

Review

Synaptic dysfunction in genetic models of Parkinson's disease: A role for autophagy?

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ABSTRACT

The past decade in Parkinson's disease (PD) research has been punctuated by numerous advances in understanding genetic factors that contribute to the disease. Common to most of the genetic models of Parkinsonian neurodegeneration are pathologic mechanisms of mitochondrial dysfunction, secretory vesicle dysfunction and oxidative stress that likely trigger common cell death mechanisms. Whereas presynaptic function is implicated in the function/dysfunction of α -synuclein, the first gene shown to contribute to PD, synaptic function has not comprised a major focus in most other genetic models. However, recent advances in understanding the impact of mutations in parkin and LRRK2 have also yielded insights into synaptic dysfunction as a possible early pathogenic mechanism. Autophagy is a common neuronal response in each of these genetic models of PD, participating in the clearance of protein aggregates and injured mitochondria. However, the potential consequences of autophagy upregulation on synaptic structure and function remain unknown. In this review, we discuss the evidence that supports a role for synaptic dysfunction in the neurodegenerative cascade in PD, and highlight unresolved questions concerning a potential role for autophagy in either pathological or compensatory synaptic remodeling. This article is part of a Special Issue entitled "Autophagy and protein degradation in neurological diseases."

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Introduction

Parkinson's disease (PD) is a progressive, debilitating neurodegenerative disease characterized clinically by deterioration of motor capacities and, in many patients, dementia. The hallmark diagnostic pathology of PD is comprised of neuronal cell death and Lewy bodies/neurites in the substantia nigra, several additional brainstem nuclei and areas of the cerebral cortex. Although our understanding of the multifactorial etiology of PD is far from complete, numerous monogenic determinants of the disease identified in kindreds with

familial PD, together with a growing number of genetic risk factors identified in large cohorts of patients with sporadic PD, have provided opportunities to investigate the functions of proteins that likely play pivotal roles in the neurodegenerative cascade (Lesage and Brice, 2009).

Protein aggregation, mitochondrial dysfunction, secretory pathway organelle dysfunction and oxidative stress represent common factors in various models of Parkinsonian neurodegeneration (Cookson and van der Brug, 2008). Synaptic dysfunction, implicated in numerous studies of the first known genetic determinant of PD, α -synuclein, is beginning to emerge as a factor in other genetic models of PD. In this review, we will discuss evidence that the PD gene products LRRK2, parkin and α -synuclein play roles in regulating synaptic (dys)function. We will also highlight emerging questions concerning possible roles for autophagy in the neuritic/synaptic compartment in LRRK2, parkin and α -synuclein

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models of PD. Whereas autophagy functions in the clearance of protein aggregates (Wong and Cuervo, 2010), and in mitochondrial quality control (Zhu and Chu, 2010), the potential impact of autophagic proteolysis on synaptic structure and function remains unknown.

The ubiquitin–proteasome system (UPS) (Yi and Ehlers, 2005, 2007) and endocytic–lysosomal pathways (Groc and Choquet, 2006; Hirling, 2009) of protein degradation play important roles in the regulation of synaptic function and plasticity. In contrast, potential roles for autophagy in modulating synaptic structure and function have remained largely unexplored. Supporting the possibility that autophagy could modify synaptic structure and function is the presence of components of the autophagic machinery localized to the synaptic compartment (Seidenbecher et al., 2004). Microtubule associated protein 1 light chain 3 (LC3) and gamma-aminobutyric acid receptor-associated protein (GABARAP) are homologs of the yeast AuTophagy protein 8 (Atg8). GABARAP regulates aspects of GABA receptor degradation in an invertebrate model of neuronal development (Bamber and Rowland, 2006; Rowland et al., 2006). Furthermore, autophagy interacts with both UPS (Kirkin et al., 2009; Korolchuk et al., 2010) and the endocytic/lysosomal system (Fader and Colombo, 2009; Lee and Gao, 2008; Rusten and Simonsen, 2008). We propose that autophagy may play compensatory or deleterious roles in shaping synaptic structure/function in PD and other neurodegenerative diseases.

Leucine-rich repeat kinase 2 (LRRK2)

Mutations in the LRRK2 gene underlie familial, autosomal dominant parkinsonism linked to the PARK8 chromosomal locus 12p11.23–q13.11 (Funayama et al., 2002; Paisan-Ruiz et al., 2004; Zimprich et al., 2004). LRRK2 is a large (2527 amino acids, ~280 kDa), multidomain protein featuring two catalytic domains: a ras of complex proteins (Roc) GTPase domain and a kinase domain separated by a C-terminal of Ras (COR) domain (Mata et al., 2006). Disease segregating mutations in LRRK2 have been reported in the kinase domain (G2019S, I2020T), the Roc domain (R1441C/G) and in the COR domain (Y1699C) (reviewed in (Mata et al., 2006)). The literature suggests that the G2019S mutation in the kinase domain increases LRRK2 kinase activity, whereas mutations in the Roc domain appear to decrease the GTPase activity of LRRK2, affect protein dimerization and may increase kinase activity (analyzed in more detail in a recent review by (Greggio and Cookson, 2009)).

