-1* could restore the connectivity

of mitochondria in cells lacking *PINK1* exposed to rotenone⁴⁵² but not in *PINK1*-deficient cells not exposed to rotenone.³⁷⁷ In short, the ability of *PINK1*/Parkin or *DJ-1* to protect mitochondrial function seems, most of the time, to be independent of the presence of the other. As noted above, there are conflicting reports as to whether *PINK1*, Parkin, and *DJ-1* form a complex,^{451,452} and it is intriguing that both *PINK1* and *DJ-1* promote the activation of AKT. But, as yet, epistasis experiments to determine if these effects on AKT activity occur through the same pathway. Finally, it is worth noting that triple knockout mice lacking *PINK1*, *parkin*, and *DJ-1* fail to show nigral degeneration even with aging.³⁷³ This somewhat surprising result could reflect action in a common pathway as with *PINK1* and *parkin*. Alternatively, it could reflect developmental compensation, the presence of which is strongly hinted at in experiments utilizing conditional adult-specific *parkin* knockout mice³⁴³ (compare with Refs. 465,466). If compensation is involved, it would of course be very interesting to know how it is brought about.

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p0390 In summary, the above observations tentatively suggest that *DJ-1* acts in parallel to *PINK1* and *parkin*, not through them. A similar conclusion is suggested by experiments in *Drosophila*. While *Drosophila DJ-1* mutants show mitochondrial defects, these are in many ways distinct from those associated with loss of *PINK1* or *parkin*.⁴⁶² In addition, while expression of *DJ-1* is capable of rescuing muscle defects due to loss of *PINK1*, it cannot rescue identical-looking muscle defects due to loss of *parkin*. Expression of either *PINK1* or *parkin* in a *DJ-1* mutant also has the surprising effect of causing organismal lethality. All of these results suggest complex, not linear, interactions.

s0175

IV. Convergent Pathways and Future Directions

p0395 Several lines of evidence suggest possible links between α -Syn and mitochondrial biology. Mice lacking *SNCA* show increased resistance to MPTP⁴⁶⁷ and reductions in the levels of the mitochondrial lipid cardiolipin.⁴⁶⁸ Mice overexpressing α -Syn also show mitochondrial damage.^{87,469–472} α -Syn has been shown to localize to mitochondria,^{87,473–476} with the levels of mitochondrially localized α -Syn increasing in the brains of PD patients.⁴⁷⁷ Recently, two groups have shown that expression of wild-type α -Syn results in increased fragmentation of the mitochondrial network, while decreased levels of α -Syn resulted in increased tubulation of the network.^{475,476} Both studies suggest that α -Syn promotes these effects through direct interactions with mitochondrial lipids. Kamp and colleagues suggest that α -Syn prevents fusion, while

Nakamura and colleagues suggest that it promotes fission, an issue that needs to be resolved. Regardless of the exact mechanism, do these observations help us understand a possible basis for α -Syn toxicity? This awaits future studies.

p0400 Mitochondrial fusion allows the sharing of proteins, lipids, and small molecules, which helps to preserve the overall mitochondrial and cellular function. In the absence of fusion, individual mitochondria are likely to diverge in terms of their requirements for these factors depending on where they are and the metabolic activities they are engaged in. This would be expected to lead, over time, to increasing numbers of dysfunctional mitochondria as individual units stochastically enter states that result in a decreased ability to maintain a hyperpolarized membrane potential, the loss of which results in increased dysfunction and/or increased ROS production, leading ultimately to cell dysfunction and death.⁴⁷⁸ In support of such a model, loss of mitochondrial fusion through mutation or loss of *mfns* results in mitochondrial depolarization and cell death (reviewed in Ref. 479). Seen in this light, α -Syn's ability to promote mitochondrial fission may tip the balance toward mitochondrial and cellular dysfunction by limiting opportunities for mitochondria to fuse with and complement each other's defects. In such a model, *PINK1* and *parkin* would still promote the removal of dysfunctional mitochondrial units as they arose, as observed by Kamp and colleagues.⁴⁷⁶ However, if compensatory biogenesis does not keep up with this removal, and/or the *PINK1/parkin* pathway becomes saturated or energy-limited in its ability to promote mitophagy (see Ref. 415 for a possible example of energy limitation), mitochondrial damage may accumulate over time, leading to cellular dysfunction and death. An important prediction of this model is that inhibitors of mitochondrial fission, which tip the balance back more toward the fused state, should act as suppressors of α -Syn toxicity. It will be interesting to determine whether inhibitors of *drp-1* recently identified⁴⁸⁰ have therapeutic benefit in situations in which α -Syn is either overexpressed or mutated.

p0405 Finally, we note that both mitochondrial quality control and α -Syn removal involve use of the cells' two major protein degradative systems, namely, the proteasome and autophagy. Importantly, inhibition of autophagy can compromise degradation of ubiquitin-proteasome substrates, and inhibition of the proteasome inhibits some autophagic processes (such as *PINK1/parkin*-dependent mitophagy) and places increased demand on the autophagic degradation system to remove misfolded proteins and to provide essential amino acids. As noted above, expression of α -Syn can disrupt proteasome function and autophagy. In consequence, increased levels of α -Syn may, through this mechanism alone, compromise the removal of damaged mitochondria. Conversely, preventing the generation and/or removal of damaged mitochondria through inhibition of *PINK1*, *parkin*, or *DJ-1* results in cells experiencing increased levels of cellular ROS-dependent protein and lipid damage, and decreased

levels of ATP. This results in increased demand for proteasome and autophagy-dependent degradation, which are energy-dependent processes, in a context in which energy may be limiting. As a result, effective removal of α -Syn (the dose dependence of which with respect to disease generation is exquisite) may be compromised, resulting in the accumulation of toxic forms of the protein. Given this interrelationship, and the fact that α -Syn accumulation and/or damage to *PINK1/parkin* pathway components are probably present in many forms of PD, therapies designed to inhibit α -Syn-dependent toxicity or defects in mitophagy may have broad therapeutic benefit.

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REFERENCES

1. Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. *Neuron* 2003;**39**:889–909.
2. Dawson TM, Dawson VL. Molecular pathways of neurodegeneration in Parkinson's disease. *Science* 2003;**302**:819–22.
3. Shulman JM, De Jager PL, Feany MB. Parkinson's disease: genetics and pathogenesis. *Annu Rev Pathol* 2011;**6**:193–222.
4. Forno LS. Neuropathology of Parkinson's disease. *J Neuropathol Exp Neurol* 1996;**55**:259–72.
5. Spillantini MG, Schmidt ML, Lee VM-Y, Trojanowski JQ, Jakes R, Goedert M. α -Synuclein in Lewy bodies. *Nature* 1997;**388**:839–40.
6. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the α -synuclein gene identified in families with Parkinson's disease. *Science* 1997;**276**:2045–7.
7. Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, et al. Ala30Pro mutation in the gene encoding α -synuclein in Parkinson's disease. *Nat Genet* 1998;**18**:106–8.
8. Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I, et al. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Ann Neurol* 2004;**55**:164–73.
9. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, et al. Alpha-synuclein locus triplication causes Parkinson's disease. *Science* 2003;**302**:841.
10. Chartier-Harlin MC, Kachergus J, Roumier C, Mouroux V, Douay X, Lincoln S, et al. Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet* 2004;**364**:1167–9.
11. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* 2000;**3**:1301–6.

12. Orth M, Schapira AH. Mitochondrial involvement in Parkinson's disease. *Neurochem Int* 2002;**40**:533–41.
13. Jenner P. Oxidative stress in Parkinson's disease. *Ann Neurol* 2003;**53**(Suppl. 3):S26–36 [discussion S36–S28].
14. Banerjee R, Starkov AA, Beal MF, Thomas B. Mitochondrial dysfunction in the limelight of Parkinson's disease pathogenesis. *Biochim Biophys Acta* 2009;**1792**:651–63.
15. Graham DG. Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. *Mol Pharmacol* 1978;**14**:633–43.
16. Abou-Sleiman PM, Muqit MM, Wood NW. Expanding insights of mitochondrial dysfunction in Parkinson's disease. *Nat Rev Neurosci* 2006;**7**:207–19.
17. Dodson MW, Guo M, Pinkl, Parkin, DJ-1 and mitochondrial dysfunction in Parkinson's disease. *Curr Opin Neurobiol* 2007;**17**:331–7.
18. Bogaerts V, Theuns J, van Broeckhoven C. Genetic findings in Parkinson's disease and translation into treatment: a leading role for mitochondria? *Genes Brain Behav* 2008;**7**:129–51.
19. Vila M, Ramonet D, Perier C. Mitochondrial alterations in Parkinson's disease: new clues. *J Neurochem* 2008;**107**:317–28.
20. Goedert M. Alpha-synuclein and neurodegenerative diseases. *Nat Rev Neurosci* 2001;**2**:492–501.
21. Beyer K, Domingo-Sabat M, Lao JI, Carrato C, Ferrer I, Ariza A. Identification and characterization of a new alpha-synuclein isoform and its role in Lewy body diseases. *Neurogenetics* 2008;**9**:15–23.
22. Weinreb PH, Zhen W, Poon AW, Conway KA, Lansbury Jr. PT. NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. *Biochemistry* 1996;**35**:13709–15.
23. Bussell Jr. R, Eliezer D. Residual structure and dynamics in Parkinson's disease-associated mutants of α -synuclein. *J Biol Chem* 2001;**276**:45996–6003.
24. Davidson WS, Jonas A, Clayton DF, George JM. Stabilization of α -synuclein secondary structure upon binding to synthetic membranes. *J Biol Chem* 1998;**273**:9443–9.
25. Jo E, McLaurin J, Yip CM, St. George-Hyslop P, Fraser PE. α -Synuclein membrane interactions and lipid specificity. *J Biol Chem* 2000;**275**:34328–34.
26. Perrin RJ, Woods WS, Clayton DF, George JM. Interaction of human α -synuclein and Parkinson's disease variants with phospholipids. Structural analysis using site-directed mutagenesis. *J Biol Chem* 2000;**275**:34393–8.
27. Eliezer D, Kutluay E, Bussell Jr. R, Browne G. Conformational properties of α -synuclein in its free and lipid-associated states. *J Mol Biol* 2001;**307**:1061–73.
28. Bussell Jr. R, Eliezer D. A structural and functional role for 11-mer repeats in α -synuclein and other exchangeable lipid binding proteins. *J Mol Biol* 2003;**329**:763–78.
29. Chandra S, Chen X, Rizo J, Jahn R, Sudhof TC. A broken α -helix in folded α -synuclein. *J Biol Chem* 2003;**278**:15313–8.
30. Zhu M, Li J, Fink AL. The association of α -synuclein with membranes affects bilayer structure, stability and fibril formation. *J Biol Chem* 2003;**278**:40186–97.
31. Jao CC, Der-Sarkissian A, Chen J, Langen R. Structure of membrane-bound alpha-synuclein studied by site-directed spin labeling. *Proc Natl Acad Sci USA* 2004;**101**:8331–6.
32. Abeliovich A, Schmitz Y, Farinas I, Choi-Lundberg D, Ho WH, Castillo PE, et al. Mice lacking α -synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron* 2000;**25**:239–52.
33. Murphy DD, Rueter SM, Trojanowski JQ, Lee VM-Y. Synucleins are developmentally expressed, and α -synuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons. *J Neurosci* 2000;**20**:3214–20.

34. Cabin DE, Shimazu K, Murphy D, Cole NB, Gottschalk W, McIlwain KL, et al. Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged repetitive stimulation in mice lacking α -synuclein. *J Neurosci* 2002;**22**:8797–807.
35. Larsen KE, Schmitz Y, Troyer MD, Mosharov E, Dietrich P, Quazi AZ, et al. Alpha-synuclein overexpression in PC12 and chromaffin cells impairs catecholamine release by interfering with a late step in exocytosis. *J Neurosci* 2006;**26**:11915–22.
36. Cheng F, Vivacqua G, Yu S. The role of alpha-synuclein in neurotransmission and synaptic plasticity. *J Chem Neuroanat* 2010;(1).
37. Nemani VM, Lu W, Berge V, Nakamura K, Onoa B, Lee MK, et al. Increased expression of alpha-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis. *Neuron* 2010;**65**:66–79.
38. Venda LL, Cragg SJ, Buchman VL, Wade-Martins R. Alpha-synuclein and dopamine at the crossroads of Parkinson's disease. *Trends Neurosci* 2010;**33**:559–68.
39. Chandra S, Gallardo G, Fernandez-Chacon R, Schluter OM, Sudhof TC. Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. *Cell* 2005;**123**:383–96.
40. Bartels T, Choi JG, Selkoe DJ. Alpha-synuclein occurs physiologically as a helically folded tetramer that resists aggregation. *Nature* 2011;**477**:107–10.
41. Wang W, Perovic I, Chittuluru J, Kaganovich A, Nguyen LT, Liao J, et al. A soluble alpha-synuclein construct forms a dynamic tetramer. *Proc Natl Acad Sci USA* 2011;**108**:17797–802.
42. Anderson JP, Walker DE, Goldstein JM, de Laat R, Banducci K, Caccavello RJ, et al. Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. *J Biol Chem* 2006;**281**:29739–52.
43. El-Agnaf OM, Bodles AM, Guthrie DJ, Harriott P, Irvine GB. The N-terminal region of non-A beta component of Alzheimer's disease amyloid is responsible for its tendency to assume beta-sheet and aggregate to form fibrils. *Eur J Biochem* 1998;**258**:157–63.
44. Narhi L, Wood SJ, Steavenson S, Jiang Y, Wu GM, Anafi D, et al. Both familial Parkinson's disease mutations accelerate α -synuclein aggregation. *J Biol Chem* 1999;**274**:9843–6.
45. Conway KA, Lee S-J, Rochet J-C, Ding TT, Williamson RE, Lansbury Jr. PT. Acceleration of oligomerization, not fibrillization, is a shared property of both α -synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. *Proc Natl Acad Sci USA* 2000;**97**:571–6.
46. Rochet JC, Conway KA, Lansbury Jr. PT. Inhibition of fibrillization and accumulation of prefibrillar oligomers in mixtures of human and mouse α -synuclein. *Biochemistry* 2000;**39**:10619–26.
47. Serpell LC, Berriman J, Jakes R, Goedert M, Crowther RA. Fiber diffraction of synthetic α -synuclein filaments shows amyloid-like cross- β conformation. *Proc Natl Acad Sci USA* 2000;**97**:4897–902.
48. Volles MJ, Lansbury Jr. PT. Zeroing in on the pathogenic form of α -synuclein and its mechanism of neurotoxicity in Parkinson's disease. *Biochemistry* 2003;**42**:7871–8.
49. Rochet JC, Outeiro TF, Conway KA, Ding TT, Volles MJ, Lashuel HA, et al. Interactions among alpha-synuclein, dopamine, and biomembranes: some clues for understanding neurodegeneration in Parkinson's disease. *J Mol Neurosci* 2004;**23**:23–34.
50. Volles MJ, Lee S-J, Rochet J-C, Shtilerman MD, Ding TT, Kessler JC, et al. Vesicle permeabilization by protofibrillar α -synuclein: implications for the pathogenesis and treatment of Parkinson's disease. *Biochemistry* 2001;**40**:7812–9.
51. Ding TT, Lee S-J, Rochet J-C, Lansbury Jr. PT. Annular α -synuclein protofibrils are produced when spherical protofibrils are incubated in solution or bound to brain-derived membranes. *Biochemistry* 2002;**41**:10209–17.
52. Lashuel HA, Petre BM, Wall J, Simon M, Nowak RJ, Walz T, et al. α -Synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils. *J Mol Biol* 2002;**322**:1089–102.

