Parkin and α-synuclein: Opponent Actions in the Pathogenesis of Parkinson's Disease

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Dominant mutations in the gene for α-synuclein, a small presynaptic protein, can cause Parkinson's disease. Although there is still substantial debate about the precise mechanisms, α-synuclein is toxic to vulnerable neurons, probably as a result of its tendency to aggregate. Opposing this is another gene product that, when mutated, causes a recessive form of parkinsonism, parkin. Parkin has been recently shown to protect cells against α-synuclein toxicity. However, the precise details of the mechanism are unclear. This review will discuss the concept that there are multiple neuronal functions that are targeted by mutant α-synuclein, and in many cases, there is evidence that parkin can protect cells against damage to the same systems. The authors will also discuss ways in which to test some of these ideas, by using newly identified genes such as *DJ-1* that cause similar phenotypes. NEUROSCIENTIST 10(1):63–72, 2004. DOI: 10.1177/1073858403260392

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How do neurodegenerative conditions such as Alzheimer's disease or Parkinson's disease (PD) arise? In most cases, we have no concrete causal factor, and we tend to group these cases together as sporadic or idiopathic. This confounds our attempts to understand the disease process and limits our abilities to develop imaginative treatments. However, in the inherited forms of the same disorders, there is unambiguous causality. Generally, it holds that one copy of a dominant gene mutation or two copies of a recessive mutation are sufficient to evoke disease, although there are some wrinkles in this simple rubric. In this review, we will discuss PD, in which there are both dominant (*α-synuclein*) and recessive (*parkin, DJ-1)* genes that can cause similar if not identical phenotypes (see Table 1). The fundamental hypothesis driving much of the work described in this article is that by understanding the overlap in mechanisms between phenotypically similar genetic disorders, we may be able to delineate the complex pathways that lead to disease.

Details of the clinical phenotype of PD have been described elsewhere (Gwinn-Hardy 2002), but there are a few very salient features that should be appreciated (Table 1). First, there is substantial neuronal loss, particularly of dopaminergic neurons in the substantia nigra pars compacta (SNpc). As this region of the brain is critically involved in the initiation of movement, cellular damage in the SNpc is related to the cardinal symptoms of the disease: bradykinesia, resting tremor, muscular rigidity, and postural instability. The behavioral symptoms of PD do not manifest until about 80% of SNpc neurons are lost. However, neuronal loss is not limited to the SNpc, nor is it necessarily true that this region is the first affected. Neuronal damage becomes more widespread throughout many areas of the CNS during the course of the disease. Although loss of dopaminergic neurons is involved in the movement-related symptoms of the disease, PD is not limited to any specific transmitter phenotype (Braak and Braak 2000). Furthermore, some groups of dopaminergic neurons are relatively resistant to the disease process. The other major pathological event in PD is the presence of structures in the surviving neurons termed Lewy bodies and Lewy neurites (Braak and Braak 2000). Historically, staining with dyes such as eosin was used to identify Lewy-type pathology. We now know that Lewy bodies are accumulations of proteins and lipids within the cytosol. There appear to be many components of Lewy bodies, but prominently these include a protein that, when mutated, causes PD: α-synuclein.

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α-*synuclein* and *Parkin*: Genes That Cause PD

The first identification of a monogenic form of PD was the discovery by Polymeropoulos and others (1997) of dominant mutations in the *SNCA* gene, which encodes α-synuclein. This gene had been cloned some years earlier as an abundant synaptic protein and as a gene upregulated in zebra finches during song learning (Clayton and George 1998). These observations suggest that α-synuclein may play a role in synaptic plasticity, although the normal role(s) are still not fully elucidated. Transgenic mice lacking α-synuclein appear phenotypically normal, indicating that either α-synuclein is not required for normal growth or that there is some redun-

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Table 1. Phenotypic Characteristics of Parkinson's Disease Caused by Mutations in *α-synuclein*, *Parkin*, or *DJ-1**

	α-synuclein	Parkin	DJ-1
Inheritance	Dominant	Recessive	Recessive
Age of onset (years)	40-50	< 40	< 40
Number of mutations	2 (in 2 families)	> 100	2 (in 2 families)
Clinical phenotypes	Parkinsonism plus dementia	Variable; parkinsonism but often with prominent signs such as dystonia	Parkinsonism with psychiatric symptoms
Lewy pathology	Yes	No (except one case)	Unknown
Protein function	Regulation of vesicle release?	E3 protein-ubiquitin ligase	Multiple
Effects of mutations	Promote damaging protein aggregation	Decrease ability to ubiquitylate targets	Knockout or destabilize protein

*Since writing this paper, our laboratory has found a third mutation in the α-synuclein gene. This is a triplication of the wild type allele that is inherited in an autosomal dominant manner (Singleton 2003).

dancy in its function and another protein may accommodate the lack of α-synuclein (Abeliovich and others 2000). On a biochemical level, the mice display a reduction in striatal dopamine levels and increased dopamine-dependent response to amphetamine, supporting the hypothesis that α-synuclein is involved in presynaptic regulation of dopamine. There are a number of homologues (β- and γ-synucleins and synoretin) that make up a gene family of small proteins with high homology (Clayton and George 1998). The general structure of these proteins is to have an N-terminal domain that mediates lipid binding, a central "repeat" region, and an acidic C-terminal tail.