A more ubiquitous role for LRRK2 in neurodegenerative diseases associated with parkinsonism is suggested by the identification of the G2019S mutation in parkinsonism patients with no family history of disease (Gilks et al., 2005; Healy et al., 2008) and identification of variations in LRRK2 as important risk factors in two genome wide association studies of sporadic PD (Simon-Sanchez et al., 2009; Satake et al., 2009). Furthermore, the neuropathology demonstrated in post-mortem brain examinations of patients with LRRK2 mutations most often involves synucleinopathy, but occasionally tauopathy, suggesting a role for LRRK2 that is upstream of protein inclusion pathology (Taymans and Cookson, 2010; Uitti et al., 2004; Wider et al., 2010; Wszolek et al., 1995).

Although mRNA expression studies show little concordance regarding the level of LRRK2 mRNA expression in the substantia nigra (Galter et al., 2006; Higashi et al., 2007; Melrose et al., 2006; Simon-Sanchez et al., 2006; Taymans et al., 2006), LRRK2 protein expression has been demonstrated in tyrosine-hydroxylase positive neurons of the substantia nigra pars compacta and in medium-sized spiny neurons of the striatum. Cortical regions that are affected in dementia associated with Parkinson's disease, including pyramidal neurons of the cerebral cortex and of Ammon's horn, also demonstrate relatively high levels of LRRK2 (Biskup et al., 2006; Higashi et al., 2007; Melrose et al., 2007; Paisan-Ruiz et al., 2004). LRRK2 immunoreactivity does not appear in Lewy bodies (Higashi et al., 2007; Melrose et al., 2007).

Functional impairments in nigrostriatal dopaminergic innervation and degeneration of the nigrostriatal projections have been demonstrated in R1441C LRRK2 homozygous knock-in mice (Tong et al., 2009) and in R1441C-LRRK2 BAC transgenic mice (Li et al., 2009), respectively. G2019S BAC transgenic mice show deficiencies in striatal dopamine release and enhanced striatal tau immunoreactivity without dopaminergic neuron loss in the substantia nigra (Li et al., 2010). Furthermore, LRRK2 overexpression appears to be a modifier of synucleinopathy in A53T transgenic mice (Lin et al., 2009). Homozygous LRRK2 knockout mice show no readily apparent brain pathology (Andres-Mateos et al., 2009; Lin et al., 2009; Tong et al., 2010). Taken together, these studies suggest a gain-of-function effect of mutant LRRK2.

In neurons and neuronal cell lines, overexpression of LRRK2 cDNAs containing PD-associated mutations are associated with a phenotype of neurite injury and retraction (Macleod et al., 2006; Plowey et al., 2008; Smith et al., 2006, 2005). Macleod et al. (2006) demonstrated that overexpression of mutant LRRK2 in primary cortical cultures results in gradual neurite injury and retraction that temporally precede apoptotic cell death in both *in vitro* and *in vivo* systems. Neurite injury is accompanied by induction of neuritic autophagy, which eventually contributes to neurite shortening (Plowey et al., 2008). This suggests that early events in the neurodegenerative cascade downstream of mutant LRRK2 likely target the neuritic/synaptic compartment.

Endogenous and overexpressed LRRK2 immunoreactivity has been demonstrated in association with membrane-bound organelles of the secretory pathway, including the endoplasmic reticulum and Golgi apparatus (Biskup et al., 2006; Gloeckner et al., 2006; Hatano et al., 2007), structures of the endocytic pathway, including lipid rafts, caveolar necks, clathrin-coated endosomes and multivesicular endosomes (Alegre-Abarrategui et al., 2009; Biskup et al., 2006; Hatano et al., 2007; Shin et al., 2008), and microtubules (Biskup et al., 2006; Gloeckner et al., 2006). Localization to amphisomes, points of intersection of endocytic and autophagic degradation pathways, has also been reported (Alegre-Abarrategui et al., 2009), although it is unclear if LRRK2 is entrapped passively as cargo or in a regulatory capacity. Collectively, these immunolocalization studies implicate potential roles for LRRK2 in regulating protein trafficking through the secretory/endocytic pathways, critical avenues of synaptic regulation.