Au4

53. Li J, Uversky VN, Fink AL. Effect of familial Parkinson's disease point mutations A30P and A53T on the structural properties, aggregation, and fibrillation of human α -synuclein. *Biochemistry* 2001;**40**:11604–13.
54. Choi W, Zibae S, Jakes R, Serpell LC, Davletov B, Crowther RA, et al. Mutation E46K increases phospholipid binding and assembly into filaments of human alpha-synuclein. *FEBS Lett* 2004;**576**:363–8.
55. Hoyer W, Cherny D, Subramaniam V, Jovin TM. Impact of the acidic C-terminal region comprising amino acids 109-140 on alpha-synuclein aggregation in vitro. *Biochemistry* 2004;**43**:16233–42.
56. Outeiro TF, Kontopoulos E, Altman S, Kufareva I, Strathearn KE, Amore AM, et al. Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of parkinson's disease. *Science* 2007;**317**:516–9.
57. Smith DP, Tew DJ, Hill AF, Bottomley SP, Masters CL, Barnham KJ, et al. Formation of a high affinity lipid-binding intermediate during the early aggregation phase of alpha-synuclein. *Biochemistry* 2008;**47**:1425–34.
58. Volles MJ, Lansbury Jr. PT. Vesicle permeabilization by protofibrillar α -synuclein is sensitive to Parkinson's disease-linked mutations and occurs by a pore-like mechanism. *Biochemistry* 2002;**41**:4595–602.
59. Giehm L, Svergun DI, Otzen DE, Vestergaard B. Low-resolution structure of a vesicle disrupting α -synuclein oligomer that accumulates during fibrillation. *Proc Natl Acad Sci USA* 2011;**108**:3246–51.
60. Kaye R, Sokolov Y, Edmonds B, McIntire TM, Milton SC, Hall JE, et al. Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. *J Biol Chem* 2004;**279**:46363–6.
61. Quist A, Doudevski I, Lin H, Azimova R, Ng D, Frangione B, et al. Amyloid ion channels: a common structural link for protein-misfolding disease. *Proc Natl Acad Sci USA* 2005;**102**:10427–32.
62. Danzer KM, Haasen D, Karow AR, Moussaïd S, Habeck M, Giese A, et al. Different species of alpha-synuclein oligomers induce calcium influx and seeding. *J Neurosci* 2007;**27**:9220–32.
63. Tsigelny IF, Bar-On P, Sharikov Y, Crews L, Hashimoto M, Miller MA, et al. Dynamics of alpha-synuclein aggregation and inhibition of pore-like oligomer development by beta-synuclein. *FEBS J* 2007;**274**:1862–77.
64. Feng LR, Federoff HJ, Vicini S, Maguire-Zeiss KA. Alpha-synuclein mediates alterations in membrane conductance: a potential role for alpha-synuclein oligomers in cell vulnerability. *Eur J Neurosci* 2010;**32**:10–7.
65. Greenbaum EA, Graves CL, Mishizen-Eberz AJ, Lupoli MA, Lynch DR, Englander SW, et al. The E46K mutation in alpha-synuclein increases amyloid fibril formation. *J Biol Chem* 2005;**280**:7800–7.
66. Fredenburg RA, Rospigliosi C, Meray RK, Kessler JC, Lashuel HA, Eliezer D, et al. The impact of the E46K mutation on the properties of alpha-synuclein in its monomeric and oligomeric states. *Biochemistry* 2007;**46**:7107–18.
67. Bertoncini CW, Jung YS, Fernandez CO, Hoyer W, Griesinger C, Jovin TM, et al. Release of long-range tertiary interactions potentiates aggregation of natively unstructured alpha-synuclein. *Proc Natl Acad Sci USA* 2005;**102**:1430–5.
68. Dedmon MM, Lindorff-Larsen K, Christodoulou J, Vendruscolo M, Dobson CM. Mapping long-range interactions in alpha-synuclein using spin-label NMR and ensemble molecular dynamics simulations. *J Am Chem Soc* 2005;**127**:476–7.
69. Salmon L, Nodet G, Ozanne V, Yin G, Jensen MR, Zweckstetter M, et al. NMR characterization of long-range order in intrinsically disordered proteins. *J Am Chem Soc* 2010;**132**:8407–18.

70. Crowther RA, Jakes R, Spillantini MG, Goedert M. Synthetic filaments assembled from C-terminally truncated alpha-synuclein. *FEBS Lett* 1998;**436**:309–12.
71. Murray IV, Giasson BI, Quinn SM, Koppaka V, Axelsen PH, Ischiropoulos H, et al. Role of alpha-synuclein carboxy-terminus on fibril formation in vitro. *Biochemistry* 2003;**42**:8530–40.
72. Li W, West N, Colla E, Pletnikova O, Troncoso JC, Marsh L, et al. Aggregation promoting C-terminal truncation of alpha-synuclein is a normal cellular process and is enhanced by the familial Parkinson's disease-linked mutations. *Proc Natl Acad Sci USA* 2005;**102**:2162–7.
73. Liu CW, Giasson BI, Lewis KA, Lee VM, Demartino GN, Thomas PJ. A precipitating role for truncated alpha-synuclein and the proteasome in alpha-synuclein aggregation: implications for pathogenesis of Parkinson disease. *J Biol Chem* 2005;**280**:22670–8.
74. Wu KP, Kim S, Fela DA, Baum J. Characterization of conformational and dynamic properties of natively unfolded human and mouse alpha-synuclein ensembles by NMR: implication for aggregation. *J Mol Biol* 2008;**378**:1104–15.
75. Bertocini CW, Fernandez CO, Griesinger C, Jovin TM, Zweckstetter M. Familial mutants of alpha-synuclein with increased neurotoxicity have a destabilized conformation. *J Biol Chem* 2005;**280**:30649–52.
76. Rospigliosi CC, McClendon S, Schmid AW, Ramlall TF, Barre P, Lashuel HA, et al. E46K Parkinson's-linked mutation enhances C-terminal-to-N-terminal contacts in alpha-synuclein. *J Mol Biol* 2009;**388**:1022–32.
77. Giasson BI, Duda JE, Murray IV, Chen Q, Souza JM, Hurtig HI, et al. Oxidative damage linked to neurodegeneration by selective α -synuclein nitration in synucleinopathy lesions. *Science* 2000;**290**:985–9.
78. Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, Masliah E, Goldberg MS, et al. α -Synuclein is phosphorylated in synucleinopathy lesions. *Nat Cell Biol* 2002;**4**:160–4.
79. Neumann M, Kahle PJ, Giasson BI, Ozmen L, Borroni E, Spooen W, et al. Misfolded proteinase K-resistant hyperphosphorylated α -synuclein in aged transgenic mice with locomotor deterioration and in human α -synucleinopathies. *J Clin Invest* 2002;**110**:1429–39.
80. Kaneko H, Kakita A, Kasuga K, Nozaki H, Ishikawa A, Miyashita A, et al. Enhanced accumulation of phosphorylated alpha-synuclein and elevated beta-amyloid 42/40 ratio caused by expression of the presenilin-1 deltaT440 mutant associated with familial Lewy body disease and variant Alzheimer's disease. *J Neurosci* 2007;**27**:13092–7.
81. Hasegawa M, Fujiwara H, Nonaka T, Wakabayashi K, Takahashi H, Lee VM, et al. Phosphorylated alpha-synuclein is ubiquitinated in alpha-synucleinopathy lesions. *J Biol Chem* 2002;**277**:49071–6.
82. Baba M, Nakajo S, Tu P-H, Tomita T, Nakaya K, Lee VM-Y, et al. Aggregation of α -synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. *Am J Pathol* 1998;**152**:879–84.
83. Chen L, Periquet M, Wang X, Negro A, McLean PJ, Hyman BT, et al. Tyrosine and serine phosphorylation of alpha-synuclein have opposing effects on neurotoxicity and soluble oligomer formation. *J Clin Invest* 2009;**119**:3257–65.
84. Mirzaei H, Schieler JL, Rochet J-C, Regnier F. Identification of rotenone-induced modifications in α -synuclein using affinity pull-down and tandem mass spectrometry. *Anal Chem* 2006;**78**:2422–31.
85. Przedborski S, Chen Q, Vila M, Giasson BI, Djaldatti R, Vukosavic S, et al. Oxidative post-translational modifications of alpha-synuclein in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease. *J Neurochem* 2001;**76**:637–40.
86. Giasson BI, Ischiropoulos H, Lee VM, Trojanowski JQ. The relationship between oxidative/nitrative stress and pathological inclusions in Alzheimer's and Parkinson's diseases. *Free Radic Biol Med* 2002;**32**:1264–75.

87. Martin LJ, Pan Y, Price AC, Sterling W, Copeland NG, Jenkins NA, et al. Parkinson's disease alpha-synuclein transgenic mice develop neuronal mitochondrial degeneration and cell death. *J Neurosci* 2006;**26**:41–50.
88. McCormack AL, Mak SK, Shenasa M, Langston WJ, Forno LS, Di Monte DA. Pathologic modifications of alpha-synuclein in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated squirrel monkeys. *J Neuropathol Exp Neurol* 2008;**67**:793–802.
89. Paxinou E, Chen Q, Weisse M, Giasson BI, Norris EH, Rueter SM, et al. Induction of alpha-synuclein aggregation by intracellular nitrative insult. *J Neurosci* 2001;**21**:8053–61.
90. Danielson SR, Held JM, Schilling B, Oo M, Gibson BW, Andersen JK. Preferentially increased nitration of alpha-synuclein at tyrosine-39 in a cellular oxidative model of Parkinson's disease. *Anal Chem* 2009;**81**:7823–8.
91. Takahashi M, Kanuka H, Fujiwara H, Koyama A, Hasegawa M, Miura M, et al. Phosphorylation of alpha-synuclein characteristic of synucleinopathy lesions is recapitulated in alpha-synuclein transgenic *Drosophila*. *Neurosci Lett* 2003;**336**:155–8.
92. Yamada M, Iwatsubo T, Mizuno Y, Mochizuki H. Overexpression of alpha-synuclein in rat substantia nigra results in loss of dopaminergic neurons, phosphorylation of alpha-synuclein and activation of caspase-9: resemblance to pathogenetic changes in Parkinson's disease. *J Neurochem* 2004;**91**:451–61.
93. Chen L, Feany MB. Alpha-synuclein phosphorylation controls neurotoxicity and inclusion formation in a *Drosophila* model of Parkinson disease. *Nat Neurosci* 2005;**8**:657–63.
94. Shults CW, Rockenstein E, Crews L, Adame A, Mante M, Larrea G, et al. Neurological and neurodegenerative alterations in a transgenic mouse model expressing human alpha-synuclein under oligodendrocyte promoter: implications for multiple system atrophy. *J Neurosci* 2005;**25**:10689–99.
95. Smith WW, Margolis RL, Li X, Troncoso JC, Lee MK, Dawson VL, et al. Alpha-synuclein phosphorylation enhances eosinophilic cytoplasmic inclusion formation in SH-SY5Y cells. *J Neurosci* 2005;**25**:5544–52.
96. Eszlamboli A, Romero-Ramos M, Burger C, Bjorklund T, Muzyczka N, Mandel RJ, et al. Long-term consequences of human alpha-synuclein overexpression in the primate ventral midbrain. *Brain* 2007;**130**:799–815.
97. Wakamatsu M, Ishii A, Ukai Y, Sakagami J, Iwata S, Ono M, et al. Accumulation of phosphorylated alpha-synuclein in dopaminergic neurons of transgenic mice that express human alpha-synuclein. *J Neurosci Res* 2007;**85**:1819–25.
98. Chau KY, Ching HL, Schapira AH, Cooper JM. Relationship between alpha synuclein phosphorylation, proteasomal inhibition and cell death: relevance to Parkinson's disease pathogenesis. *J Neurochem* 2009;**110**:1005–13.
99. Schell H, Hasegawa T, Neumann M, Kahle PJ. Nuclear and neuritic distribution of serine-129 phosphorylated alpha-synuclein in transgenic mice. *Neuroscience* 2009;**160**:796–804.
100. Riedel M, Goldbaum O, Wille M, Richter-Landsberg C. Membrane lipid modification by docosahexaenoic acid (DHA) promotes the formation of alpha-synuclein inclusion bodies immunopositive for SUMO-1 in oligodendroglial cells after oxidative stress. *J Mol Neurosci* 2011;**43**:290–302.
101. Tofaris GK, Razaq A, Ghetti B, Lilley KS, Spillantini MG. Ubiquitination of alpha-synuclein in Lewy bodies is a pathological event not associated with impairment of proteasome function. *J Biol Chem* 2003;**278**:44405–11.
102. Choi DH, Kim YJ, Kim YG, Joh TH, Beal MF, Kim YS. Role of matrix metalloproteinase 3-mediated alpha-synuclein cleavage in dopaminergic cell death. *J Biol Chem* 2011;**286**:14168–77.
103. Paleologou KE, Oueslati A, Shakked G, Rospigliosi CC, Kim HY, Lamberto GR, et al. Phosphorylation at S87 is enhanced in synucleinopathies, inhibits alpha-synuclein oligomerization, and influences synuclein-membrane interactions. *J Neurosci* 2010;**30**:3184–98.