Subsequently, it has been shown that α-synuclein is one of a number of toxic proteins associated with neurodegenerative conditions in which mutations promote protein aggregation (Taylor and others 2002). In Alzheimer's disease, amyotrophic lateral sclerosis, and polyglutamine disorders such as Huntington's disease, there are proteins that aggregate and are present in pathological structures. The major pathological structures in PD, Lewy bodies, contain large amounts of α-synuclein. This is true for sporadic cases and those with α-synuclein mutations, which reinforces the similarity between inherited and noninherited forms of this disease. The aggregation of α-synuclein, which is mediated by sequences within the central repeat domain, is key to understanding PD. This is shown diagrammatically in Figure 1 and is discussed in greater detail below.

The first described mutations in *parkin* were described in a set of Japanese patients with autosomal recessive juvenile parkinsonism (Kitada and others 1998). However, *parkin* mutations have been found in many cases, including some phenotypes indistinguishable from idiopathic PD. A salient difference is that whereas autosomal recessive juvenile parkinsonism patients display many of the clinical features of idiopathic PD, most individuals with homozygous mutations in parkin lack Lewy bodies.

The protein encoded by parkin is an E3 ubiquitin ligase (see Fig. 2), one of a number of enzymes that controls protein degradation by the proteasome (reviewed in Cookson 2003). E3 ligases catalyze the addition of a chain of ubiquitin molecules (ubiquitylation) to the target before it can be degraded. Generally, mutations in parkin impair its E3 ligase function. Parkin is neuroprotective in a number of different model systems and, importantly, can protect against α-synuclein toxicity in vitro (Petrucelli and others 2002) and in vivo (Yang and others 2003). This leads to the concept that parkin and α-synuclein both affect cell survival pathways that may involve the toxicity of cellular proteins. These "opponent actions" will be discussed along with those mechanistic details that have been identified to date.

Opponent Actions of α-synuclein and Parkin

There are a number of model systems that show that overexpression of α-synuclein can damage certain vulnerable types of neurons. These range from in vitro studies to both invertebrate and vertebrate systems. Transgenic animals have been engineered with the aim to mimic the features of PD due to either dominant mutations in α-synuclein or a lack of parkin. Mice, *Drosophila*, and *C. elegans* have all been used to this end (for review, see Lotharius and Brundin 2002).

There are some inconsistencies, but in general mutant α-synuclein is more toxic than wild-type human protein, and rodent homologues are generally nontoxic. The critical dependency of protein concentration has been elegantly illustrated by experiments using inducible promoters to drive expression of α-synuclein variants in human cell lines (Xu and others 2002). Although mutant α-synuclein is toxic at lower expression levels, the wild-type protein becomes equally toxic as protein levels increase. This demonstrates that the difference between wild-type and mutant α-synuclein is a quantitative rather than qualitative one. However, it also shows that α-synuclein mutations work by a gain of toxic function. The properties that make α-synuclein toxic are present in the wild-type protein but are exaggerated in the mutant forms, leading to greatly increased incidence of disease.

But what are the properties of α-synuclein that make it toxic? To explain this, we need to understand the nature of this protein and its conformational flexibility.

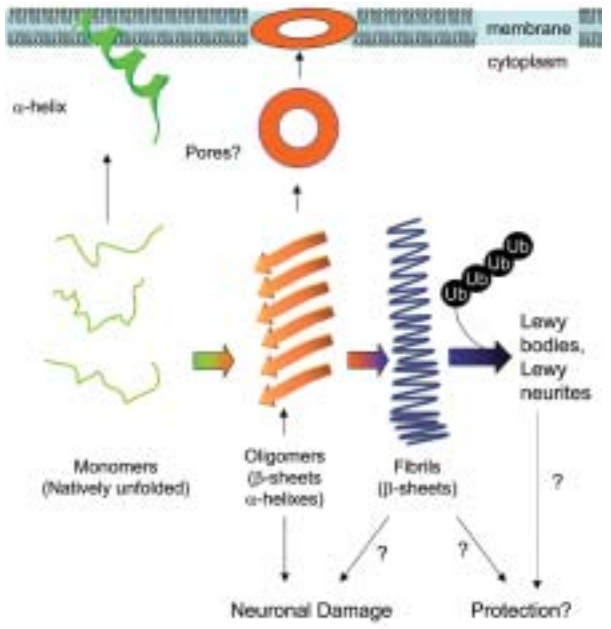

Fig. 1. The many structural faces of α-synuclein. Monomeric α-synuclein (green) is natively unfolded, although it can interact with the surface of lipid membranes by forming α-helices. A transition to oligomeric species stabilized by β-sheets, as shown, or α-helices is associated with neurotoxicity. Annular pores can also be formed, which may insert into the membrane. Oligomers may also convert to fibrillar species, also stabilized by β-sheets but much larger and less soluble, that are the presumed substrate for formation of Lewy bodies and Lewy neurites. The decrease in solubility due to increasing aggregation is represented by darker colors (from green to black on the arrows). Whether fibrils are damaging to cells or protective is unclear. Adapted in part from Volles and Lansbury (2003) and Uversky (2003).