Several recent studies have provided some suggestion that LRRK2 impacts secretory and endocytic pathway function. Shin et al. (2008) demonstrated that LRRK2 interacts with Rab5b, a regulator of endocytic vesicle trafficking, by yeast two-hybrid and co-immunoprecipitation techniques. In their primary neuron experiments, overexpression of wild-type LRRK2, LRRK2 PD mutants and kinase impaired 1906M-LRRK2, as well as siRNA knockdown of LRRK2, were associated with decreases in presynaptic vesicle endocytosis rates in primary rat hippocampal neurons (Shin et al., 2008), prompting the authors to surmise that any change in LRRK2 expression or function may alter the balance of homeostatic interactions with other endocytosis proteins. More recently, Xiong et al. (2010) overexpressed wild-type LRRK2 in primary neuronal cultures and documented reduced rates of synaptic vesicle endocytosis and exocytosis in hippocampal neurons (Xiong et al., 2010). LRK-1 – a homolog of human LRRK2 in *Caenorhabditis elegans* that is localized to the Golgi apparatus (Sakaguchi-Nakashima et al., 2007) – is necessary for appropriate axonal localization of synaptic vesicle proteins, suggesting a role for LRK-1 in Golgi cargo sorting (Sakaguchi-Nakashima et al., 2007). Although additional evidence is needed, an attractive hypothesis is that LRRK2 modifies synaptic structure and function through effects on synaptic protein trafficking and potentially degradation.

Mutant LRRK2 induced degeneration was first reported to be associated with membrane-bound, phospho-tau immunoreactive accumulations in dystrophic neurites (Macleod et al., 2006). Our group subsequently demonstrated a link between mutant LRRK2

induced neurite degeneration and autophagy: mutant LRRK2 expression in differentiated SH-SY5Y cells is associated with elevated neurite autophagosomes (Plowey et al., 2008). Furthermore, neuritic autophagy is responsible for neurite retraction because inhibition of the autophagic machinery via siRNA knockdown of LC3 or Atg7 reversed the mutant LRRK2 induced neurite retraction (Plowey et al., 2008). Other manipulations that suppress autophagy, such as phosphorylation of LC3, also prevent mutant LRRK2-mediated neurite retraction in primary cortical neuronal cultures (Cherra et al., 2010b). These data suggest that autophagy, which is thought to be protective in many neuronal contexts, may induce neurite withdrawal in the process of promoting survival (Cherra et al., 2010a). An alternative hypothesis is that autophagy induced by mutant LRRK2 contributes to neuronal death as has been demonstrated in a MPP+ cell culture model of PD (Zhu et al., 2007).

Additional studies delineating whether the increase in neurite autophagy is due to direct or indirect effects of mutant LRRK2 overexpression may help in resolving these questions. Our group rarely sees colocalization of GFP-LC3 labeled autophagosomes with HA-tagged LRRK2 (unpublished data), and protein aggregation is not a prominent ultrastructural feature in these cells. Thus, it is less likely that autophagy is induced as a nonspecific cellular response to degrade an overexpressed, dysfunctional protein. While LRRK2 has been suggested to function as a direct regulator of autophagy (Alegre-Abarrategui et al., 2009), a role in modifying the activity of autophagy proteins by direct interaction or phosphorylation has yet to be demonstrated. It is also possible that autophagy is elicited as a compensatory response to upstream injury. Nevertheless, over time, even compensatory autophagy responses can result in pathologic dendrite remodeling.

In summary, several lines of emerging evidence suggest that mutant LRRK2 impacts the morphology and possibly the function of the neuritic/synaptic compartment in a potentially reversible manner that precedes neuronal cell death. These effects may be mediated through mutant LRRK2 effects on the secretory/endocytic pathways, through direct effects on autophagic neurite catabolism or via secondary autophagic responses to neurite injury. We favor a model in which neurite autophagy is a response to an as yet undefined alteration in dendritic/synaptic function (Fig. 1), although a direct effect on the autophagy machinery cannot be excluded. To date, few potential LRRK2 substrates have been reported: LRRK2 (autophosphorylation) (Gloeckner et al., 2006; Greggio et al., 2009, 2008; Luzon-Toro et al., 2007; West et al., 2005), moesin (Jaleel et al., 2007; Parisiadou et al., 2009) and 4E-BP (Imai et al., 2008; Kumar et al., 2010). It will be of critical interest to determine which regulatory proteins in the secretory, endocytic or autophagic pathways, if any, are LRRK2 kinase substrates to lend insight into this issue.