Au5

104. Waxman EA, Giasson BI. Specificity and regulation of casein kinase-mediated phosphorylation of alpha-synuclein. *J Neuropathol Exp Neurol* 2008;**67**:402–16.
105. Hashimoto M, Hsu LJ, Xia Y, Takeda A, Sisk A, Sundsmo M, et al. Oxidative stress induces amyloid-like aggregate formation of NACP/ α -synuclein in vitro. *Neuroreport* 1999;**10**:717–21.
106. Paik SR, Shin HJ, Lee JH. Metal-catalyzed oxidation of α -synuclein in the presence of Copper (II) and hydrogen peroxide. *Arch Biochem Biophys* 2000;**378**:269–77.
107. Souza JM, Giasson BI, Chen Q, Lee VM, Ischiropoulos H. Dityrosine cross-linking promotes formation of stable alpha-synuclein polymers. Implication of nitritative and oxidative stress in the pathogenesis of neurodegenerative synucleinopathies. *J Biol Chem* 2000;**275**:18344–9.
108. Takahashi T, Yamashita H, Nakamura T, Nagano Y, Nakamura S. Tyrosine 125 of alpha-synuclein plays a critical role for dimerization following nitritative stress. *Brain Res* 2002;**938**:73–80.
109. Krishnan S, Chi EY, Wood SJ, Kendrick BS, Li C, Garzon-Rodriguez W, et al. Oxidative dimer formation is the critical rate-limiting step for Parkinson's disease α -synuclein fibrillogenesis. *Biochemistry* 2003;**42**:829–37.
110. Cole NB, Murphy DD, Lebowitz J, Di Noto L, Levine RL, Nussbaum RL. Metal-catalyzed oxidation of alpha synuclein: helping to define the relationship between oligomers, protofilaments and filaments. *J Biol Chem* 2005;**280**:9678–90.
111. Qin Z, Hu D, Han S, Hong DP, Fink AL. Role of different regions of alpha-synuclein in the assembly of fibrils. *Biochemistry* 2007;**46**:13322–30.
112. Shamoto-Nagai M, Maruyama W, Hashizume Y, Yoshida M, Osawa T, Riederer P, et al. In parkinsonian substantia nigra, alpha-synuclein is modified by acrolein, a lipid-peroxidation product, and accumulates in the dopamine neurons with inhibition of proteasome activity. *J Neural Transm* 2007;**114**:1559–67.
113. Bosco DA, Fowler DM, Zhang Q, Nieva J, Powers ET, Wentworth Jr. P, et al. Elevated levels of oxidized cholesterol metabolites in Lewy body disease brains accelerate alpha-synuclein fibrilization. *Nat Chem Biol* 2006;**2**:249–53.
114. Uversky VN, Yamin G, Souillac PO, Goers J, Glaser CB, Fink AL. Methionine oxidation inhibits fibrillation of human α -synuclein in vitro. *FEBS Lett* 2002;**517**:239–44.
115. Zhou W, Long C, Reaney SH, Di Monte DA, Fink AL, Uversky VN. Methionine oxidation stabilizes non-toxic oligomers of alpha-synuclein through strengthening the auto-inhibitory intra-molecular long-range interactions. *Biochim Biophys Acta* 2010;**1802**:322–30.
116. Hokenson MJ, Uversky VN, Goers J, Yamin G, Munishkina LA, Fink AL. Role of individual methionines in the fibrillation of methionine-oxidized alpha-synuclein. *Biochemistry* 2004;**43**:4621–33.
117. Yamin G, Glaser CB, Uversky VN, Fink AL. Certain metals trigger fibrillation of methionine-oxidized α -synuclein. *J Biol Chem* 2003;**278**:27630–5.
118. Conway KA, Rochet J-C, Bieganski RM, Lansbury Jr. PT. Kinetic stabilization of the α -synuclein protofibril by a dopamine- α -synuclein adduct. *Science* 2001;**294**:1346–9.
119. Glaser CB, Yamin G, Uversky VN, Fink AL. Methionine oxidation, alpha-synuclein and Parkinson's disease. *Biochim Biophys Acta* 2005;**1703**:157–69.
120. Cappai R, Leck SL, Tew DJ, Williamson NA, Smith DP, Galatis D, et al. Dopamine promotes alpha-synuclein aggregation into SDS-resistant soluble oligomers via a distinct folding pathway. *FASEB J* 2005;**19**:1377–9.
121. Li HT, Lin DH, Luo XY, Zhang F, Ji LN, Du HN, et al. Inhibition of alpha-synuclein fibrillization by dopamine analogs via reaction with the amino groups of alpha-synuclein. Implication for dopaminergic neurodegeneration. *FEBS J* 2005;**272**:3661–72.
122. Bisaglia M, Mammi S, Bubacco L. Kinetic and structural analysis of the early oxidation products of dopamine. Analysis of the interactions with alpha-synuclein. *J Biol Chem* 2007;**282**:15597–605.

123. Mazzulli JR, Armakola M, Dumoulin M, Parastatidis I, Ischiropoulos H. Cellular oligomerization of alpha-synuclein is determined by the interaction of oxidized catechols with a C-terminal sequence. *J Biol Chem* 2007;**282**:31621–30.
124. Pham CL, Leong SL, Ali FE, Kenche VB, Hill AF, Gras SL, et al. Dopamine and the dopamine oxidation product 5,6-dihydroxyindole promote distinct on-pathway and off-pathway aggregation of alpha-synuclein in a pH-dependent manner. *J Mol Biol* 2009;**387**:771–85.
125. Bisaglia M, Tosatto L, Munari F, Tessari I, de Laureto PP, Mammi S, et al. Dopamine quinones interact with alpha-synuclein to form unstructured adducts. *Biochem Biophys Res Commun* 2010;**394**:424–8.
126. Li J, Zhu M, Manning-Bog AB, Di Monte DA, Fink AL. Dopamine and L-dopa disaggregate amyloid fibrils: implications for Parkinson's and Alzheimer's disease. *FASEB J* 2004;**18**:962–4.
127. Norris EH, Giasson BI, Hodara R, Xu S, Trojanowski JQ, Ischiropoulos H, et al. Reversible inhibition of alpha-synuclein fibrillization by dopaminochrome-mediated conformational alterations. *J Biol Chem* 2005;**280**:21212–9.
128. Follmer C, Romao L, Einsiedler CM, Porto TC, Lara FA, Moncores M, et al. Dopamine affects the stability, hydration, and packing of protofibrils and fibrils of the wild type and variants of alpha-synuclein. *Biochemistry* 2007;**46**:472–82.
129. Leong SL, Pham CL, Galatis D, Fodero-Tavoletti MT, Perez K, Hill AF, et al. Formation of dopamine-mediated alpha-synuclein-soluble oligomers requires methionine oxidation. *Free Radic Biol Med* 2009;**46**:1328–37.
130. Conway KA, Harper JD, Lansbury Jr. PT. Fibrils formed *in vitro* from α -synuclein and two mutant forms linked to Parkinson's disease are typical amyloid. *Biochemistry* 2000;**39**:2552–63.
131. Rekas A, Knott RB, Sokolova A, Barnham KJ, Perez KA, Masters CL, et al. The structure of dopamine induced alpha-synuclein oligomers. *Eur Biophys J* 2010;**39**:1407–19.
132. Herrera FE, Chesi A, Paleologou KE, Schmid A, Munoz A, Vendruscolo M, et al. Inhibition of alpha-synuclein fibrillization by dopamine is mediated by interactions with five C-terminal residues and with E83 in the NAC region. *PLoS One* 2008;**3**:e3394.
133. Mazzulli JR, Mishizen AJ, Giasson BI, Lynch DR, Thomas SA, Nakashima A, et al. Cytosolic catechols inhibit alpha-synuclein aggregation and facilitate the formation of intracellular soluble oligomeric intermediates. *J Neurosci* 2006;**26**:10068–78.
134. Xu J, Kao SY, Lee FJ, Song W, Jin LW, Yankner BA. Dopamine-dependent neurotoxicity of α -synuclein: a mechanism for selective neurodegeneration in Parkinson disease. *Nat Med* 2002;**8**:600–6.
135. Caudle WM, Richardson JR, Wang MZ, Taylor TN, Guillot TS, McCormack AL, et al. Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. *J Neurosci* 2007;**27**:8138–48.
136. Mosharov EV, Larsen KE, Kanter E, Phillips KA, Wilson K, Schmitz Y, et al. Interplay between cytosolic dopamine, calcium, and alpha-synuclein causes selective death of substantia nigra neurons. *Neuron* 2009;**62**:218–29.
137. Norris EH, Giasson BI, Ischiropoulos H, Lee VM. Effects of oxidative and nitrative challenges on α -synuclein fibrillogenesis involve distinct mechanisms of protein modifications. *J Biol Chem* 2003;**278**:27230–40.
138. Yamin G, Uversky VN, Fink AL. Nitration inhibits fibrillation of human α -synuclein *in vitro* by formation of soluble oligomers. *FEBS Lett* 2003;**542**:147–52.
139. Hodara R, Norris EH, Giasson BI, Mishizen-Eberz AJ, Lynch DR, Lee VM, et al. Functional consequences of alpha-synuclein tyrosine nitration: diminished binding to lipid vesicles and increased fibril formation. *J Biol Chem* 2004;**279**:47746–53.

140. Yu Z, Xu X, Xiang Z, Zhou J, Zhang Z, Hu C, et al. Nitrated alpha-synuclein induces the loss of dopaminergic neurons in the substantia nigra of rats. *PLoS One* 2010;**5**:e9956.
141. Ross MF, Filipovska A, Smith RA, Gait MJ, Murphy MP. Cell-penetrating peptides do not cross mitochondrial membranes even when conjugated to a lipophilic cation: evidence against direct passage through phospholipid bilayers. *Biochem J* 2004;**383**:457–68.
142. Al-Taei S, Penning NA, Simpson JC, Futaki S, Takeuchi T, Nakase I, et al. Intracellular traffic and fate of protein transduction domains HIV-1 TAT peptide and octaarginine. Implications for their utilization as drug delivery vectors. *Bioconjug Chem* 2006;**17**:90–100.
143. Okochi M, Walter J, Koyama A, Nakajo S, Baba M, Iwatsubo T, et al. Constitutive phosphorylation of the Parkinson's disease associated alpha-synuclein. *J Biol Chem* 2000;**275**:390–7.
144. Paleologou KE, Schmid AW, Rospigliosi CC, Kim HY, Lamberto GR, Fredenburg RA, et al. Phosphorylation at Ser-129 but not the phosphomimics S129E/D inhibits the fibrillation of alpha-synuclein. *J Biol Chem* 2008;**283**:16895–905.
145. Hoyer W, Antony T, Cherny D, Heim G, Jovin TM, Subramaniam V. Dependence of alpha-synuclein aggregate morphology on solution conditions. *J Mol Biol* 2002;**322**:383–93.
146. Gorbatyuk OS, Li S, Sullivan LF, Chen W, Kondrikova G, Manfredsson FP, et al. The phosphorylation state of Ser-129 in human alpha-synuclein determines neurodegeneration in a rat model of Parkinson disease. *Proc Natl Acad Sci USA* 2008;**105**:763–8.
147. Azeredo da Silveira S, Schneider BL, Cifuentes-Diaz C, Sage D, Abbas-Terki T, Iwatsubo T, et al. Phosphorylation does not prompt, nor prevent, the formation of alpha-synuclein toxic species in a rat model of Parkinson's disease. *Hum Mol Genet* 2009;**18**:872–87.
148. McFarland NR, Fan Z, Xu K, Schwarzschild MA, Feany MB, Hyman BT, et al. Alpha-synuclein S129 phosphorylation mutants do not alter nigrostriatal toxicity in a rat model of Parkinson disease. *J Neuropathol Exp Neurol* 2009;**68**:515–24.
149. Takahashi M, Ko LW, Kulathingal J, Jiang P, Sevelev D, Yen SH. Oxidative stress-induced phosphorylation, degradation and aggregation of alpha-synuclein are linked to upregulated CK2 and cathepsin D. *Eur J Neurosci* 2007;**26**:863–74.
150. Sugeno N, Takeda A, Hasegawa T, Kobayashi M, Kikuchi A, Mori F, et al. Serine 129 phosphorylation of alpha-synuclein induces unfolded protein response-mediated cell death. *J Biol Chem* 2008;**283**:23179–88.
151. Kragh CL, Lund LB, Febbraro F, Hansen HD, Gai WP, El-Agnaf O, et al. α -Synuclein aggregation and Ser-129 phosphorylation-dependent cell death in oligodendroglial cells. *J Biol Chem* 2009;**284**:10211–22.
152. Pronin AN, Morris AJ, Surguchov A, Benovic JL. Synucleins are a novel class of substrates for G protein-coupled receptor kinases. *J Biol Chem* 2000;**275**:26515–22.
153. Arawaka S, Wada M, Goto S, Karube H, Sakamoto M, Ren CH, et al. The role of G-protein-coupled receptor kinase 5 in pathogenesis of sporadic Parkinson's disease. *J Neurosci* 2006;**26**:9227–38.
154. Inglis KJ, Chereau D, Brigham EF, Chiou SS, Schobel S, Frigon NL, et al. Polo-like kinase 2 (PLK2) phosphorylates alpha-synuclein at serine 129 in central nervous system. *J Biol Chem* 2009;**284**:2598–602.
155. Mbefo MK, Paleologou KE, Boucharaba A, Oueslati A, Schell H, Fournier M, et al. Phosphorylation of synucleins by members of the Polo-like kinase family. *J Biol Chem* 2010;**285**:2807–22.
156. Lee KW, Chen W, Junn E, Im JY, Grosso H, Sonsalla PK, et al. Enhanced phosphatase activity attenuates [alpha]-synucleinopathy in a mouse model. *J Neurosci* 2011;**31**:6963–71.
157. Gitler AD, Chesni A, Geddie ML, Strathearn KE, Hamamichi S, Hill KJ, et al. Alpha-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. *Nat Genet* 2009;**41**:308–15.