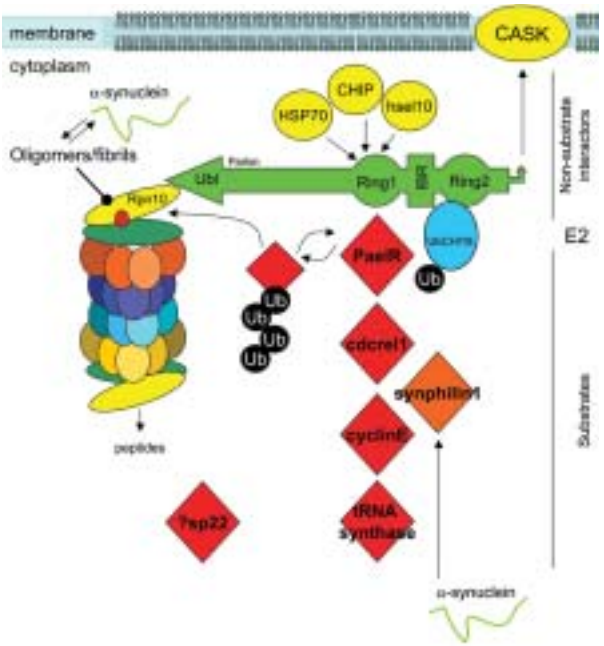

Fig. 2. Parkin and the ubiquitin-proteasome system. Parkin, drawn in green, has a distinct structure with an ubiquitin-like domain (Ubl) at the N-terminus and two RING fingers at the C-terminus separated by an in-between ring (IBR) domain. Parkin binds to a number of substrates—red diamonds aligned approximately with the domains that they are thought to bind. Note that the glycosylated α-synuclein species, sp22, is separated from the other putative substrates, which mainly bind at RING1. Synphilin-1, one of the proposed parkin substrates, is also reported to bind to α-synuclein. The RING-IBR-RING region also binds several nonsubstrate interactors (yellow), including the 70kDa heat shock protein (Hsp70), the C-terminal Hsp interacting protein (CHIP), and the human homologue of sel10 (hsel10). Other nonsubstrate interactors include the membrane protein CASK/lin10, which interacts with the extreme C-terminus of parkin, and a protein in the proteasome cap, Rpn10. The N-terminal Ubl domain appears to mediate binding of parkin to the proteasome. RING2 is responsible for recruitment of E2 enzymes (blue), which carry activated ubiquitin. Several rounds of addition of ubiquitin result in the addition of a polyubiquitin chain on the substrate, which is then degraded by the proteasome. Mutant and, to a lesser extent, wild-type α-synuclein also inhibit the proteasome; although a monomer of α-synuclein is shown for clarity, it is not clear which form of this protein mediates inhibition. Adapted from Cookson (2003).

In its soluble form, α-synuclein is natively unfolded, having little or no ordered structure that can be readily detected (reviewed in detail in Uversky 2003). The protein can adopt a more structured helical form when it binds to lipids, such as presynaptic vesicles, where a proportion of the protein is found in the brain. As stated above, α-synuclein is also found in Lewy bodies and other pathological structures in the PD brain (Braak and Braak 2000). Lewy bodies are characterized at the ultrastructural level by the presence of fibrillar material, and, by inference, α-synuclein is likely to be the major building block of these fibrils. Although this has not been formally proven, α-synuclein can be induced to form similar structures in the test tube (Volles and Lansbury 2003). Such fibrils formed in vitro are stabilized by intermolecular β-sheets and are much more ordered than the monomeric form. Thus, α-synuclein is probably in a heavily aggregated and relatively insoluble form in the disease state. Interestingly, β- and γ-synucleins are resistant to aggregation, probably due to sequence differences in the central domain of the proteins. An outline of the pathways of aggregation of α-synuclein is shown in Figure 1.

However, there is some evidence that fibrils are not the toxic species in PD. The formation of insoluble fibrils is not a common effect of both mutations; although A53T can promote fibril formation, A30P in fact retards this effect. Both A30P and A53T mutations promote the formation of relatively small and soluble aggregated forms referred to as oligomers or protofibrils. From this, it can be inferred that oligomers or protofibrils rather than fibrils mediate toxicity to neurons (Volles and Lansbury 2003). In support of this idea, toxicity of α-synuclein is correlated with the presence of relatively soluble species in cultured cells, whose molecular weight approximates small oligomers (Xu and others 2002). There is evidence of oligomeric species of α-synuclein in membrane fractions from human brain (Sharon and others 2003). Furthermore, the presence of lipids promotes the rate of oligomer formation.

One interpretation of these observations is that Lewy bodies could be beneficial to neurons, moving the partially aggregated species into a relatively safe and sequestered form. However, formation of sufficient amounts of fibrillar, insoluble α-synuclein deposits is associated with damage to specific groups of neurons in the spinal cord of two different lines of transgenic mice (Lotharius and Brundin 2002 and references therein). Hence, there is still debate about whether soluble oligomeric species, fibrils, or both cause neuronal damage in PD.

A refinement to the oligomer hypothesis comes from observations in vitro of porelike structures formed when α-synuclein is incubated with membranes (Volles and Lansbury 2003). The presence of a pore in close juxtaposition to cellular membranes (Fig. 1) would be predicted to have a number of deleterious effects, depending on which membrane system in the cell was damaged. For example, damage at the plasma membrane might decrease the ability of the cell to maintain a homeostatic environment. If such pores formed on presynaptic vesicles, damage to regulated neurotransmitter release might result (see below). However, whether such porelike structures are actually formed in the context of the whole cell remains to be proven.