Parkin

Parkin mutations underlie disease in kindreds with autosomal recessive juvenile parkinsonism linked to the PARK2 locus on chromosome 6 (Kitada et al., 1998). Parkin is an E3 ubiquitin ligase (Shimura et al., 2000; Zhang et al., 2000), one of numerous ubiquitin ligases expressed in neurons that likely impact synaptic function via the UPS (Helton and Ehlers, 2008). Parkin mutations found in PD patients confer either decrements in ubiquitin ligase activity (Hampe et al., 2006; Shimura et al., 2000) or disrupt its localization, solubility or interactions with substrates (Hampe et al., 2006; Sriram et al., 2005; Wang et al., 2005). Most mouse models of parkin-deficient mice have demonstrated nigrostriatal synaptic deficits, mitochondrial dysfunction and deficits in learning without nigral dopaminergic cell loss (Goldberg et al., 2003; Itier et al., 2003; Kitada et al., 2009; Palacino et al., 2004). However, a recent report demonstrates hallmark Parkinson's disease features in BAC mutant Parkin-Q311X mice (Hart and Xu, 2009), including accumulation of protease

resistant α -synuclein. Although synucleinopathy is not a typical feature of autosomal recessive juvenile parkinsonism in humans (Kitada et al., 1998), Lewy bodies have been observed in rare families (Farrer et al., 2001; Pramstaller et al., 2005).

In the post-synaptic compartment, parkin has been reported to function as a PDZ-binding protein via its C-terminus. Its association with CASK, a post-synaptic multidomain scaffolding protein, results in synaptic localization of parkin in cultured cortical neurons (Fallon et al., 2002). Loss of the C-terminus, as occurs with a common truncating mutation of parkin, could thus be hypothesized to reduce parkin localization to the post-synaptic scaffold and reduce ubiquitination of parkin's post-synaptic substrates. The C-terminus of parkin also appears to be important for parkin interactions with the BAR-SH3 domain of the endocytic protein endophilin-A1, suggesting a role for parkin in regulating membrane protein ubiquitination and endocytosis (Trempe et al., 2009). Immunoelectron microscopy experiments in rat brain demonstrate neuronal parkin expression to be localized prominently to membranous organelles (ER, Golgi) and to vesicular structures of dendrites and presynaptic nerve terminals (Mouatt-Prigent et al., 2004). Taken together, these findings suggest potential roles for parkin in regulating the trafficking of synaptic proteins through the secretory and endocytic pathways.

Several electrophysiologic studies in parkin knockout mice have suggested decreased excitatory post-synaptic responsiveness and plasticity in the striatum or CA1 hippocampal neurons (Goldberg et al., 2003; Hanson et al., 2010; Itier et al., 2003; Kitada et al., 2009). These studies were conducted in brain slices from animals ranging from 2 to 16 months old, during which compensatory mechanisms or chronic injury may counteract the loss of parkin function on excitatory synapse function. Furthermore, other possibilities should be considered. Upregulation of other ubiquitin ligases (Helton and Ehlers, 2008), decreased de-ubiquitination or decreases in parkin substrate synthesis may compensate for lifelong loss of parkin activity.

In contrast, acute modulation of parkin expression by knockdown of endogenous parkin or overexpression of mutant parkin potentiates glutamatergic post-synaptic function in primary hippocampal cultures with enhanced vulnerability to synaptic glutamate stress (Helton et al., 2008). Enhanced functional expression of excitatory synapses was observed, suggesting that one function of parkin may relate to removal of glutamatergic synapses. Acute overexpression of parkin has been shown to protect, and acute parkin knockdown has been shown to exacerbate, kainate-induced excitotoxicity in cultured cerebellar granule neurons and dopaminergic midbrain neurons (Staropoli et al., 2003). The anti-apoptotic effect was attributed to modulation of the levels of the candidate parkin substrate and potential pro-apoptotic factor cyclin E (Staropoli et al., 2003). A further investigation of the effects of parkin on kainate receptor expression in this model would be very interesting given the above discussion. Inhibitory synapse activity and density were unaffected by altering parkin expression (Helton et al., 2008). Since LRRK2 and parkin interact via the COR domain of LRRK2 and the C-terminal RING2 domain of Parkin (Smith et al., 2005), it is possible that LRRK2 and parkin function as part of a complex to regulate glutamatergic synapse formation/elimination and/or excitatory synapse protein trafficking and degradation (Fig. 1).

Mutant parkin effects on glutamate toxicity may be adversely complemented by decreased ubiquitination and degradation of phospholipase C γ 1 resulting in enhanced Ca²⁺ levels emanating from ryanodine-sensitive stores (Sandebring et al., 2009). Parkin also monoubiquitinates PICK1, resulting in reduced potentiation of acid-sensing ion channel (ASIC) currents by PICK1; loss of parkin function results in potentiation of excitotoxic ASIC currents via interactions with PICK1 (Joch et al., 2007). PICK1 is also implicated in activity-dependent internalization and retention of the GluR2 subunit of AMPA receptors (Lin and Huganir, 2007) and may represent a mechanism through which parkin impacts