158. Kim EJ, Sung JY, Lee HJ, Rhim H, Hasegawa M, Iwatsubo T, et al. Dyrk1A phosphorylates alpha-synuclein and enhances intracellular inclusion formation. *J Biol Chem* 2006;**281**:33250–7.
159. Negro A, Brunati AM, Donella-Deana A, Massimino ML, Pinna LA. Multiple phosphorylation of α -synuclein by protein tyrosine kinase Syk prevents eosin-induced aggregation. *FASEB J* 2002;**16**:210–2.
160. Lewis KA, Yaeger A, DeMartino GN, Thomas PJ. Accelerated formation of alpha-synuclein oligomers by concerted action of the 20S proteasome and familial Parkinson mutations. *J Bioenerg Biomembr* 2010;**42**:85–95.
161. Periquet M, Fulga T, Myllykangas L, Schlossmacher MG, Feany MB. Aggregated alpha-synuclein mediates dopaminergic neurotoxicity in vivo. *J Neurosci* 2007;**27**:3338–46.
162. Tofaris GK, Garcia Reitböck P, Humby T, Lambourne SL, O'Connell M, Ghetti B, et al. Pathological changes in dopaminergic nerve cells of the substantia nigra and olfactory bulb in mice transgenic for truncated human alpha-synuclein(1-120): implications for Lewy body disorders. *J Neurosci* 2006;**26**:3942–50.
163. Daher JP, Ying M, Banerjee R, McDonald RS, Hahn MD, Yang L, et al. Conditional transgenic mice expressing C-terminally truncated human alpha-synuclein (alphaSyn119) exhibit reduced striatal dopamine without loss of nigrostriatal pathway dopaminergic neurons. *Mol Neurodegener* 2009;**4**:34.
164. Wakamatsu M, Ishii A, Iwata S, Sakagami J, Ukai Y, Ono M, et al. Selective loss of nigral dopamine neurons induced by overexpression of truncated human alpha-synuclein in mice. *Neurobiol Aging* 2008;**29**:574–85.
165. Ulusoy A, Febbraro F, Jensen PH, Kirik D, Romero-Ramos M. Co-expression of C-terminal truncated alpha-synuclein enhances full-length alpha-synuclein-induced pathology. *Eur J Neurosci* 2010;**32**:409–22.
166. Mishizen-Eberz AJ, Guttman RP, Giasson BI, Day 3rd GA, Hodara R, Ischiropoulos H, et al. Distinct cleavage patterns of normal and pathologic forms of alpha-synuclein by calpain I in vitro. *J Neurochem* 2003;**86**:836–47.
167. Mishizen-Eberz AJ, Norris EH, Giasson BI, Hodara R, Ischiropoulos H, Lee VM, et al. Cleavage of alpha-synuclein by calpain: potential role in degradation of fibrillized and nitrated species of alpha-synuclein. *Biochemistry* 2005;**44**:7818–29.
168. Duffy BM, Warner LR, Hou ST, Jiang SX, Gomez-Isla T, Leenhouts KM, et al. Calpain-cleavage of alpha-synuclein: connecting proteolytic processing to disease-linked aggregation. *Am J Pathol* 2007;**170**:1725–38.
169. Seveler D, Jiang P, Yen SH. Cathepsin D is the main lysosomal enzyme involved in the degradation of alpha-synuclein and generation of its carboxy-terminally truncated species. *Biochemistry* 2008;**47**:9678–87.
170. Lee H-J, Choi C, Lee S-J. Membrane-bound α -synuclein has a high aggregation propensity and the ability to seed the aggregation of the cytosolic form. *J Biol Chem* 2002;**277**:671–8.
171. Cole NB, Murphy DD, Grider T, Rueter S, Brasaemle D, Nussbaum RL. Lipid droplet binding and oligomerization properties of the Parkinson's disease protein α -synuclein. *J Biol Chem* 2002;**277**:6344–52.
172. Perrin RJ, Woods WS, Clayton DF, George JM. Exposure to long chain polyunsaturated fatty acids triggers rapid multimerization of synucleins. *J Biol Chem* 2001;**276**:41958–62.
173. Sharon R, Bar-Joseph I, Frosch MP, Walsh DM, Hamilton JA, Selkoe DJ. The formation of highly soluble oligomers of α -synuclein is regulated by fatty acids and enhanced in Parkinson's disease. *Neuron* 2003;**37**:583–95.
174. De Franceschi G, Frare E, Pivato M, Relini A, Penco A, Greggio E, et al. Structural and morphological characterization of aggregated species of [alpha]-synuclein induced by docosahexaenoic acid. *J Biol Chem* 2011;**286**:22262–74.

175. Necula M, Chirita CN, Kuret J. Rapid anionic micelle-mediated alpha-synuclein fibrillization in vitro. *J Biol Chem* 2003;**278**:46674–80.
176. Zhu M, Fink AL. Lipid binding inhibits α -synuclein fibril formation. *J Biol Chem* 2003;**278**:16873–7.
177. Jo E, Darabie AA, Han K, Tandon A, Fraser PE, McLaurin J. alpha-Synuclein-synaptosomal membrane interactions: implications for fibrillogenesis. *Eur J Biochem* 2004;**271**:3180–9.
178. Giehm L, Oliveira CL, Christiansen G, Pedersen JS, Otzen DE. SDS-induced fibrillation of alpha-synuclein: an alternative fibrillation pathway. *J Mol Biol* 2010;**401**:115–33.
179. Narayanan V, Scarlata S. Membrane binding and self-association of alpha-synucleins. *Biochemistry* 2001;**40**:9927–34.
180. Bisaglia M, Tessari I, Pinato L, Bellanda M, Giraud S, Fasano M, et al. A topological model of the interaction between alpha-synuclein and sodium dodecyl sulfate micelles. *Biochemistry* 2005;**44**:329–39.
181. Ulmer TS, Bax A, Cole NB, Nussbaum RL. Structure and dynamics of micelle-bound human alpha-synuclein. *J Biol Chem* 2005;**280**:9595–603.
182. Bodner CR, Dobson CM, Bax A. Multiple tight phospholipid-binding modes of alpha-synuclein revealed by solution NMR spectroscopy. *J Mol Biol* 2009;**390**:775–90.
183. Ferreon AC, Gambin Y, Lemke EA, Deniz AA. Interplay of alpha-synuclein binding and conformational switching probed by single-molecule fluorescence. *Proc Natl Acad Sci USA* 2009;**106**:5645–50.
184. Trexler A, Rhoades E. alpha-Synuclein binds large unilamellar vesicles as an extended helix. *Biochemistry* 2009;**48**:2304–6. [Au5]
185. Mihajlovic M, Lazaridis T. Membrane-bound structure and energetics of alpha-synuclein. *Proteins* 2008;**70**:761–78.
186. Abedini A, Raleigh DP. A role for helical intermediates in amyloid formation by natively unfolded polypeptides? *Phys Biol* 2009;**6**:15005.
187. Abedini A, Raleigh DP. A critical assessment of the role of helical intermediates in amyloid formation by natively unfolded proteins and polypeptides. *Protein Eng Des Sel* 2009;**22**:453–9.
188. Anderson VL, Ramlall TF, Rospigliosi CC, Webb WW, Eliezer D. Identification of a helical intermediate in trifluoroethanol-induced alpha-synuclein aggregation. *Proc Natl Acad Sci USA* 2010;**107**:18850–5.
189. Aisenbrey C, Borowik T, Bystrom R, Bokvist M, Lindstrom F, Misiak H, et al. How is protein aggregation in amyloidogenic diseases modulated by biological membranes? *Eur Biophys J* 2008;**37**:247–55.
190. Bystrom R, Aisenbrey C, Borowik T, Bokvist M, Lindstrom F, Sani MA, et al. Disordered proteins: biological membranes as two-dimensional aggregation matrices. *Cell Biochem Biophys* 2008;**52**:175–89.
191. Pandey AP, Haque F, Rochet JC, Hovis JS. Clustering of alpha-synuclein on supported lipid bilayers: role of anionic lipid, protein, and divalent ion concentration. *Biophys J* 2009;**96**:540–51.
192. Haque F, Pandey AP, Cambrea LR, Rochet JC, Hovis JS. Adsorption of alpha-synuclein on lipid bilayers: modulating the structure and stability of protein assemblies. *J Phys Chem B* 2010;**114**:4070–81.
193. Pandey AP, Haque F, Rochet JC, Hovis JS. alpha-Synuclein-induced tubule formation in lipid bilayers. *J Phys Chem B* 2011;**115**:5886–93.
194. Bodner CR, Maltsev AS, Dobson CM, Bax A. Differential phospholipid binding of alpha-synuclein variants implicated in Parkinson's disease revealed by solution NMR spectroscopy. *Biochemistry* 2010;**49**:862–71.

195. Bartels T, Ahlstrom LS, Leftin A, Kamp F, Haass C, Brown MF, et al. The N-terminus of the intrinsically disordered protein alpha-synuclein triggers membrane binding and helix folding. *Biophys J* 2010;**99**:2116–24.
196. Vamvaca K, Volles MJ, Lansbury Jr. PT. The first N-terminal amino acids of alpha-synuclein are essential for alpha-helical structure formation in vitro and membrane binding in yeast. *J Mol Biol* 2009;**389**:413–24.
197. Riekkinen P, Rinne UK, Pelliniemi TT, Sonninen V. Interaction between dopamine and phospholipids. Studies of the substantia nigra in Parkinson disease patients. *Arch Neurol* 1975;**32**:25–7.
198. Sevcsik E, Trexler AJ, Dunn JM, Rhoades E. Allosteric in a disordered protein: oxidative modifications to alpha-synuclein act distally to regulate membrane binding. *J Am Chem Soc* 2011;**133**:7152–8.
199. Tamamizu-Kato S, Kosaraju MG, Kato H, Raussens V, Ruyschaert JM, Narayanaswami V. Calcium-triggered membrane interaction of the alpha-synuclein acidic tail. *Biochemistry* 2006;**45**:10947–56.
200. Der-Sarkissian A, Jao CC, Chen J, Langen R. Structural organization of alpha-synuclein fibrils studied by site-directed spin labeling. *J Biol Chem* 2003;**278**:37530–5.
201. Chen M, Margittai M, Chen J, Langen R. Investigation of alpha-synuclein fibril structure by site-directed spin labeling. *J Biol Chem* 2007;**282**:24970–9.
202. Del Mar C, Greenbaum EA, Mayne L, Englander SW, Woods Jr. VL. Structure and properties of alpha-synuclein and other amyloids determined at the amino acid level. *Proc Natl Acad Sci USA* 2005;**102**:15477–82.
203. Heise H, Hoyer W, Becker S, Andronesi OC, Riedel D, Baldus M. Molecular-level secondary structure, polymorphism, and dynamics of full-length alpha-synuclein fibrils studied by solid-state NMR. *Proc Natl Acad Sci USA* 2005;**102**:15871–6.
204. Vilar M, Chou HT, Luhrs T, Maji SK, Riek-Loher D, Verel R, et al. The fold of alpha-synuclein fibrils. *Proc Natl Acad Sci USA* 2008;**105**:8637–42.
205. Comellas G, Lemkau LR, Nieuwkoop AJ, Kloepper KD, Lador DT, Ebisu R, et al. Structured regions of alpha-synuclein fibrils include the early-onset Parkinson's disease mutation sites. *J Mol Biol* 2011;**411**:881–95.
206. Sato T, Kienlen-Campard P, Ahmed M, Liu W, Li H, Elliott JI, et al. Inhibitors of amyloid toxicity based on beta-sheet packing of Abeta40 and Abeta42. *Biochemistry* 2006;**45**:5503–16.
207. Sciarretta KL, Boire A, Gordon DJ, Meredith SC. Spatial separation of beta-sheet domains of beta-amyloid: disruption of each beta-sheet by N-methyl amino acids. *Biochemistry* 2006;**45**:9485–95.
208. Sievers SA, Karanicolas J, Chang HW, Zhao A, Jiang L, Zirafi O, et al. Structure-based design of non-natural amino-acid inhibitors of amyloid fibril formation. *Nature* 2011;**475**:96–100.
209. Karpinar DP, Baliya MB, Kugler S, Opazo F, Rezaei-Ghaleh N, Wender N, et al. Pre-fibrillar alpha-synuclein variants with impaired beta-structure increase neurotoxicity in Parkinson's disease models. *EMBO J* 2009;**28**:3256–68.
210. Winner B, Jappelli R, Maji SK, Desplats PA, Boyer L, Aigner S, et al. In vivo demonstration that alpha-synuclein oligomers are toxic. *Proc Natl Acad Sci USA* 2011;**108**:4194–9.
211. Lee SJ, Lim HS, Masliah E, Lee HJ. Protein aggregate spreading in neurodegenerative diseases: problems and perspectives. *Neurosci Res* 2011;**70**:339–48.
212. Lee HJ, Patel S, Lee SJ. Intravesicular localization and exocytosis of alpha-synuclein and its aggregates. *J Neurosci* 2005;**25**:6016–24.
213. Emmanouilidou E, Melachroinou K, Roumeliotis T, Garbis SD, Ntzouni M, Margaritis LH, et al. Cell-produced alpha-synuclein is secreted in a calcium-dependent manner by exosomes and impacts neuronal survival. *J Neurosci* 2010;**30**:6838–51.