Derived from the above considerations is the concept that multiple neuronal functions might be affected by mutations in α-synuclein. Once α-synuclein begins to aggregate (whether into oligomers or fibrils), there are multiple downstream targets, and there may be additive effects of disruptions in several pathways that could severely damage neurons. Later sections of this article will discuss synaptic transmission, protein degradation, mitochondrial function, and gene expression and, thus, will explicitly address the concept that α-synuclein has multiple toxicities at the subcellular level.

What is the role of parkin in these schemes? As stated above, parkin protects against α-synuclein toxicity, along with other stresses. A simple hypothesis might be that α-synuclein is a substrate for parkin-mediated degradation. Shimura and others (2001) described an *O*-glycosylated form of α-synuclein (αSP22) that can be ubiquitylated by parkin. This result, however, has not been replicated in other labs, and the relevance of αSP22 to PD remains to be elucidated. Parkin does not regulate the steady-state levels of the bulk of monomeric α-synuclein in most model systems. There is the possibility that an adaptor protein may be involved. Synphilin-1 has been shown to be associated with α-synuclein and reported to be a target for parkin's E3 ligase activity, although whether its turnover is increased has not been demonstrated (Chung and others 2001). Overall, the balance of evidence that parkin protects α-synuclein toxicity by controlling steady-state α-synuclein levels is weak, although it is possible that there are specifics of α-synuclein turnover or aggregation that have not yet been addressed.

There are a number of other mechanisms that might explain parkin's ability to protect neurons that do not invoke increased α-synuclein turnover. Parkin has been suggested to have E3 ligase activity against a number of different substrates (reviewed in Cookson 2003). These are scattered throughout the cell and may have many divergent functions. Therefore, it is possible that there are multiple ways in which parkin may protect cells. Like the multiple subcellular targets for α-synuclein toxicity, parkin appears to have multiple neuroprotective actions at the subcellular level. To clarify this complex set of interactions, we will compare the effects of α-synuclein and parkin one by one.

Cellular Targets of Parkin and α-synuclein

Synaptic Transmission

As discussed above, PD is characterized in part by the loss of dopaminergic projections from SNpc to striatum. It has been suggested that an imbalance between the rate of oxidation of dopamine or other catecholamines and inactivation of their metabolites may contribute to toxicity in selected groups of neurons. Therefore, synaptic function may be a contributor to cell death. Furthermore, alterations in synaptic transmission may occur prior to cell loss, although the evidence of these presymptomatic changes is fragmentary. In part, this is because of the difficulty in identifying presymptomatic PD patients. However, PET brain imaging of otherwise asymptomatic members of PD families has demonstrated an interesting relationship between the synaptic "dopaminergic phenotype" and clinical parkinsonism. For example, heterozygous parkin mutation carriers who are clinically unaffected (as expected for a recessive disease) show some evidence for dopaminergic dysfunction (Khan and others 2002). Furthermore, dystonia is a prominent feature in many parkin patients and may be related to damage to the dopamine system.

What is known about the role of parkin and α-synuclein in synaptic transmission, specifically in the dopaminergic system? Some of the proposed interactions are highlighted in Figure 3. The clearest link for α-synuclein to the synaptic transmission of dopamine stems from the original localization of this protein to synaptic terminals (Clayton and George 1998). This localization has been confirmed in cultured primary neurons (McLean and others 2000) and brain tissue (Iwai and others 1995; Irizarry and others 1996). The gene for α-synuclein is highly expressed in several human brain regions, including SNpc (Solano and others 2000), and the protein is abundant in the striatal terminals of nigrostriatal dopaminergic projections (Iwai and others 1995; Irizarry and others 1996).

α-synuclein has been hypothesized to have an important function in vesicular neurotransmitter release (Clayton and George 1998), due to its ability to bind artificial phospholipid membranes (Davidson and others 1998) and synaptic vesicles (Jensen and others 1998). However, α-synuclein knockout mice display unaltered levels of synaptic vesicle proteins (Abeliovich and oth-