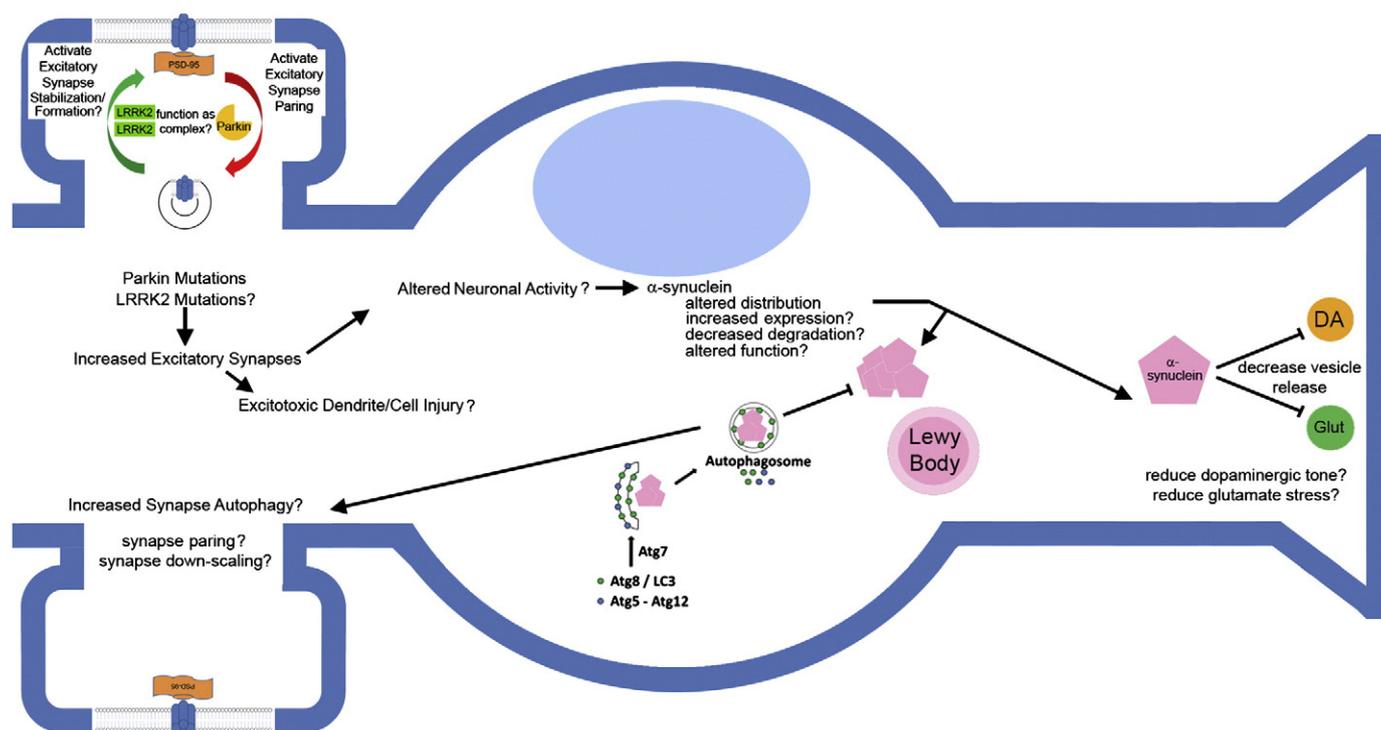


Fig. 1. Schematic diagram showing hypothetical links for LRRK2, parkin and α -synuclein in synaptic dysregulation. Parkin mutations have been reported by Helton et al. (2008) to interrupt the ability of wild-type parkin to pare excitatory synapses. Association of parkin and LRRK2 with elements of the secretory and endocytic pathways (see text) raise the possibility that they affect synaptic structure and function by regulating synaptic protein trafficking. LRRK2, which has been reported to interact with parkin (Smith et al., 2005), is hypothesized to induce the formation or stabilization of excitatory synapses. Downstream effects of LRRK2 and parkin mutations would thus increase vulnerability to synaptic glutamate stress (Helton et al., 2008) and could influence neuronal activity. Alterations in neuronal activity induce alterations in the distribution of α -synuclein, and thus may affect α -synuclein effects in the presynaptic terminal. Hypothetically, alterations in α -synuclein distribution and function could decrease presynaptic vesicle cycling to decrease dopaminergic oxidative stress and synaptic glutamate stress, but this function may be undermined by the propensity for α -synuclein to form cytosolic aggregates. The speculative linkages among these elements are indicated with question marks.

glutamate receptor trafficking. Parkin overexpression has been shown to potentiate ATP-induced inward currents conducted by P2X receptors in PC12 cells, an effect that is not seen with overexpression of parkin mutants (Sato et al., 2006).