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214. Jang A, Lee HJ, Suk JE, Jung JW, Kim KP, Lee SJ. Non-classical exocytosis of alpha-synuclein is sensitive to folding states and promoted under stress conditions. *J Neurochem* 2010;**113**:1263–74.
215. Alvarez-Erviti L, Seow Y, Schapira AH, Gardiner C, Sargent IL, Wood MJ, et al. Lysosomal dysfunction increases exosome-mediated alpha-synuclein release and transmission. *Neurobiol Dis* 2011;**42**:360–7.
216. Danzer KM, Ruf WP, Putcha P, Joyner D, Hashimoto T, Glabe C, et al. Heat-shock protein 70 modulates toxic extracellular alpha-synuclein oligomers and rescues trans-synaptic toxicity. *FASEB J* 2011;**25**:326–36.
217. Hansen C, Angot E, Bergstrom AL, Steiner JA, Pieri L, Paul G, et al. alpha-Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. *J Clin Invest* 2011;**121**:715–25.
218. Lee HJ, Suk JE, Bae EJ, Lee JH, Paik SR, Lee SJ. Assembly-dependent endocytosis and clearance of extracellular alpha-synuclein. *Int J Biochem Cell Biol* 2008;**40**:1835–49.
219. Desplats P, Lee HJ, Bae EJ, Patrick C, Rockenstein E, Crews L, et al. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. *Proc Natl Acad Sci USA* 2009;**106**:13010–5.
220. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* 2003;**24**:197–211.
221. Zhang W, Wang T, Pei Z, Miller DS, Wu X, Block ML, et al. Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. *FASEB J* 2005;**19**:533–42.
222. Zhang W, Dallas S, Zhang D, Guo JP, Pang H, Wilson B, et al. Microglial PHOX and Mac-1 are essential to the enhanced dopaminergic neurodegeneration elicited by A30P and A53T mutant alpha-synuclein. *Glia* 2007;**55**:1178–88.
223. Lee SJ. Origins and effects of extracellular alpha-synuclein: implications in Parkinson's disease. *J Mol Neurosci* 2008;**34**:17–22.
224. Reynolds AD, Glanzer JG, Kadiu I, Ricardo-Dukelow M, Chaudhuri A, Ciborowski P, et al. Nitrated alpha-synuclein-activated microglial profiling for Parkinson's disease. *J Neurochem* 2008;**104**:1504–25.
225. Su X, Federoff HJ, Maguire-Zeiss KA. Mutant alpha-synuclein overexpression mediates early proinflammatory activity. *Neurotox Res* 2009;**16**:238–54.
226. Mattson MP. Neuronal life-and-death signaling, apoptosis, and neurodegenerative disorders. *Antioxid Redox Signal* 2006;**8**:1997–2006.
227. Rochet JC. Novel therapeutic strategies for the treatment of protein-misfolding diseases. *Expert Rev Mol Med* 2007;**9**:1–34.
228. Wassef R, Haenold R, Hansel A, Brot N, Heinemann SH, Hoshi T. Methionine sulfoxide reductase A and a dietary supplement S-methyl-L-cysteine prevent Parkinson's-like symptoms. *J Neurosci* 2007;**27**:12808–16.
229. Liu F, Hindupur J, Nguyen JL, Ruf KJ, Zhu J, Schieler JL, et al. Methionine sulfoxide reductase A protects dopaminergic cells from Parkinson's disease-related insults. *Free Radic Biol Med* 2008;**45**:242–55.
230. Levine RL, Moskovitz J, Stadtman ER. Oxidation of methionine in proteins: roles in antioxidant defense and cellular regulation. *IUBMB Life* 2000;**50**:301–7.
231. Zhang XH, Weissbach H. Origin and evolution of the protein-repairing enzymes methionine sulphoxide reductases. *Biol Rev Camb Philos Soc* 2008;**83**:249–57.
232. Zhu M, Qin ZJ, Hu D, Munishkina LA, Fink AL. Alpha-synuclein can function as an antioxidant preventing oxidation of unsaturated lipid in vesicles. *Biochemistry* 2006;**45**:8135–42.

233. Moskovitz J, Weissbach H, Brot N. Cloning the expression of a mammalian gene involved in the reduction of methionine sulfoxide residues in proteins. *Proc Natl Acad Sci USA* 1996;**93**:2095–9.
234. Petropoulos I, Mary J, Perichon M, Friguet B. Rat peptide methionine sulphoxide reductase: cloning of the cDNA, and down-regulation of gene expression and enzyme activity during aging. *Biochem J* 2001;**355**:819–25.
235. Botella JA, Bayersdorfer F, Schneuwly S. Superoxide dismutase overexpression protects dopaminergic neurons in a Drosophila model of Parkinson's disease. *Neurobiol Dis* 2008;**30**:65–73.
236. Kensler TW, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu Rev Pharmacol Toxicol* 2007;**47**:89–116.
237. Vargas MR, Johnson JA. The Nrf2-ARE cytoprotective pathway in astrocytes. *Expert Rev Mol Med* 2009;**11**:e17.
238. Sykietis GP, Bohmann D. Stress-activated cap'n'collar transcription factors in aging and human disease. *Sci Signal* 2010;**3**:re3.
239. Barone MC, Sykietis GP, Bohmann D. Genetic activation of Nrf2 signaling is sufficient to ameliorate neurodegenerative phenotypes in a Drosophila model of Parkinson's disease. *Dis Model Mech* 2011;**4**:701–7.
240. Muchowski PJ, Wacker JL. Modulation of neurodegeneration by molecular chaperones. *Nat Rev Neurosci* 2005;**6**:11–22.
241. Dedmon MM, Christodoulou J, Wilson MR, Dobson CM. Heat shock protein 70 inhibits alpha-synuclein fibril formation via preferential binding to prefibrillar species. *J Biol Chem* 2005;**280**:14733–40.
242. Huang C, Cheng H, Hao S, Zhou H, Zhang X, Gao J, et al. Heat shock protein 70 inhibits alpha-synuclein fibril formation via interactions with diverse intermediates. *J Mol Biol* 2006;**364**:323–36.
243. Luk KC, Mills IP, Trojanowski JQ, Lee VM. Interactions between Hsp70 and the hydrophobic core of alpha-synuclein inhibit fibril assembly. *Biochemistry* 2008;**47**:12614–25.
244. Roodveldt C, Bertoncini CW, Andersson A, van der Goot AT, Hsu ST, Fernandez-Montesinos R, et al. Chaperone proteostasis in Parkinson's disease: stabilization of the Hsp70/alpha-synuclein complex by Hip. *EMBO J* 2009;**28**:3758–70.
245. Scherzer CR, Eklund AC, Morse LJ, Liao Z, Locascio JJ, Fefer D, et al. Molecular markers of early Parkinson's disease based on gene expression in blood. *Proc Natl Acad Sci USA* 2007;**104**:955–60.
246. Hinault MP, Cuendet AF, Mattoo RU, Mensi M, Dietler G, Lashuel HA, et al. Stable alpha-synuclein oligomers strongly inhibit chaperone activity of the Hsp70 system by weak interactions with J-domain co-chaperones. *J Biol Chem* 2010;**285**:38173–82.
247. Auluck PK, Chan HY, Trojanowski JQ, Lee VM, Bonini NM. Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. *Science* 2002;**295**:865–8.
248. Auluck PK, Meulener MC, Bonini NM. Mechanisms of suppression of [alpha]-synuclein neurotoxicity by geldanamycin in Drosophila. *J Biol Chem* 2005;**280**:2873–8.
249. Klucken J, Shin Y, Masliah E, Hyman BT, McLean PJ. Hsp70 reduces alpha-synuclein aggregation and toxicity. *J Biol Chem* 2004;**279**:25497–502.
250. Outeiro TF, Putcha P, Tetzlaff JE, Spoelgen R, Koker M, Carvalho F, et al. Formation of toxic oligomeric alpha-synuclein species in living cells. *PLoS One* 2008;**3**:e1867.
251. Liu F, Nguyen JL, Hulleman JD, Li L, Rochet J-C. Mechanisms of DJ-1 neuroprotection in a cellular model of Parkinson's disease. *J Neurochem* 2008;**105**:2435–53.
252. Shimshek DR, Mueller M, Wiessner C, Schweizer T, van der Putten PH. The HSP70 molecular chaperone is not beneficial in a mouse model of alpha-synucleinopathy. *PLoS One* 2010;**5**:e10014.

Au5

253. Rekas A, Adda CG, Andrew Aquilina J, Barnham KJ, Sunde M, Galatis D, et al. Interaction of the molecular chaperone alphaB-crystallin with alpha-synuclein: effects on amyloid fibril formation and chaperone activity. *J Mol Biol* 2004;**340**:1167–83.
254. Rekas A, Jankova L, Thorn DC, Cappai R, Carver JA. Monitoring the prevention of amyloid fibril formation by alpha-crystallin. Temperature dependence and the nature of the aggregating species. *FEBS J* 2007;**274**:6290–304.
255. Waudby CA, Knowles TP, Devlin GL, Skepper JN, Ecroyd H, Carver JA, et al. The interaction of alphaB-crystallin with mature alpha-synuclein amyloid fibrils inhibits their elongation. *Biophys J* 2010;**98**:843–51.
256. Ghosh JG, Houck SA, Clark JI. Interactive sequences in the molecular chaperone, human alphaB crystallin modulate the fibrillation of amyloidogenic proteins. *Int J Biochem Cell Biol* 2008;**40**:954–67.
257. Ahmad MF, Raman B, Ramakrishna T, Rao Ch M. Effect of phosphorylation on alpha B-crystallin: differences in stability, subunit exchange and chaperone activity of homo and mixed oligomers of alpha B-crystallin and its phosphorylation-mimicking mutant. *J Mol Biol* 2008;**375**:1040–51.
258. Zourlidou A, Payne Smith MD, Latchman DS. HSP27 but not HSP70 has a potent protective effect against alpha-synuclein-induced cell death in mammalian neuronal cells. *J Neurochem* 2004;**88**:1439–48.
259. Outeiro TF, Klucken J, Strathearn KE, Liu F, Nguyen P, Rochet JC, et al. Small heat shock proteins protect against alpha-synuclein-induced toxicity and aggregation. *Biochem Biophys Res Commun* 2006;**351**:631–8.
260. Pountney DL, Treweek TM, Chataway T, Huang Y, Chegini F, Blumbergs PC, et al. Alpha B-crystallin is a major component of glial cytoplasmic inclusions in multiple system atrophy. *Neurotox Res* 2005;**7**:77–85.
261. Abou-Sleiman PM, Healy DG, Quinn N, Lees AJ, Wood NW. The role of pathogenic DJ-1 mutations in Parkinson's disease. *Ann Neurol* 2003;**54**:283–6.
262. Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, et al. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science* 2003;**299**:256–9.
263. Hague S, Rogaeva E, Hernandez D, Gulick C, Singleton A, Hanson M, et al. Early-onset Parkinson's disease caused by a compound heterozygous DJ-1 mutation. *Ann Neurol* 2003;**54**:271–4.
264. Clark LN, Afridi S, Mejia-Santana H, Harris J, Louis ED, Cote LJ, et al. Analysis of an early-onset Parkinson's disease cohort for DJ-1 mutations. *Mov Disord* 2004;**19**:796–800.
265. Hering R, Strauss KM, Tao X, Bauer A, Voitalla D, Mietz EM, et al. Novel homozygous p. E64D mutation in DJ1 in early onset Parkinson disease (PARK7). *Hum Mutat* 2004;**24**:321–9.
266. Nuytemans K, Theuns J, Cruts M, Van Broeckhoven C. Genetic etiology of Parkinson disease associated with mutations in the SNCA, PARK2, PINK1, PARK7, and LRRK2 genes: a mutation update. *Hum Mutat* 2010;**31**:763–80.
267. Honbou K, Suzuki NN, Horiuchi M, Niki T, Taira T, Ariga H, et al. The crystal structure of DJ-1, a protein related to male fertility and Parkinson's disease. *J Biol Chem* 2003;**278**:31380–4 [Epub 32003 Jun 31388].
268. Huai Q, Sun Y, Wang H, Chin LS, Li L, Robinson H, et al. Crystal structure of DJ-1/RS and implication on familial Parkinson's disease. *FEBS Lett* 2003;**549**:171–5.
269. Lee SJ, Kim SJ, Kim IK, Ko J, Jeong CS, Kim GH, et al. Crystal structures of human DJ-1 and Escherichia coli Hsp31, which share an evolutionarily conserved domain. *J Biol Chem* 2003;**278**:44552–9.
270. Tao X, Tong L. Crystal structure of human DJ-1, a protein associated with early onset Parkinson's disease. *J Biol Chem* 2003;**278**:31372–9.

271. Wilson MA, Collins JL, Hod Y, Ringe D, Petsko GA. The 1.1-Å resolution crystal structure of DJ-1, the protein mutated in autosomal recessive early onset Parkinson's disease. *Proc Natl Acad Sci USA* 2003;**100**:9256–61.
272. Bandopadhyay R, Kingsbury AE, Cookson MR, Reid AR, Evans IM, Hope AD, et al. The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinson's disease. *Brain* 2004;**127**:420–30.
273. Lucas JI, Marin I. A new evolutionary paradigm for the Parkinson disease gene DJ-1. *Mol Biol Evol* 2007;**24**:551–61.
274. Lev N, Roncevic D, Ickowicz D, Melamed E, Offen D. Role of DJ-1 in Parkinson's disease. *J Mol Neurosci* 2006;**29**:215–25.
275. Kahle PJ, Waak J, Gasser T. DJ-1 and prevention of oxidative stress in Parkinson's disease and other age-related disorders. *Free Radic Biol Med* 2009;**47**:1354–61.
276. Canet-Aviles RM, Wilson MA, Miller DW, Ahmad R, McLendon C, Bandyopadhyay S, et al. The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfenic acid-driven mitochondrial localization. *Proc Natl Acad Sci USA* 2004;**101**:9103–8.
277. Taira T, Saito Y, Niki T, Iguchi-Arigo SM, Takahashi K, Ariga H. DJ-1 has a role in antioxidative stress to prevent cell death. *EMBO Rep* 2004;**5**:213–8.
278. Shendelman S, Jonason A, Martinat C, Leete T, Abeliovich A. DJ-1 is a redox-dependent molecular chaperone that inhibits α -synuclein aggregate formation. *PLoS Biol* 2004;**2**:e362.
279. Zhou W, Zhu M, Wilson MA, Petsko GA, Fink AL. The oxidation state of DJ-1 regulates its chaperone activity toward alpha-synuclein. *J Mol Biol* 2006;**356**:1036–48.
280. Logan T, Clark L, Ray SS. Engineered disulfide bonds restore chaperone-like function of DJ-1 mutants linked to familial Parkinson's disease. *Biochemistry* 2010;**49**:5624–33.
281. Witt AC, Lakshminarasimhan M, Remington BC, Hasim S, Pozharski E, Wilson MA. Cysteine pKa depression by a protonated glutamic acid in human DJ-1. *Biochemistry* 2008;**47**:7430–40.
282. Macedo MG, Anar B, Bronner IF, Cannella M, Squitieri F, Bonifati V, et al. The DJ-1L166P mutant protein associated with early onset Parkinson's disease is unstable and forms higher-order protein complexes. *Hum Mol Genet* 2003;**12**:2807–16.
283. Moore DJ, Zhang L, Dawson TM, Dawson VL. A missense mutation (L166P) in DJ-1, linked to familial Parkinson's disease, confers reduced protein stability and impairs homo-oligomerization. *J Neurochem* 2003;**87**:1558–67.
284. Gorner K, Holtorf E, Odoy S, Nuscher B, Yamamoto A, Regula JT, et al. Differential effects of Parkinson's disease-associated mutations on stability and folding of DJ-1. *J Biol Chem* 2004;**279**:6943–51.
285. Olzmann JA, Brown K, Wilkinson KD, Rees HD, Huai Q, Ke H, et al. Familial Parkinson's disease-associated L166P mutation disrupts DJ-1 protein folding and function. *J Biol Chem* 2004;**279**:8506–15.
286. Hulleman JD, Mirzaei H, Guigard E, Taylor KL, Ray SS, Kay CM, et al. Destabilization of DJ-1 by familial substitution and oxidative modifications: implications for Parkinson's disease. *Biochemistry* 2007;**46**:5776–89.
287. Lakshminarasimhan M, Maldonado MT, Zhou W, Fink AL, Wilson MA. Structural impact of three Parkinsonism-associated missense mutations on human DJ-1. *Biochemistry* 2008;**47**:1381–92.
288. Malgieri G, Eliezer D. Structural effects of Parkinson's disease linked DJ-1 mutations. *Protein Sci* 2008;**17**:855–68.
289. Choi J, Sullards MC, Olzmann JA, Rees HD, Weintraub ST, Bostwick DE, et al. Oxidative damage of DJ-1 is linked to sporadic Parkinson and Alzheimer diseases. *J Biol Chem* 2006;**281**:10816–24.