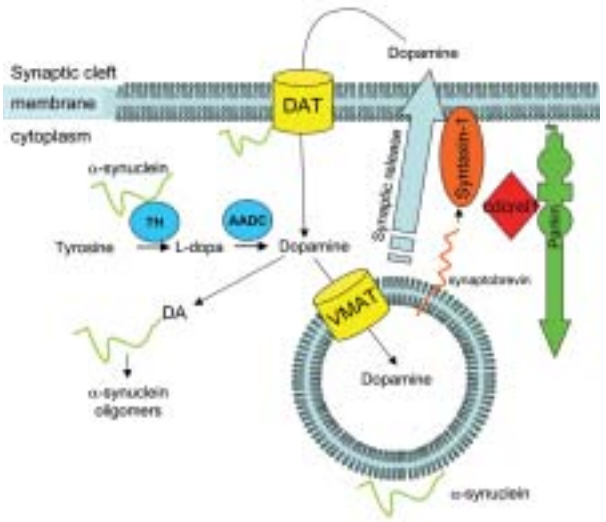

Fig. 3. Parkin and α-synuclein at synapses. This diagram shows a schematic of a presynaptic dopaminergic neuron as an example of how α-synuclein and parkin may affect synaptic transmission. Dopamine (DA) is synthesized by the sequential action of tyrosine hydroxylase (TH) and aromatic acid decarboxylase (AADC) in the cytosol. The vesicular monoamine transporter (VMAT) then sequesters DA into vesicles. After stimulation, release of neurotransmitter into the synaptic cleft is mediated by fusion of the vesicle at the plasma membrane. Proteins on the vesicle surface (synaptobrevin is shown) or at the plasma membrane (syntaxin-1; there are actually several proteins in a complex) interact to elicit release of the vesicle's contents. The released transmitter is taken back into the neuron by the dopamine transporter (DAT). α-synuclein interacts with several of these components, including TH, DAT, and the lipid vesicles. Adducts of α-synuclein and DA may form to promote oligomerization of α-synuclein into toxic species. Although there is no evidence yet that parkin directly interacts with this system, the parkin substrate CDCrel-1 can be associated with syntaxin-1.

ers 2000) and general synaptic specialization proteins (Cabin and others 2002). The morphology and distribution of synaptic vesicles are unaltered in striatum of α-synuclein knockout mice (Abeliovich and others 2000). However, there is a reduction of vesicles from the reserve-resting vesicle pool in hippocampal neurons of α-synuclein knockout mice (Cabin and others 2002). Similarly, a lowering of wild-type α-synuclein via antisense oligonucleotide treatment in cultured hippocampal neurons results in a marked depletion of vesicles from the distal vesicle pool, whereas the docked vesicle pool remains unchanged (Murphy and others 2000). Together, these results suggest that α-synuclein plays an important role in anchoring and regulating the undocked pool of presynaptic vesicles.

The vesicular dopamine transporter (VMAT2) regulates storage of dopamine by transporting dopamine from the cytosol, where it is synthesized, into presynaptic vesicles ready for release at the synapse. VMAT2 is down-regulated by A53T mutant α-synuclein in human embryonic mesencephalic cells (Lotharius and others 2002), but the kinetics of this transporter are normal in α-synuclein–null mice (Dauer and others 2002). The reduction in depolarization-induced dopamine release by A53T α-synuclein suggests that vesicular storage of dopamine is impaired due to lowered VMAT2 levels (Lotharius and Brundin 2002 and references therein). Stimulated dopamine release from striatal slice preparations is normal in one line of α-synuclein knockout mice, indicating that α-synuclein is not required for dopamine release (Dauer and others 2002). However, striatal tissue dopamine is significantly decreased in another knockout mouse line (Abeliovich and others 2000). Overexpression of A53T α-synuclein results in increased amphetamine-induced dopamine release, which supports the theory of elevated cytosolic dopamine (Lotharius and others 2002). Increased cytosolic dopamine may promote cellular toxicity, through the formation of dopamine-quinones and reactive oxygen species (reviewed in Stokes and others 1999). Whether oxidative stress is a key early event in pathogenesis of PD is arguable, as the correlation between expression of a dopaminergic phenotype and cellular susceptibility to PD is poor. However, cytosolic dopamine levels may contribute to α-synuclein toxicity by stabilizing toxic intermediates of aggregation (Conway and others 2001), as discussed above. Therefore, if α-synuclein does promote accumulation of dopamine in specific cellular compartments, its aggregation and toxicity may be enhanced.

The evidence for involvement of parkin in vesicular function is less direct than for α-synuclein. One of parkin's substrates, CDCrel-1 (Zhang and others 2000), is a synaptic vesicle associated GTPase that interacts with the SNARE protein syntaxin (Beites and others 1999). CDCrel-1 reduces the exocytosis of human growth factor from cultured cells via this interaction with syntaxin (Beites and others 1999). Thus, parkin may indirectly affect synaptic vesicle events by regulating CDCrel-1 protein levels and syntaxin activity. However, CDCrel-1 levels are unaffected in mice lacking parkin (Goldberg and others 2003), implying that parkin may not be the only E3 ligase responsible for regulating this substrate and that ablation of parkin is not sufficient to disrupt the activity of its substrates. Moreover, CDCrel-1 is not essential for neurotransmitter release (Peng and others 2002). Consistent with these results, there are no alterations in either tissue or extracellular dopamine levels in parkin knockout mice (Goldberg and others 2003). Interestingly, VMAT2 levels are also reduced in parkin-null mice (Itier and others 2003), which suggests that α-synuclein and parkin both have regulatory effects on VMAT2 levels.

α-synuclein might also affect dopamine neurotransmission by altering dopamine synthesis and efflux via an interaction with tyrosine hydroxylase (TH) (Perez and others 2002), the rate-limiting enzyme in dopamine biosynthesis. This interaction with TH may be mediated in part by α-synuclein's shared homology with 14-3-3, a chaperone that is known to bind and activate TH (Ichimura and others 1988). Perez and others (2002) propose that a loss of α–synuclein in PD, via decreased

expression or aggregation, may lead to overactivity of TH leading to excessive cytosolic dopamine. We have shown that α-synuclein has effects on transcription of several components in the dopamine synthetic pathway (Baptista and others 2003). None of the enzymes in the biosynthetic pathway for dopamine are known to be substrates of parkin's E3 ligase activity. However, parkin-null mice display a shift toward increased dopamine metabolism, which is thought to represent increased monoamine oxidase activity (Itier and others 2003).