Deficiencies in presynaptic dopamine release in parkin knockout mice (Goldberg et al., 2003; Itier et al., 2003; Kitada et al., 2009) have driven research into presynaptic parkin substrates. CDCrel-1, a septin GTPase that interacts with syntaxin to inhibit vesicle exocytosis, is ubiquitinated by Parkin (Zhang et al., 2000). CDCrel-1 was shown not to be essential for neuronal development or function in homozygotic CDCrel-1 null mice (Peng et al., 2002). However, dysfunctional mutant Parkin might therefore be hypothesized to lead to increased levels of CDCrel-1 and increased inhibition of presynaptic vesicle exocytosis, leading to inhibition of presynaptic dopaminergic function. Alternatively, as supported by the work of Son et al. (2005), this could lead to accumulation of CDCrel-1 as has been documented in proteomic studies of parkin knockout mice (Periquet et al., 2005). Overexpression of Septin 4, the *Drosophila* homologue of human CDCrel-1, is toxic to dopaminergic neurons (Munoz-Soriano and Paricio, 2007). Parkin-mediated ubiquitination of synaptotagmin XI (Huynh et al., 2003) and the orphan G protein-coupled receptor 37 (GPR37) (Marazziti et al., 2007), may impact presynaptic function through their effects on neurotransmitter release and dopamine transporter function, respectively. Finally, in addition to documented effects on protein accumulation and inclusion pathology, parkin ubiquitination of the glycosylated form of α -synuclein, Sp22 (Shimura et al., 2001), and the α -synuclein interacting protein synphilin-1 (Chung et al., 2001) also may impact presynaptic functions of these proteins.

In addition to catalyzing the formation of K48 polyubiquitin chains that target substrates for proteasomal degradation, parkin has garnered recent attention for its potential roles in targeting substrates

for autophagy via K63-linked polyubiquitin tagging (Doss-Pepe et al., 2005). Parkin recruits injured mitochondria for degradation via autophagy (mitophagy) as a mechanism of mitochondrial quality control (Narendra et al., 2008, 2009; Dagda et al., 2009). Parkin targets the damaged mitochondria for p62-dependent mitophagy via K63 and K27 polyubiquitin linkages (Geisler et al., 2010). Therefore, loss of parkin function may affect synaptic function indirectly by affecting the energy generating capacity in dendrites. Parkin has also been shown target some proteins, including the synaptic protein synphilin-1, for inclusion formation via K63-linked polyubiquitin linkages (Lim et al., 2005). Furthermore, K63 polyubiquitin linkages mediated by parkin or other ubiquitin ligases suggests the possibility of synaptic modulation by autophagic proteolysis.

α -Synuclein

α -Synuclein has been a major focus of PD genetic research since point mutations (Polymeropoulos et al., 1997) (Kruger et al., 1998) (Zarranz et al., 2004) and genetic locus multiplication (Singleton et al., 2003) in the α -synuclein gene were found to underlie rare forms of familial PD. Furthermore, the discovery that ubiquitinated aggregates of α -synuclein are a prominent component of Lewy bodies in post-mortem PD brains (Spillantini et al., 1997) has fueled the notion that α -synuclein aggregation plays a central role in the sporadic PD. α -Synuclein is a 19 kDa, 140 amino acid protein containing three domains including an N-terminal alpha-helical lipid binding domain that confers its ability to loosely bind to synaptic vesicles. The A30P substitution negates or reduces the ability to bind membranes (Cole et al., 2002; Fortin et al., 2004; Jensen et al., 1998; Jo et al., 2002; Perrin et al., 2000). The propensity of α -synuclein and its mutants to form toxic soluble oligomers and insoluble aggregates and the potential

downstream disruption of various critical cell processes likely represents a common pathway of neurodegeneration in PD and are the subjects of several excellent reviews over the past decade (Cookson and van der Brug, 2008; Lotharius and Brundin, 2002; Sidhu et al., 2004a,b). In our discussion, we will focus on evidence implicating roles for α -synuclein in synaptic regulation.