290. Meulener MC, Xu K, Thomson L, Ischiropoulos H, Bonini NM. Mutational analysis of DJ-1 in *Drosophila* implicates functional inactivation by oxidative damage and aging. *Proc Natl Acad Sci USA* 2006;**103**:12517–22.
291. Zhou W, Freed CR. DJ-1 up-regulates glutathione synthesis during oxidative stress and inhibits A53T alpha-synuclein toxicity. *J Biol Chem* 2005;**280**:43150–8.
292. Batelli S, Albani D, Rametta R, Polito L, Prato F, Pesaresi M, et al. DJ-1 modulates alpha-synuclein aggregation state in a cellular model of oxidative stress: relevance for Parkinson's disease and involvement of HSP70. *PLoS One* 2008;**3**:e1884.
293. Li L, Prabhakaran K, Zhang X, Zhang L, Liu H, Borowitz JL, et al. 1Alpha,25-dihydroxyvitamin D3 attenuates cyanide-induced neurotoxicity by inhibiting uncoupling protein-2 up-regulation. *J Neurosci Res* 2008;**86**:1397–408.
294. Deeg S, Gralle M, Sroka K, Bahr M, Wouters FS, Kermer P. BAG1 restores formation of functional DJ-1 L166P dimers and DJ-1 chaperone activity. *J Cell Biol* 2010;**188**:505–13.
295. Bennett MC, Bishop JF, Leng Y, Chock PB, Chase TN, Mouradian MM. Degradation of alpha-synuclein by proteasome. *J Biol Chem* 1999;**274**:33855–8.
296. Tofaris GK, Layfield R, Spillantini MG. Alpha-synuclein metabolism and aggregation is linked to ubiquitin-independent degradation by the proteasome. *FEBS Lett* 2001;**509**:22–6.
297. McNaught KS, Mytilineou C, Jnobaptiste R, Yabut J, Shashidharan P, Jennert P, et al. Impairment of the ubiquitin-proteasome system causes dopaminergic cell death and inclusion body formation in ventral mesencephalic cultures. *J Neurochem* 2002;**81**:301–6.
298. Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC. Alpha-synuclein is degraded by both autophagy and the proteasome. *J Biol Chem* 2003;**278**:25009–13.
299. Machiya Y, Hara S, Arawaka S, Fukushima S, Sato H, Sakamoto M, et al. Phosphorylated alpha-synuclein at Ser-129 is targeted to the proteasome pathway in a ubiquitin-independent manner. *J Biol Chem* 2010;**285**:40732–44.
300. Ancolio K, Alves da Costa C, Ueda K, Checler F. Alpha-synuclein and the Parkinson's disease-related mutant Ala53Thr-alpha-synuclein do not undergo proteasomal degradation in HEK293 and neuronal cells. *Neurosci Lett* 2000;**285**:79–82.
301. Petrucelli L, O'Farrell C, Lockhart PJ, Baptista M, Kehoe K, Vink L, et al. Parkin protects against the toxicity associated with mutant α -synuclein: proteasome dysfunction selectively affects catecholaminergic neurons. *Neuron* 2002;**36**:1007–19.
302. Vogiatzi T, Xilouri M, Vekrellis K, Stefanis L. Wild type α -synuclein is degraded by chaperone mediated autophagy and macroautophagy in neuronal cells. *J Biol Chem* 2008;**283**:23542–56. Au5
303. Stefanis L, Larsen KE, Rideout HJ, Sulzer D, Greene LA. Expression of A53T mutant but not wild-type alpha-synuclein in PC12 cells induces alterations of the ubiquitin-dependent degradation system, loss of dopamine release, and autophagic cell death. *J Neurosci* 2001;**21**:9549–60.
304. Tanaka Y, Engelender S, Igarashi S, Rao RK, Wanner T, Tanzi RE, et al. Inducible expression of mutant alpha-synuclein decreases proteasome activity and increases sensitivity to mitochondria-dependent apoptosis. *Hum Mol Genet* 2001;**10**:919–26.
305. Snyder H, Mensah K, Theisler C, Lee J, Matouschek A, Wolozin B. Aggregated and monomeric alpha-synuclein bind to the S6' proteasomal protein and inhibit proteasomal function. *J Biol Chem* 2003;**278**:11753–9.
306. Lindersson E, Beedholm R, Hojrup P, Moos T, Gai W, Hendil KB, et al. Proteasomal inhibition by alpha-synuclein filaments and oligomers. *J Biol Chem* 2004;**279**:12924–34.
307. Emmanouilidou E, Stefanis L, Vekrellis K. Cell-produced alpha-synuclein oligomers are targeted to, and impair, the 26S proteasome. *Neurobiol Aging* 2008;**31**:953–68. Au5
308. Zhang NY, Tang Z, Liu CW. Alpha-synuclein protofibrils inhibit 26S proteasome-mediated protein degradation understanding the cytotoxicity of protein protofibrils in neurodegenerative diseases pathogenesis. *J Biol Chem* 2008;**283**:20288–98.

309. Lee FK, Wong AK, Lee YW, Wan OW, Chan HY, Chung KK. The role of ubiquitin linkages on alpha-synuclein induced-toxicity in a *Drosophila* model of Parkinson's disease. *J Neurochem* 2009;**110**:208–19.
310. Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. *Science* 2004;**305**:1292–5.
311. Mak SK, McCormack AL, Manning-Bog AB, Cuervo AM, Di Monte DA. Lysosomal degradation of alpha-synuclein in vivo. *J Biol Chem* 2010;**285**:13621–9.
312. Martinez-Vicente M, Tallozy Z, Kaushik S, Massey AC, Mazzulli J, Mosharov EV, et al. Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. *J Clin Invest* 2008;**118**:777–88.
313. Xilouri M, Vogiatzi T, Vekrellis K, Park D, Stefanis L. Abberant alpha-synuclein confers toxicity to neurons in part through inhibition of chaperone-mediated autophagy. *PLoS One* 2009;**4**:e5515.
314. Ebrahimi-Fakhari D, Cantuti-Castelvetri I, Fan Z, Rockenstein E, Masliah E, Hyman BT, et al. Distinct roles in vivo for the ubiquitin-proteasome system and the autophagy-lysosomal pathway in the degradation of {alpha}-synuclein. *J Neurosci* 2011;**31**:14508–20.
315. Winslow AR, Chen CW, Corrochano S, Acevedo-Arozena A, Gordon DE, Peden AA, et al. alpha-Synuclein impairs macroautophagy: implications for Parkinson's disease. *J Cell Biol* 2010;**190**:1023–37.
316. Yu WH, Dorado B, Figueroa HY, Wang L, Planel E, Cookson MR, et al. Metabolic activity determines efficacy of macroautophagic clearance of pathological oligomeric alpha-synuclein. *Am J Pathol* 2009;**175**:736–47.
317. Sarkar S, Davies JE, Huang Z, Tunnacliffe A, Rubinsztein DC. Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and {alpha}-synuclein. *J Biol Chem* 2007;**282**:5641–52.
318. Sarkar S, Floto RA, Berger Z, Imarisio S, Cordenier A, Pasco M, et al. Lithium induces autophagy by inhibiting inositol monophosphatase. *J Cell Biol* 2005;**170**:1101–11.
319. Sarkar S, Perlstein EO, Imarisio S, Pineau S, Cordenier A, Maglathlin RL, et al. Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. *Nat Chem Biol* 2007;**3**:331–8.
320. Crews L, Spencer B, Desplats P, Patrick C, Paulino A, Rockenstein E, et al. Selective molecular alterations in the autophagy pathway in patients with Lewy body disease and in models of alpha-synucleinopathy. *PLoS One* 2010;**5**:e9313.
321. Riedel M, Goldbaum O, Schwarz L, Schmitt S, Richter-Landsberg C. 17-AAG induces cytoplasmic alpha-synuclein aggregate clearance by induction of autophagy. *PLoS One* 2010;**5**:e8753.
322. Ishikawa A, Tsuji S. Clinical analysis of 17 patients in 12 Japanese families with autosomal-recessive type juvenile parkinsonism. *Neurology* 1996;**47**:160–6.
323. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 1998;**392**:605–8.
324. van Nuenen BF, Weiss MM, Bloem BR, Reetz K, van Eimeren T, Lohmann K, et al. Heterozygous carriers of a Parkin or PINK1 mutation share a common functional endophenotype. *Neurology* 2009;**72**:1041–7.
325. Wang C, Ma H, Feng X, Xie S, Chan P. Parkin dosage mutations in patients with early-onset sporadic and familial Parkinson's disease in Chinese: an independent pathogenic role. *Brain Res* 2010;**1358**:30–8.
326. Deshaies RJ, Joazeiro CA. RING domain E3 ubiquitin ligases. *Annu Rev Biochem* 2009;**78**:399–434.
327. Shimura H, Hattori N, Kubo S, Mizuno Y, Asakawa S, Minoshima S, et al. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nat Genet* 2000;**25**:302–5.

Au6

328. Zhang Y, Gao J, Chung KK, Huang H, Dawson VL, Dawson TM. Parkin functions as an E2-dependent ubiquitin-protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1. *Proc Natl Acad Sci USA* 2000;**97**:13354–9.
329. Imai Y, Soda M, Takahashi R. Parkin suppresses unfolded protein stress-induced cell death through its E3 ubiquitin-protein ligase activity. *J Biol Chem* 2000;**275**:35661–4.
330. West AB, Dawson VL, Dawson TM. The role of Parkin in Parkinson's disease. In: Dawson TM, editor. *Parkinson's disease: genetics and pathogenesis*. New York: Informa Healthcare; 2007. pp. 199–218.
331. Dawson TM, Dawson VL. The role of parkin in familial and sporadic Parkinson's disease. *Mov Disord* 2010;**25**(Suppl. 1):S32–9.
332. de la Torre ER, Gomez-Suaga P, Martinez-Salvador M, Hilfiker S. Posttranslational modifications as versatile regulators of parkin function. *Curr Med Chem* 2011;**18**:2477–85.
333. Winklhofer KF, Henn IH, Kay-Jackson PC, Heller U, Tatzelt J. Inactivation of parkin by oxidative stress and C-terminal truncations: a protective role of molecular chaperones. *J Biol Chem* 2003;**278**:47199–208.
334. Chung KK, Thomas B, Li X, Pletnikova O, Troncoso JC, Marsh L, et al. S-nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function. *Science* 2004;**304**:1328–31.
335. LaVoie MJ, Ostaszewski BL, Weihofen A, Schlossmacher MG, Selkoe DJ. Dopamine covalently modifies and functionally inactivates parkin. *Nat Med* 2005;**11**:1214–21.
336. Meng F, Yao D, Shi Y, Kabakoff J, Wu W, Reicher J, et al. Oxidation of the cysteine-rich regions of parkin perturbs its E3 ligase activity and contributes to protein aggregation. *Mol Neurodegener* 2011;**6**:34.
337. Moszczynska A, Yamamoto BK. Methamphetamine oxidatively damages parkin and decreases the activity of 26S proteasome in vivo. *J Neurochem* 2011;**116**:1005–17. [Au6]
338. Wong ES, Tan JM, Wang C, Zhang Z, Tay SP, Zaiden N, et al. Relative sensitivity of parkin and other cysteine-containing enzymes to stress-induced solubility alterations. *J Biol Chem* 2007;**282**:12310–8.
339. Kawahara K, Hashimoto M, Bar-On P, Ho GJ, Crews L, Mizuno H, et al. alpha-Synuclein aggregates interfere with Parkin solubility and distribution: role in the pathogenesis of Parkinson disease. *J Biol Chem* 2008;**283**:6979–87.
340. Corti O, Hampe C, Koutnikova H, Darios F, Jacquier S, Prigent A, et al. The p38 subunit of the aminoacyl-tRNA synthetase complex is a Parkin substrate: linking protein biosynthesis and neurodegeneration. *Hum Mol Genet* 2003;**12**:1427–37.
341. Ko HS, von Coelln R, Sriram SR, Kim SW, Chung KK, Pletnikova O, et al. Accumulation of the authentic parkin substrate aminoacyl-tRNA synthetase cofactor, p38/JTV-1, leads to catecholaminergic cell death. *J Neurosci* 2005;**25**:7968–78.
342. Ko HS, Kim SW, Sriram SR, Dawson VL, Dawson TM. Identification of far upstream element-binding protein-1 as an authentic Parkin substrate. *J Biol Chem* 2006;**281**:16193–6.
343. Shin JH, Ko HS, Kang H, Lee Y, Lee YI, Pletnikova O, et al. PARIS (ZNF746) repression of PGC-1alpha contributes to neurodegeneration in Parkinson's disease. *Cell* 2011;**144**:689–702.
344. Zheng B, Liao Z, Locascio JJ, Lesniak KA, Roderick SS, Watt ML, et al. PGC-1alpha, a potential therapeutic target for early intervention in Parkinson's disease. *Sci Transl Med* 2010;**2**:52ra73. [Au6]
345. Pacelli C, De Rasmio D, Signorile A, Grattagliano I, di Tullio G, D'Orazio A, et al. Mitochondrial defect and PGC-1alpha dysfunction in parkin-associated familial Parkinson's disease. *Biochim Biophys Acta* 2011;**1812**:1041–53.
346. Finck BN, Kelly DP. PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. *J Clin Invest* 2006;**116**:615–22.