Another critical mechanism for inactivating dopamine is through presynaptic uptake via the dopamine transporter (DAT), which plays an important role in dopamine neurotransmission by removing extracellular dopamine from the synaptic cleft. The C-terminal region of DAT has been shown to interact with the central domain of α-synuclein (Lee and others 2001; Wersinger and Sidhu 2003). In this way, α-synuclein can directly affect DAT activity. In fact, membrane-bound DAT is decreased by cotransfection of wild-type α-synuclein (Wersinger and others 2003). This altered distribution or activity of DAT may alter cellular susceptibility to the toxin MPP^+ (see section on mitochondria), which requires uptake via DAT. A number of studies report altered MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium) toxicity in the presence of mutant α-synuclein or the absence of wild-type α-synuclein. For instance, resistance to MPTP-toxicity resistance in two different lines of α-synuclein knockout mice is explained either by decreased DAT activity (Abeliovich and others 2000) or by an inability of the toxin to inhibit complex I in the absence of DAT abnormalities (Dauer and others 2002). Similarly, DAT-mediated MPP^+ toxicity is enhanced by mutant α-synuclein in vitro (Lehmensiek and others 2002). Another in vitro study reveals that overexpression of A53T α-synuclein in cultured human mesencephalic neuroblastoma cells results in decreased DAT (Lotharius and others 2002).

Parkin-null mice display decreased DAT levels and corresponding decreased dopamine uptake without a loss in dopamine neurons (Itier and others 2003). Deficits in dopamine function, and in particular the DAT, were noted in parkin-null mice (Itier and others 2003). Goldberg and others (2003) also generated a parkin-null mouse and noted an increase in extracellular dopamine and some behavioral deficits. These authors did, however, note that no loss of dopaminergic neurons was observed in aged mice. Similarly, no loss of dopaminergic neurons was found in a parkin-null *Drosophila* (Greene and others 2003). Hence, loss of function of parkin alone is not sufficient to cause nigral degeneration in either of these species, but its absence does affect dopaminergic neurotransmission.

In summary, α-synuclein is clearly involved in the presynaptic regulation of dopamine and is generally found to negatively regulate dopamine release. α-synuclein also seems to regulate undocked pools of vesicles that are critical for neurotransmission during extended low-frequency neuronal firing (Cabin and others 2002). There is also evidence from two recent knockout mouse studies that parkin may also modulate dopamine release. Although the mechanistic details are unclear, parkin may be indirectly involved in vesicle regulation via its interactions with CDCrel-1. Such interactions between α-synuclein, parkin, and synaptic vesicles would provide a pivotal role in dopamine neurotransmission because vesicular storage of cytosolic dopamine is required prior to classic vesicular exocytosis of dopamine. Such an interaction could also affect the neurotoxic potential of cytosolic dopamine (Stokes and others 1999). One caveat to this discussion is that many laboratories have focused on dopamine because of the involvement of the SNpc in the movement-related symptoms of PD. Consequently, there is less focus on whether parkin or α-synuclein has effects on other transmitter systems, and our knowledge in this area is therefore likely to be incomplete.

Protein Clearance/Degradation Mechanism

One possibility for the observations of antagonistic properties of α-synuclein and parkin mutations is that α-synuclein inhibits the degradation of some or all of the parkin substrates. This idea comes from observations of other neurodegenerative diseases in which aggregating proteins are associated with cellular toxicity. For example, aggregates of expanded forms of huntingtin, the HD gene, can inhibit the proteasome (Bence and others 2001). This has turned out to be a general rule for many proteins that aggregate; the presence of aggregates within neurons as either biochemical entities or microscopically visible inclusion bodies can inhibit the ability of cells to degrade other proteasomal substrates.

In the case of α-synuclein, there is some available information about the mechanism involved. In in vitro experiments, fibrillar forms of wild-type α-synuclein can inhibit proteasome function, measured biochemically (Snyder and others 2003). The source of this inhibition may be the interaction of aggregated α-synuclein with specific proteins in the proteasomal cap. A little caution is required here if we assume that the true toxic species are intermediates rather than the fully formed fibrillar species, but the possibility that aggregated proteins directly block the proteasome is worth discussing. The cellular correlate of this mechanism is the observation that cell lines transfected with mutant α-synuclein are deficient in proteasomal activity using several independent measures of proteasome function in different cellular models (Tanaka and others 2001; Petrucelli and others 2002). Generally, mutant α-synuclein has a more convincing effect than the wild-type protein, although a small effect of wild type has been noted (Petrucelli and others 2002), supporting the concept of quantitative rather than qualitative differences.

Wild-type parkin, on the other hand, protects cells either against proteasome dysfunction directly (Petrucelli and others 2002) or against the presence of triggers of "ER stress" (Yang and others 2003). As proteins are matured through the biosynthetic machinery of

the ER, inevitably a proportion of them is improperly folded. The presence of large amounts of misfolded proteins triggers the unfolded-protein response such that the cell can correctly refold or degrade these proteins. In some models, parkin can protect against ER stress, which is often mimicked by adding inhibitors of glycosylation such as tunicamycin. However, not all laboratories have been able to reproduce this effect (Darios and others 2003), and it remains to be seen whether this is a consistent finding. Conceptually, parkin might protect against ER stress by promoting the degradation of specific substrates that would otherwise be toxic. A candidate is the parkin interactor Pael-R (parkin-associated endothelin-like receptor), which when overexpressed in flies causes neuronal damage that can be ameliorated by wild-type parkin (Yang and others 2003).