Consideration of the potential roles of α -synuclein in synaptic function has traditionally been focused on presynaptic function since several studies have demonstrated that the normal localization of α -synuclein is in the presynaptic terminal. α -Synuclein was first cloned from the rat brain cDNA library by Maroteaux et al. (1988) who showed that endogenous α -synuclein is localized to presynaptic vesicles in cholinergic fibers innervating the electric organ of *Torpedo californica*. Ultrastructurally, immunoreactivity for endogenous α -synuclein in rat brain has been demonstrated in close association with the undocked, distal synaptic vesicle pool (Clayton and George, 1999; Iwai et al., 1995). Differential centrifugation experiments have also demonstrated the localization of α -synuclein expression to synaptosomal fractions (Kahle et al., 2000). Recombinant wild-type α -synuclein (Specht et al., 2005) and transgenic wild-type α -synuclein expression (Kahle et al., 2000) also localize to presynaptic terminals. Presynaptic localization appears to be dependent on large portions of the N-terminal and core domains but is independent of the C-terminus (Specht et al., 2005). Neural activity (Fortin et al., 2005) and depolarization (Tao-Cheng, 2006) have been reported to affect α -synuclein distribution in the presynaptic terminal albeit in opposite directions. Overexpression of α -synuclein mutants does not disturb delivery to presynaptic terminals (Kahle et al., 2000) despite abnormal cellular accumulations. A clue to the possible function of synuclein in regulating vesicle release comes from the CSP α knockout mice, a mouse with a severe neurodegenerative phenotype caused by dysfunction of SNARE complexes that mediate vesicle fusion/release (Chandra et al., 2005). Chandra et al. (2005) found that crossing transgenic wild-type α -synuclein mice, but not A30P α -synuclein transgenic mice, with CSP α knockout mice restores SNARE complex formation and rescues the progeny from the severe neurodegenerative phenotype, suggesting a role for α -synuclein in maintaining SNARE complexes. These studies suggest that α -synuclein impacts presynaptic function by regulating some aspect of synaptic vesicle fusion/release or the dynamics of the reserve pool of synaptic vesicles.

Studies of neurotransmitter release in catecholaminergic cells have lent support to the hypothesis that α -synuclein functions in the regulation of presynaptic neurotransmitter vesicle pools. Murphy et al. (2000) showed a robust decrease of vesicles in the distal synaptic pool but not in the docked pool in primary rat hippocampal cultures following a 50% knockdown in endogenous α -synuclein levels. In an α -synuclein knockout mouse, Abeliovich et al. (2000) reported the effect of increasing stimulated DA release in knockout mice striatal slices using a paired-pulse stimuli, suggesting that α -synuclein normally acts as activity-dependent negative regulator of DA neurotransmission. They found that knockout mice demonstrated reduced striatal DA content and decreased amphetamine-stimulated locomotion and proposed that the lack of negative α -synuclein regulation led to a decrease in the readily releasable pool of DA vesicles, although no apparent change in presynaptic vesicle pools in electron micrographs of striatum were seen (Abeliovich et al., 2000). Cabin et al. (2002) did show in a separate α -synuclein knockout mouse attenuated CA1 dendritic field EPSCs with stimulation of Schaffer collaterals in hippocampal slices of mice lacking α -synuclein and ultrastructural evidence of decreased presynaptic vesicles, suggesting decreased reserve storage pool of vesicles. In a double-knockout model of α - and β -synuclein, no changes in presynaptic vesicle density or any other presynaptic morphologic parameter was seen, nor were there any changes in tests of hippocampal synaptic plasticity, yet a modest 20% reduction in striatal dopamine content was reported (Chandra et al., 2004). Yavich et al. (2004) employed *in vivo* voltammetry in α -synuclein null mice to document an increased readily releasable pool (altered dopamine compartmentalization) and increased facilitation, suggestive of

increased refilling rate of the reserve pool, as a compensatory mechanism to maintain stable dopamine release during stimulation. Most recently, α - and γ -synuclein double-knockout mice were reported to show increased evoked release of striatal dopamine that was not attributable to increased striatal DA or to decreased DA reuptake (Senior et al., 2008). Overexpression of wild-type or A30P synuclein in chromaffin cells and PC12 cells is associated with impairment of dopamine secretion despite robustly increased numbers of morphologically docked dense core vesicles and unaltered sensitivity to Ca^{+2} , suggestive of inhibition of a step in exocytosis before Ca^{+2} induced fusion (Larsen et al., 2006). Overall, these studies support a model in which α -synuclein functions to inhibit DA vesicle release by downregulating vesicle access to the presynaptic terminal membrane.

In dopaminergic cells, α -synuclein has also been observed to suppress tyrosine-hydroxylase activity, improve dopamine storage into vesicles and reduce the activity of the dopamine transporter, whereas mutant α -synuclein is impaired in many of these interactions (Sidhu et al., 2004a,b). The cumulative effect of α -synuclein may therefore be to decrease the levels of cytoplasmic dopamine and oxidative stress in a neuroprotective manner (Sidhu et al., 2004a,b). In consideration of recent findings in which afferent glutamatergic activity is enhanced in neurons with mutant parkin expression, we envision a model in which the function of α -synuclein may be to decrease synaptic dopamine turnover and oxidative stress, but which is undermined by the propensity of α -synuclein to aggregate (Fig. 1).