347. St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jager S, et al. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* 2006;**127**:397–408.
348. Wu Z, Boss O. Targeting PGC-1 alpha to control energy homeostasis. *Expert Opin Ther Targets* 2007;**11**:1329–38.
349. Lin JD. Minireview: the PGC-1 coactivator networks: chromatin-remodeling and mitochondrial energy metabolism. *Mol Endocrinol* 2009;**23**:2–10.
350. Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, et al. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol* 2010;**12**:119–31.
351. Chew KC, Matsuda N, Saisho K, Lim GG, Chai C, Tan HM, et al. Parkin mediates apparent e2-independent monoubiquitination in vitro and contains an intrinsic activity that catalyzes polyubiquitination. *PLoS One* 2011;**6**:e19720.
352. Mukhopadhyay D, Riezman H. Proteasome-independent functions of ubiquitin in endocytosis and signaling. *Science* 2007;**315**:201–5.
353. Komander D. The emerging complexity of protein ubiquitination. *Biochem Soc Trans* 2009;**37**:937–53.
354. Sakata E, Yamaguchi Y, Kurimoto E, Kikuchi J, Yokoyama S, Yamada S, et al. Parkin binds the Rpn10 subunit of 26S proteasomes through its ubiquitin-like domain. *EMBO Rep* 2003;**4**:301–6.
355. Um JW, Im E, Lee HJ, Min B, Yoo L, Yoo J, et al. Parkin directly modulates 26S proteasome activity. *J Neurosci* 2010;**30**:11805–14.
356. Petrucelli L, O'Farrell C, Lockhart PJ, Baptista M, Kehoe K, Vink L, et al. Parkin protects against the toxicity associated with mutant alpha-synuclein: proteasome dysfunction selectively affects catecholaminergic neurons. *Neuron* 2002;**36**:1007–19.
357. Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* 2004;**304**:1158–60.
358. Samaranch L, Lorenzo-Betancor O, Arbelo JM, Ferrer I, Lorenzo E, Irigoyen J, et al. PINK1-linked parkinsonism is associated with Lewy body pathology. *Brain* 2010;**133**:1128–42.
359. Farrer M, Chan P, Chen R, Tan L, Lincoln S, Hernandez D, et al. Lewy bodies and parkinsonism in families with parkin mutations. *Ann Neurol* 2001;**50**:293–300.
360. Pramstaller PP, Schlossmacher MG, Jacques TS, Scaravilli F, Eskelson C, Pepivani I, et al. Lewy body Parkinson's disease in a large pedigree with 77 Parkin mutation carriers. *Ann Neurol* 2005;**58**:411–22.
361. Dagda RK, Zhu J, Chu CT. Mitochondrial kinases in Parkinson's disease: converging insights from neurotoxin and genetic models. *Mitochondrion* 2009;**9**:289–98.
362. Guo M. What have we learned from Drosophila models of Parkinson's disease? *Prog Brain Res* 2010;**184**:3–16.
363. Whitworth AJ, Pallanck LJ. The PINK1/Parkin pathway: a mitochondrial quality control system? *J Bioenerg Biomembr* 2009;**41**:499–503.
364. Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, Pallanck LJ. Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. *Proc Natl Acad Sci USA* 2003;**100**:4078–83.
365. Pesah Y, Pham T, Burgess H, Middlebrooks B, Verstreken P, Zhou Y, et al. Drosophila parkin mutants have decreased mass and cell size and increased sensitivity to oxygen radical stress. *Development* 2004;**131**:2183–94.
366. Whitworth AJ, Theodore DA, Greene JC, Benes H, Wes PD, Pallanck LJ. Increased glutathione S-transferase activity rescues dopaminergic neuron loss in a Drosophila model of Parkinson's disease. *Proc Natl Acad Sci USA* 2005;**102**:8024–9.

Au6

367. Palacino JJ, Sagi D, Goldberg MS, Krauss S, Motz C, Wacker M, et al. Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. *J Biol Chem* 2004;**279**:18614–22.
368. Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Seol JH, et al. Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature* 2006;**441**:1162–6.
369. Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, et al. Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. *Nature* 2006;**441**:1157–61.
370. Yang Y, Gehrke S, Imai Y, Huang Z, Ouyang Y, Wang JW, et al. Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of Drosophila Pink1 is rescued by Parkin. *Proc Natl Acad Sci USA* 2006;**103**:10793–8.
371. Yun J, Cao JH, Dodson MW, Clark IE, Kapahi P, Chowdhury RB, et al. Loss-of-function analysis suggests that Omi/HtrA2 is not an essential component of the PINK1/PARKIN pathway in vivo. *J Neurosci* 2008;**28**:14500–10.
372. Ibanez P, Lesage S, Lohmann E, Thobois S, De Michele G, Borg M, et al. Mutational analysis of the PINK1 gene in early-onset parkinsonism in Europe and North Africa. *Brain* 2006;**129**:686–94.
373. Kitada T, Tong Y, Gautier CA, Shen J. Absence of nigral degeneration in aged parkin/DJ-1/PINK1 triple knockout mice. *J Neurochem* 2009;**111**:696–702.
374. Muftuoglu M, Elilib B, Dalmizrak O, Ercan A, Kulaksiz G, Ogus H, et al. Mitochondrial complex I and IV activities in leukocytes from patients with parkin mutations. *Mov Disord* 2004;**19**:544–8.
375. Hoepken HH, Gispert S, Morales B, Wingerter O, Del Turco D, Mulsch A, et al. Mitochondrial dysfunction, peroxidation damage and changes in glutathione metabolism in PARK6. *Neurobiol Dis* 2007;**25**:401–11.
376. Stichel CC, Zhu XR, Bader V, Linnartz B, Schmidt S, Lubbert H. Mono- and double-mutant mouse models of Parkinson's disease display severe mitochondrial damage. *Hum Mol Genet* 2007;**16**:2377–93.
377. Exner N, Treske B, Paquet D, Holmstrom K, Schiesling C, Gispert S, et al. Loss-of-function of human PINK1 results in mitochondrial pathology and can be rescued by parkin. *J Neurosci* 2007;**27**:12413–8.
378. Gautier CA, Kitada T, Shen J. Loss of PINK1 causes mitochondrial functional defects and increased sensitivity to oxidative stress. *Proc Natl Acad Sci USA* 2008;**105**:11364–9.
379. Wood-Kaczmar A, Gandhi S, Yao Z, Abramov AY, Miljan EA, Keen G, et al. PINK1 is necessary for long term survival and mitochondrial function in human dopaminergic neurons. *PLoS One* 2008;**3**:e2455.
380. Poole AC, Thomas RE, Andrews LA, McBride HM, Whitworth AJ, Pallanck LJ. The PINK1/Parkin pathway regulates mitochondrial morphology. *Proc Natl Acad Sci USA* 2008;**105**:1638–43.
381. Gegg ME, Cooper JM, Schapira AH, Taanman JW. Silencing of PINK1 expression affects mitochondrial DNA and oxidative phosphorylation in dopaminergic cells. *PLoS One* 2009;**4**:e4756.
382. Sandebring A, Thomas KJ, Beilina A, van der Brug M, Cleland MM, Ahmad R, et al. Mitochondrial alterations in PINK1 deficient cells are influenced by calcineurin-dependent dephosphorylation of dynamin-related protein 1. *PLoS One* 2009;**4**:e5701.
383. Dagda RK, Cherra 3rd SJ, Kulich SM, Tandon A, Park D, Chu CT. Loss of PINK1 function promotes mitophagy through effects on oxidative stress and mitochondrial fission. *J Biol Chem* 2009;**284**:13843–55.
384. Morais VA, Verstreken P, Roethig A, Smet J, Snellinx A, Vanbrabant M, et al. Parkinson's disease mutations in PINK1 result in decreased Complex I activity and deficient synaptic function. *EMBO Mol Med* 2009;**1**:99–111.

385. Grunewald A, Voges L, Rakovic A, Kasten M, Vandebona H, Hemmelmann C, et al. Mutant Parkin impairs mitochondrial function and morphology in human fibroblasts. *PLoS One* 2010;**5**:e12962.
386. Shim JH, Yoon SH, Kim KH, Han JY, Ha JY, Hyun DH, et al. The antioxidant Trolox helps recovery from the familial Parkinson's disease-specific mitochondrial deficits caused by PINK1- and DJ-1-deficiency in dopaminergic neuronal cells. *Mitochondrion* 2011;**11**:707–15. Au5
387. Billia F, Hauck L, Konecny F, Rao V, Shen J, Mak TW. PTEN-inducible kinase 1 (PINK1)/Park6 is indispensable for normal heart function. *Proc Natl Acad Sci USA* 2011;**108**:9572–7. Au6
388. Schmidt S, Linnartz B, Mendritzki S, Szczepan T, Lubbert M, Stichel CC, et al. Genetic mouse models for Parkinson's disease display severe pathology in glial cell mitochondria. *Hum Mol Genet* 2011;**20**:1197–211. Au6
389. Chen H, Chan DC. Mitochondrial dynamics—fusion, fission, movement, and mitophagy—in neurodegenerative diseases. *Hum Mol Genet* 2009;**18**:R169–76.
390. Gandre-Babbe S, van der Blik AM. The novel tail-anchored membrane protein Mff controls mitochondrial and peroxisomal fission in mammalian cells. *Mol Biol Cell* 2008;**19**:2402–12.
391. Otera H, Wang C, Cleland MM, Setoguchi K, Yokota S, Youle RJ, et al. Mff is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells. *J Cell Biol* 2011;**191**:1141–58.
392. Fuller MT. Spermatogenesis. In: Martinez-Arias A, Bate M, editors. *The development of Drosophila melanogaster*. Cold Spring Harbor, NY: Cold Spring Harbor Press; 1993. pp. 71–147.
393. Deng H, Dodson MW, Huang H, Guo M. The Parkinson's disease genes pink1 and parkin promote mitochondrial fission and/or inhibit fusion in Drosophila. *Proc Natl Acad Sci USA* 2008;**105**:14503–8.
394. Riparbelli MG, Callaini G. The Drosophila parkin homologue is required for normal mitochondrial dynamics during spermiogenesis. *Dev Biol* 2007;**303**:108–20.
395. Yang Y, Ouyang Y, Yang L, Beal MF, McQuibban A, Vogel H, et al. Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery. *Proc Natl Acad Sci USA* 2008;**105**:7070–5.
396. Park J, Lee G, Chung J. The PINK1-Parkin pathway is involved in the regulation of mitochondrial remodeling process. *Biochem Biophys Res Commun* 2009;**378**:518–23.
397. Mortiboys H, Thomas KJ, Koopman WJ, Klaffke S, Abou-Sleiman P, Olpin S, et al. Mitochondrial function and morphology are impaired in parkin-mutant fibroblasts. *Ann Neurol* 2008;**64**:555–65.
398. Yu W, Sun Y, Guo S, Lu B. The PINK1/Parkin pathway regulates mitochondrial dynamics and function in mammalian hippocampal and dopaminergic neurons. *Hum Mol Genet* 2011;**20**:3227–40. Au7
399. Lutz AK, Exner N, Fett ME, Schlehe JS, Kloos K, Lammermann K, et al. Loss of parkin or PINK1 function increases Drp1-dependent mitochondrial fragmentation. *J Biol Chem* 2009;**284**:22938–51.
400. Dimmer KS, Fritz S, Fuchs F, Messerschmitt M, Weimbach N, Neupert W, et al. Genetic basis of mitochondrial function and morphology in *Saccharomyces cerevisiae*. *Mol Biol Cell* 2002;**13**:847–53.
401. Altmann K, Westermann B. Role of essential genes in mitochondrial morphogenesis in *Saccharomyces cerevisiae*. *Mol Biol Cell* 2005;**16**:5410–7.
402. Ichishita R, Tanaka K, Sugiura Y, Sayano T, Mihara K, Oka T. An RNAi screen for mitochondrial proteins required to maintain the morphology of the organelle in *Caenorhabditis elegans*. *J Biochem* 2008;**143**:449–54.
403. Goldman SJ, Taylor R, Zhang Y, Jin S. Autophagy and the degradation of mitochondria. *Mitochondrion* 2010;**10**:309–15.

404. Twig G, Shirihai OS. The interplay between mitochondrial dynamics and mitophagy. *Antioxid Redox Signal* 2011;**14**:1939–51.
405. Hyde BB, Twig G, Shirihai OS. Organellar vs cellular control of mitochondrial dynamics. *Semin Cell Dev Biol* 2010;**21**:575–81.
406. Twig G, Graf SA, Wikstrom JD, Mohamed H, Haigh SE, Elorza A, et al. Tagging and tracking individual networks within a complex mitochondrial web with photoactivatable GFP. *Am J Physiol Cell Physiol* 2006;**291**:C176–84.
407. Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, et al. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J* 2008;**27**:433–46.
408. Acin-Perez R, Hoyos B, Zhao F, Vinogradov V, Fischman DA, Harris RA, et al. Control of oxidative phosphorylation by vitamin A illuminates a fundamental role in mitochondrial energy homeostasis. *FASEB J* 2010;**24**:627–36.
409. Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, et al. PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. *J Cell Biol* 2010;**189**:211–21.
410. Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, et al. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol* 2010;**8**:e1000298.
411. Vives-Bauza C, Zhou C, Huang Y, Cui M, de Vries RL, Kim J, et al. PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proc Natl Acad Sci USA* 2010;**107**:378–83.
412. Rakovic A, Grunewald A, Seibler P, Ramirez A, Kock N, Orolicki S, et al. Effect of endogenous mutant and wild-type PINK1 on Parkin in fibroblasts from Parkinson disease patients. *Hum Mol Genet* 2010;**19**:3124–37.
413. Ziviani E, Tao RN, Whitworth AJ. Drosophila parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. *Proc Natl Acad Sci USA* 2010;**107**:5018–23.
414. Kawajiri S, Saiki S, Sato S, Sato F, Hatano T, Eguchi H, et al. PINK1 is recruited to mitochondria with parkin and associates with LC3 in mitophagy. *FEBS Lett* 2010;**584**:1073–9.
415. Van Laar VS, Arnold B, Cassady SJ, Chu CT, Burton EA, Berman SB. Bioenergetics of neurons inhibit the translocation response of Parkin following rapid mitochondrial depolarization. *Hum Mol Genet* 2011;**20**:927–40.
416. Seibler P, Graziotto J, Jeong H, Simunovic F, Klein C, Krainc D. Mitochondrial Parkin recruitment is impaired in neurons derived from mutant PINK1 induced pluripotent stem cells. *J Neurosci* 2011;**31**:5970–6.
417. Zhou C, Huang Y, Shao Y, May J, Prou D, Perier C, et al. The kinase domain of mitochondrial PINK1 faces the cytoplasm. *Proc Natl Acad Sci USA* 2008;**105**:12022–7.
418. Whitworth AJ, Lee JR, Ho VM, Flick R, Chowdhury R, McQuibban GA. Rhomboid-7 and HtrA2/Omi act in a common pathway with the Parkinson's disease factors Pink1 and Parkin. *Dis Model Mech* 2008;**1**:168–74 [discussion 173].
419. McQuibban GA, Lee JR, Zheng L, Juusola M, Freeman M. Normal mitochondrial dynamics requires rhomboid-7 and affects Drosophila lifespan and neuronal function. *Curr Biol* 2006;**16**:982–9.
420. Jin SM, Lazarou M, Wang C, Kane LA, Narendra DP, Youle RJ. Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. *J Cell Biol* 2010;**191**:933–42.
421. Deas E, Plun-Favreau H, Gandhi S, Desmond H, Kjaer S, Loh SH, et al. PINK1 cleavage at position A103 by the mitochondrial protease PARL. *Hum Mol Genet* 2011;**20**:867–79.
422. Shi G, Lee JR, Grimes DA, Racacho L, Ye D, Yang H, et al. Functional alteration of PARL contributes to mitochondrial dysregulation in Parkinson's disease. *Hum Mol Genet* 2011;**20**:1966–74.