There are other ways in which parkin may protect against proteasome inhibition. Some of parkin's substrates have the potential to be especially damaging to postmitotic cells, such as neurons, when they accumulate. An example is cyclinE, which can trigger cell death if allowed to accumulate and hence is tightly regulated by ubiquitin-mediated degradation (Staropoli and others 2003). There are similar arguments for each of parkin's substrates, and it is possible that the problem lies in net regulation of multiple substrates rather than a specific example.

However, there is a difficulty with the idea that proteasomal dysfunction is at the heart of PD, which is a lack of specificity. As mentioned above, decreased proteasomal function occurs in several neurodegenerative disorders and hence does not seem to be specific for PD. So, are we looking at events further downstream from aggregation of synuclein, given that parkin patients have a PD-specific phenotype and a relatively mild course? It is of interest that parkin is also able to protect against other aggregating proteins that also happen to inhibit the proteasome, such as expanded polyglutamine proteins (Tsai and others 2003). Therefore, parkin may have a generally beneficial effect against those disorders in which protein aggregation and proteasome inhibition are features by maintaining the ubiquitylation and degradation of a few key substrates, whereas others begin to accumulate.

A related point is that increasing the ability of neurons to handle misfolded proteins, by expression of chaperones such as HSP70, can also protect against these types of cellular stresses (e.g., Auluck and others 2002). Overall, it seems that these data point to a central but perhaps downstream role of the proteasome in PD.

Mitochondrial Function

There is abundant literature on the role of mitochondria in PD. Several years ago, Langston and others identified a group of patients with very rapid onset severe parkinsonism that had resulted from the injection of a synthetic heroin substitute. In the synthesis of the opiate, the by-product MPTP had been formed. Isolation of this agent has provided a powerful tool for many years to induce selective dopaminergic neuronal damage in the substantia nigra (reviewed in Di Monte 2003). Among other things, MPTP is a strong inhibitor of mitochondrial complex I. Rotenone, another complex I inhibitor, also induces cell damage in the nigra (Di Monte 2003).

Both α-synuclein and parkin are thought to have effects on mitochondria. Overexpression of α-synuclein, especially the mutant forms, increases mitochondrial depolarization (Tanaka and others 2001). Furthermore, proteasome inhibitor-induced cell death in the same cell lines could be inhibited by cyclosporinA, which closes the mitochondrial permeability transition pore. Lowering expression of α-synuclein seems to have the opposite effect, as α-synuclein knockout mice are notably resistant to MPTP toxicity (Dauer and others 2002). There is evidence that α-synuclein up-regulation occurs in some toxin models of PD, and it has been suggested that aggregation or other modification of α-synuclein is a required event for toxicity (Di Monte 2003). Therefore, α-synuclein generally has a detrimental effect on mitochondrial function.

Parkin has recently been reported to protect cells against ceramide-induced cell death, which includes a mitochondrial component, preventing release of cytochrome C into the cytosol, which can trigger apoptosis (Darios and others 2003). Parkin-null *Drosophila* (Greene and others 2003) have mitochondrial abnormalities in flight muscles, which are highly ATP-dependent organs in the fly. Therefore, parkin may also protect against mitochondrial damage, which can also be linked to α-synuclein toxicity.

Signal Transduction and Gene Expression

The presence of α-synuclein impinges on a number of cellular signal transduction pathways, and hence, there is evidence for changes in the regulation of gene expression. Because a detailed analysis of gene expression in relation to PD in the living human brain is currently impossible, most of these data come from different model systems. These have included some of the animal models of α-synuclein as well as studies on human postmortem brain and cell culture models.

α-synuclein has major effects on the mitogen-activated protein kinase (MAPK) and stress-activated protein kinase (SAPK) pathways, which have critical roles in regulating cell survival (Fig. 4). These are complex pathways with substantial cross-talk between them that work through the actions of a series of protein kinases to modulate transcription. Overexpression of α-synuclein can result in an inappropriate activation of the MAPK pathway. This is mediated via an interaction between α-synuclein and ERK-2, which in turn interacts with and phosphorylates Elk-1 (Iwata and others 2001). The phosphorylation of Elk-1 was attenuated in the presence of the A53T mutation. Further evidence of an interaction between α-synuclein and ERK signaling comes from observations that overexpression of α-synuclein results in up-regulation of caveolin-1 (Hashimoto and others 2003). The caveolin family has been characterized to inactivate a

number of signaling pathways, including PLD, PKC, and ERK. Hashimoto and others showed that through caveolin-1, α-synuclein suppresses MEK-1 and ERK-2 components of the ERK signaling pathway, resulting in decreased neuritic outgrowth. ERK is also known to play a central role in synaptic plasticity (Impey and others 2002); hence, dysregulation of this pathway may be a crucial step toward neurodegeneration. Excess wild-type α-synuclein can protect against oxidative stress by inactivating JNK, part of the SAPK pathway (Hashimoto and others 2002) or by activating the PI3K/Akt pathway, resulting in the expression of anti-apoptotic members of the Bcl-2 family (Seo and others 2002). Other signaling pathways including phospholipase D (probably by a direct interaction) (Ahn and others 2002) and PKC are also suppressed as a result of α-synuclein accumulation.