Potential functions of α -synuclein in cortical glutamatergic neurons are important to consider given proposed roles for α -synuclein in synaptic plasticity and the involvement of cortical synucleinopathy in patients with Parkinson's disease with dementia and dementia with Lewy bodies. Synelfin, a songbird homologue of α -synuclein, is expressed in the glutamatergic circuits that govern song learning (Mooney and Konishi, 1991), during the critical juvenile learning period (George et al., 1995), suggesting a role in learning and plasticity. Wu et al. (2009) reported decreased miniature EPSCs in striatal medium-sized spiny neurons and increased thresholds for stimulating EPSCs from the corticostriatal afferents in transgenic mice overexpressing human WT α -synuclein. Overexpression of α -synuclein was also reported to inhibit the exocytosis of VGLUT1-expressing glutamatergic vesicles from both hippocampal and midbrain dopaminergic neurons secondary to decreased clustering of vesicles in the presynaptic terminal and reduction of the recycling pool (Nemani et al., 2010). From a presynaptic plasticity standpoint, however, Gureviciene et al. (2007) reported enhancement of LTP, a presynaptic phenomenon in mossy fiber-CA3 synapses with overexpression of α -synuclein, whereas KO mice also showed an LTP deficit in response to high frequency stimulation. α -Synuclein enhancement of glutamate vesicle release from the presynaptic terminal was also reported by Liu et al. (2007, 2004), who showed enhancement of mEPSC amplitudes and rates and amplitudes of evoked EPSCs in the post-synaptic cell of monosynaptically-connected pairs in hippocampal neuron cultures when they injected α -synuclein into the presynaptic neuron. Thus, whereas some studies recapitulate an inhibitory effect of α -synuclein on presynaptic vesicle release in glutamatergic synapses, a full understanding of α -synuclein in cortical synaptic plasticity is probably more complex and is important for our understanding of Lewy body dementia.

Autophagy contributes to the degradation of α -synuclein (Webb et al., 2003; Yu et al., 2009), providing another avenue through which autophagy may impact synaptic function. Expression of mutant α -synuclein increases cellular autophagosomes (Stefanis et al., 2001). Furthermore, chaperone mediated autophagy (CMA), which involves targeting proteins for lysosomal translocation by the LAMP2A receptor by cytosolic chaperone proteins, also contributes to α -synuclein degradation (Mak et al., 2010; Vogiatzi et al., 2008) and is impaired in models of synucleinopathy (Cuervo et al., 2004; Martinez-Vicente et al., 2008; Xilouri et al., 2009). Macroautophagy is induced to compensate for defective CMA, although this regulation may be altered in patients

with synucleinopathy due to reduced levels of autophagy regulatory proteins Atg7 and mTOR (Crews et al., 2010). These findings raise the possibilities that autophagy may influence or regulate synaptic function through α -synuclein degradation.

Methods to enhance cellular autophagy as a means to dispose of accumulating toxic synuclein oligomers have been suggested as potential therapies for synucleinopathies and other neurodegenerative diseases characterized by protein aggregation (Ravikumar et al., 2008; Sarkar et al., 2007a,b; Spencer et al., 2009). However, the potential for adverse functional effects of pharmacologically inducing autophagy needs to be carefully considered as well, particularly if synaptic remodeling may occur as an unintended side-effect of increased autophagic activity (Cherra et al., 2010a), or if there is relative impairment in autophagosome maturation, fusion or lysosomal degradation, as has been described in Alzheimer's disease (Boland et al., 2008) and several other neurodegenerative diseases (Wong and Cuervo, 2010).

Conclusions

Although a complete understanding of the functions of α -synuclein has not been achieved, it is implicated in inhibiting presynaptic vesicle release. Recent studies also suggest potential mechanisms by which parkin and LRRK2 could impact synaptic structure and function. Given these trends, construction of a unifying concept of Parkinsonian neurodegeneration should consider possible roles for these proteins in synaptic function and dysfunction as early events in the neurodegenerative cascade. Critical issues that remain to be addressed include: (1) differentiating effects on post-synaptic function versus presynaptic function, (2) whether observed effects are context dependent (acute knockdown/overexpression versus chronic compensatory effects), and (3) whether these effects are similar or dissimilar in dopaminergic, glutamatergic and other synapse types. Furthermore, the impact on synaptic function needs to be confirmed as a primary early neurodegenerative event, versus secondary to other documented PD phenomena of protein aggregation, mitochondrial dysfunction or oxidative stress. Dysregulation of mitochondrial dynamics, trafficking or quality control, as implicated in autosomal recessive PD pathogenesis (Dagda and Chu, 2009), could indirectly affect synaptic maintenance and function as well. Lastly, as autophagy-modulating drugs garner increased attention as potential therapeutic agents in neurodegenerative diseases, it becomes critically important to study the potential impact of autophagy modulation on synaptic structure and function.

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