423. Meissner C, Lorenz H, Weihofen A, Selkoe DJ, Lemberg MK. The mitochondrial intramembrane protease PARL cleaves human Pink1 to regulate Pink1 trafficking. *J Neurochem* 2011;**117**:856–67.
424. Haucke V, Ocana CS, Honlinger A, Tokatlidis K, Pfanner N, Schatz G. Analysis of the sorting signals directing NADH-cytochrome b5 reductase to two locations within yeast mitochondria. *Mol Cell Biol* 1997;**17**:4024–32.
425. Gegg ME, Cooper JM, Chau KY, Rojo M, Schapira AH, Taanman JW. Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy. *Hum Mol Genet* 2010;**19**:4861–70.
426. Poole AC, Thomas RE, Yu S, Vincow ES, Pallanck L. The mitochondrial fusion-promoting factor mitofusin is a substrate of the PINK1/parkin pathway. *PLoS One* 2010;**5**:e10054.
427. Tanaka A, Cleland MM, Xu S, Narendra DP, Suen DF, Karbowski M, et al. Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. *J Cell Biol* 2011;**191**:1367–80.
428. Chan NC, Salazar AM, Pham AH, Sweredoski MJ, Kolawa NJ, Graham RL, et al. Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. *Hum Mol Genet* 2011;**20**:1726–37.
429. Glauser L, Sonnay S, Stafa K, Moore DJ. Parkin promotes the ubiquitination and degradation of the mitochondrial fusion factor mitofusin 1. *J Neurochem* 2011;**118**:636–45. Au5
430. Rakovic A, Grunewald A, Kottwitz J, Bruggemann N, Pramstaller PP, Lohmann K, et al. Mutations in PINK1 and Parkin impair ubiquitination of Mitofusins in human fibroblasts. *PLoS One* 2011;**6**:e16746.
431. Chen D, Gao F, Li B, Wang H, Xu Y, Zhu C, et al. Parkin mono-ubiquitinates Bcl-2 and regulates autophagy. *J Biol Chem* 2010;**285**:38214–23. Au6
432. Yoshii SR, Kishi C, Ishihara N, Mizushima N. Parkin mediates proteasome-dependent protein degradation and rupture of the outer mitochondrial membrane. *J Biol Chem* 2011;**286**:19630–40. Au5
433. Narendra D, Kane LA, Hauser DN, Fearnley IM, Youle RJ. p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. *Autophagy* 2010;**6**:1090–106.
434. Wang H, Song P, Du L, Tian W, Yue W, Liu M, et al. Parkin ubiquitinates Drp1 for proteasome-dependent degradation: implication of dysregulated mitochondrial dynamics in Parkinson disease. *J Biol Chem* 2011;**286**:11649–58.
435. Head B, Griparic L, Amiri M, Gandre-Babbe S, van der Bliek AM. Inducible proteolytic inactivation of OPA1 mediated by the OMA1 protease in mammalian cells. *J Cell Biol* 2009;**187**:959–66.
436. Raasi S, Wolf DH. Ubiquitin receptors and ERAD: a network of pathways to the proteasome. *Semin Cell Dev Biol* 2007;**18**:780–91.
437. Lee JY, Nagano Y, Taylor JP, Lim KL, Yao TP. Disease-causing mutations in parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy. *J Cell Biol* 2010;**189**:671–9. Au6
438. Ding WX, Ni HM, Li M, Liao Y, Chen X, Stolz DB, et al. Nix is critical to two distinct phases of mitophagy, reactive oxygen species-mediated autophagy induction and Parkin-ubiquitin-p62-mediated mitochondrial priming. *J Biol Chem* 2010;**285**:27879–90. Au6
439. Okatsu K, Saisho K, Shimanuki M, Nakada K, Shitara H, Sou YS, et al. p62/SQSTM1 cooperates with Parkin for perinuclear clustering of depolarized mitochondria. *Genes Cells* 2010;**15**:887–900.
440. Gandhi S, Wood-Kaczmar A, Yao Z, Plun-Favreau H, Deas E, Klupsch K, et al. PINK1-associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death. *Mol Cell* 2009;**33**:627–38.

441. Akundi RS, Huang Z, Eason J, Pandya JD, Zhi L, Cass WA, et al. Increased mitochondrial calcium sensitivity and abnormal expression of innate immunity genes precede dopaminergic defects in Pink1-deficient mice. *PLoS One* 2011;**6**:e16038.
442. Heeman B, Van den Haute C, Aelvoet SA, Valsecchi F, Rodenburg RJ, Reumers V, et al. Depletion of PINK1 affects mitochondrial metabolism, calcium homeostasis and energy maintenance. *J Cell Sci* 2011;**124**:1115–25.
443. Pridgeon JW, Olzmann JA, Chin LS, Li L. PINK1 protects against oxidative stress by phosphorylating mitochondrial chaperone TRAP1. *PLoS Biol* 2007;**5**:e172.
444. Beilina A, Van Der Brug M, Ahmad R, Kesavapany S, Miller DW, Petsko GA, et al. Mutations in PTEN-induced putative kinase 1 associated with recessive parkinsonism have differential effects on protein stability. *Proc Natl Acad Sci USA* 2005;**102**:5703–8.
445. Lin W, Kang UJ. Characterization of PINK1 processing, stability, and subcellular localization. *J Neurochem* 2008;**106**:464–74.
446. Takatori S, Ito G, Iwatsubo T. Cytoplasmic localization and proteasomal degradation of N-terminally cleaved form of PINK1. *Neurosci Lett* 2008;**430**:13–7.
447. Haque ME, Thomas KJ, D'Souza C, Callaghan S, Kitada T, Slack RS, et al. Cytoplasmic Pink1 activity protects neurons from dopaminergic neurotoxin MPTP. *Proc Natl Acad Sci USA* 2008;**105**:1716–21.
448. Lin W, Kang UJ. Structural determinants of PINK1 topology and dual subcellular distribution. *BMC Cell Biol* 2010;**11**:90.
449. Murata H, Sakaguchi M, Jin Y, Sakaguchi Y, Futami J, Yamada H, et al. A new cytosolic pathway from a Parkinson disease-associated kinase, BRPK/PINK1: activation of AKT via mTORC2. *J Biol Chem* 2011;**286**:7182–9.
450. Aleyasin H, Rousseaux MW, Marcogliese PC, Hewitt SJ, Irrcher I, Joselin AP, et al. DJ-1 protects the nigrostriatal axis from the neurotoxin MPTP by modulation of the AKT pathway. *Proc Natl Acad Sci USA* 2010;**107**:3186–91.
451. Xiong H, Wang D, Chen L, Choo YS, Ma H, Tang C, et al. Parkin, PINK1, and DJ-1 form a ubiquitin E3 ligase complex promoting unfolded protein degradation. *J Clin Invest* 2009;**119**:650–60.
452. Thomas KJ, McCoy MK, Blackinton J, Beilina A, van der Brug M, Sandebring A, et al. DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. *Hum Mol Genet* 2011;**20**:40–50.
453. Nagakubo D, Taira T, Kitauro H, Ikeda M, Tamai K, Iguchi-Ariga SM, et al. DJ-1, a novel oncogene which transforms mouse NIH3T3 cells in cooperation with ras. *Biochem Biophys Res Commun* 1997;**231**:509–13.
454. Wilson MA. The role of cysteine oxidation in DJ-1 function and dysfunction. *Antioxid Redox Signal* 2011;**15**:111–22.
455. Blackinton J, Lakshminarasimhan M, Thomas KJ, Ahmad R, Greggio E, Raza AS, et al. Formation of a stabilized cysteine sulfinic acid is critical for the mitochondrial function of the parkinsonism protein DJ-1. *J Biol Chem* 2009;**284**:6476–85.
456. Waak J, Weber SS, Gorner K, Schall C, Ichijo H, Stehle T, et al. Oxidizable residues mediating protein stability and cytoprotective interaction of DJ-1 with apoptosis signal-regulating kinase 1. *J Biol Chem* 2009;**284**:14245–57.
457. Zhang L, Shimoji M, Thomas B, Moore DJ, Yu SW, Marupudi NI, et al. Mitochondrial localization of the Parkinson's disease related protein DJ-1: implications for pathogenesis. *Hum Mol Genet* 2005;**14**:2063–73.
458. Ooe H, Taira T, Iguchi-Ariga SM, Ariga H. Induction of reactive oxygen species by bisphenol A and abrogation of bisphenol A-induced cell injury by DJ-1. *Toxicol Sci* 2005;**88**:114–26.

459. Blackinton J, Ahmad R, Miller DW, van der Brug MP, Canet-Aviles RM, Hague SM, et al. Effects of DJ-1 mutations and polymorphisms on protein stability and subcellular localization. *Brain Res Mol Brain Res* 2005;**134**:76–83.
460. Lev N, Ickowicz D, Melamed E, Offen D. Oxidative insults induce DJ-1 upregulation and redistribution: implications for neuroprotection. *Neurotoxicology* 2008;**29**:397–405.
461. Junn E, Jang WH, Zhao X, Jeong BS, Mouradian MM. Mitochondrial localization of DJ-1 leads to enhanced neuroprotection. *J Neurosci Res* 2009;**87**:123–9.
462. Hao LY, Giasson BI, Bonini NM. DJ-1 is critical for mitochondrial function and rescues PINK1 loss of function. *Proc Natl Acad Sci USA* 2010;**107**:9747–52.
463. Kriebieh G, Ruckerbauer S, Burbulla LF, Kieper N, Maurer B, Waak J, et al. Reduced basal autophagy and impaired mitochondrial dynamics due to loss of Parkinson's disease-associated protein DJ-1. *PLoS One* 2010;**5**:e9367.
464. Irrcher I, Aleyasin H, Seifert EL, Hewitt SJ, Chhabra S, Phillips M, et al. Loss of the Parkinson's disease-linked gene DJ-1 perturbs mitochondrial dynamics. *Hum Mol Genet* 2010;**19**:3734–46.
465. Goldberg MS, Fleming SM, Palacino JJ, Cepeda C, Lam HA, Bhatnagar A, et al. Parkinson-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. *J Biol Chem* 2003;**278**:43628–35.
466. Von Coelln R, Thomas B, Savitt JM, Lim KL, Sasaki M, Hess EJ, et al. Loss of locus coeruleus neurons and reduced startle in parkin null mice. *Proc Natl Acad Sci USA* 2004;**101**:10744–9.
467. Dauer W, Kholodilov N, Vila M, Trillat AC, Goodchild R, Larsen KE, et al. Resistance of alpha-synuclein null mice to the parkinsonian neurotoxin MPTP. *Proc Natl Acad Sci USA* 2002;**99**:14524–9.
468. Ellis CE, Murphy EJ, Mitchell DC, Golovko MY, Scaglia F, Barcelo-Coblijn GC, et al. Mitochondrial lipid abnormality and electron transport chain impairment in mice lacking alpha-synuclein. *Mol Cell Biol* 2005;**25**:10190–201.
469. Hsu LJ, Sagara Y, Arroyo A, Rockenstein E, Sisk A, Mallory M, et al. Alpha-synuclein promotes mitochondrial deficit and oxidative stress. *Am J Pathol* 2000;**157**:401–10.
470. Orth M, Tabrizi SJ, Schapira AH, Cooper JM. Alpha-synuclein expression in HEK293 cells enhances the mitochondrial sensitivity to rotenone. *Neurosci Lett* 2003;**351**:29–32.
471. Parihar MS, Parihar A, Fujita M, Hashimoto M, Ghafourifar P. Mitochondrial association of alpha-synuclein causes oxidative stress. *Cell Mol Life Sci* 2008;**65**:1272–84.
472. Smith WW, Jiang H, Pei Z, Tanaka Y, Morita H, Sawa A, et al. Endoplasmic reticulum stress and mitochondrial cell death pathways mediate A53T mutant alpha-synuclein-induced toxicity. *Hum Mol Genet* 2005;**14**:3801–11.
473. Shavali S, Brown-Borg HM, Ebadi M, Porter J. Mitochondrial localization of alpha-synuclein protein in alpha-synuclein overexpressing cells. *Neurosci Lett* 2008;**439**:125–8.
474. Nakamura K, Nemani VM, Wallender EK, Kaehlcke K, Ott M, Edwards RH. Optical reporters for the conformation of alpha-synuclein reveal a specific interaction with mitochondria. *J Neurosci* 2008;**28**:12305–17.
475. Nakamura K, Nemani VM, Azarbal F, Skibinski G, Levy JM, Egami K, et al. Direct membrane association drives mitochondrial fission by the Parkinson disease-associated protein [alpha]-synuclein. *J Biol Chem* 2011;**286**:20710–26.
476. Kamp F, Exner N, Lutz AK, Wender N, Hegemann J, Brunner B, et al. Inhibition of mitochondrial fusion by alpha-synuclein is rescued by PINK1, Parkin and DJ-1. *EMBO J* 2011;**29**:3571–89.
477. Devi L, Raghavendran V, Prabhu BM, Avadhani NG, Anandatheerthavarada HK. Mitochondrial import and accumulation of alpha-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. *J Biol Chem* 2008;**283**:9089–100.

478. Kowald A, Kirkwood TB. Evolution of the mitochondrial fusion-fission cycle and its role in aging. *Proc Natl Acad Sci USA* 2011;**108**:10237–42.
479. Chen H, Chan DC. Physiological functions of mitochondrial fusion. *Ann N Y Acad Sci* 2010;**1201**:21–5.
480. Cassidy-Stone A, Chipuk JE, Ingerman E, Song C, Yoo C, Kuwana T, et al. Chemical inhibition of the mitochondrial division dynamin reveals its role in Bax/Bak-dependent mitochondrial outer membrane permeabilization. *Dev Cell* 2008;**14**:193–204.

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