Therefore, α-synuclein can have beneficial effects, suppressing pro-apoptotic pathways, or detrimental effects, decreasing neuritic outgrowth and altering synaptic plasticity, depending on the model used. Presumably, this is a function of the amount of expression, given that high levels of wild-type α-synuclein can damage cells whereas lower levels may be beneficial. Aberrant forms of α-synuclein due to mutation may act detrimentally on these same pathways, as discussed above. To date, there are few examples of whether parkin affects signal transduction, although it is notable that the SAPK and MAPK pathways regulate apoptosis, whereas parkin also affects this mode of cell death (Darios and others 2003; Staropoli and others 2003).

The above examples arise from hypothesis-driven tests of how synuclein might affect gene expression. An alternate approach is to measure thousands of changes in mRNA expression simultaneously across a large portion of the transcribed genome. Such large-scale approaches, such as gene expression microarrays, may yield novel targets of gene expression. We have overexpressed α-synuclein (wild type and both mutations) in human-derived neuroblastoma cell lines and monitored alterations in gene expression using cDNA microarrays (Baptista and others 2003). Unexpectedly, we identified coordinate differential regulation of genes involved in the dopamine synthesis pathway by wild-type α-synuclein. The mechanism involved has not been resolved, but it may be mediated through an interaction with the transcription factor Nurr1, which controls the expression of several dopamine genes. Scherzer and others (2003) obtained similar results using *Drosophila* that overexpress A30P mutant α-synuclein, suggesting that this result can be extrapolated to in vivo situations. These results seem to reinforce a possible contribution of dopamine to α-synuclein aggregation and toxicity. This type of approach holds great promise to uncover novel transcriptional pathways in PD.

Newly Discovered Genes; DJ-1

Bonifati and others (2003) recently identified a new gene causing a familial form of PD. The *DJ-1* mutations identified to date are, like parkin, recessive. One is a large genomic deletion that effectively stops all protein expression. The L166P point mutation destabilizes the protein, which is normally dimeric (Miller and others 2003). At the time of writing, the role of DJ-1 in mammalian cells or its relationship to either parkin or α-synuclein is unclear. DJ-1 is part of a large superfamily of proteins whose members include proteases, kinases, and chaperones. What is known is that DJ-1 responds to oxidative stress by a shift in pI, but the physiological significance of this observation is not understood.

The advantage to finding another gene, albeit in a small number of families, is that it allows us to test some of the ideas about the relationships between parkin and α-synuclein that have been discussed above. It is reasonable to expect that DJ-1 will have some beneficial effects on cells, perhaps by protecting against α-synuclein toxicity, but we do not have proof of this at the current time.

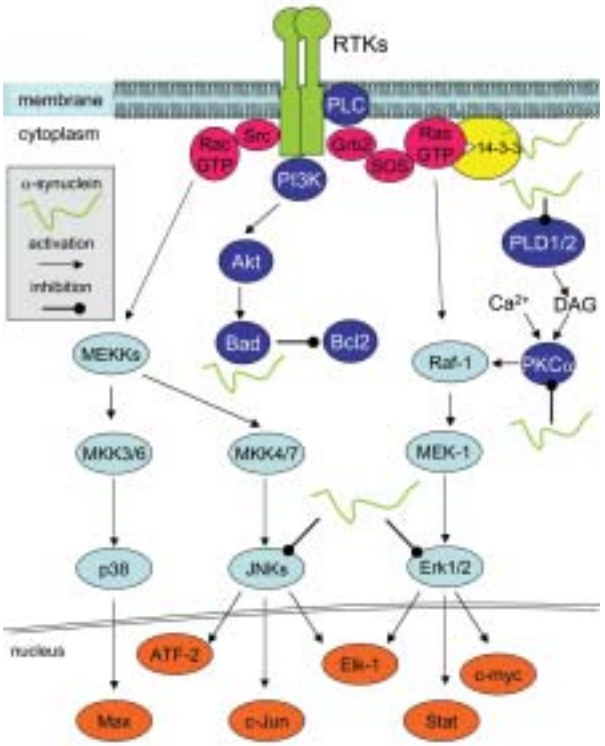

Fig. 4. Signal transduction pathways affected by α-synuclein. Activation of different receptor tyrosine kinases (RTKs) at the cell surface triggers activation of different mitogen-activated protein kinase (MAPK) pathways (shown in light blue) via interaction with a number of adaptor proteins (red). The outputs of this pathway include a number of transcription factors (orange). Also shown are a number of related signaling pathways (dark blue) that are not part of the MAPK pathways but are affected by α-synuclein, such as phospholipase D (PLD), protein kinase C (PKC), and Bad.

Summary and Conclusion

This review has highlighted the concept that the actions of α-synuclein and parkin are generally on opposing sides regarding neuronal survival. There are multiple targets for α-synuclein toxicity, downstream of its tenden-

cy to aggregate. Likewise, parkin has multiple protective effects in cells. At this time, we cannot be certain that these are all the interactions between parkin and α-synuclein, but it is clear that the major role of parkin in vulnerable neurons is to protect against α-synuclein toxicity. Testing some of these ideas, and identifying which of these pathways makes crucial contributions to neuronal damage, is greatly helped by the identification of additional genes that cause PD.

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