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Mitochondrial function and autophagy: integrating proteotoxic, redox, and metabolic stress in Parkinson's disease

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Abstract

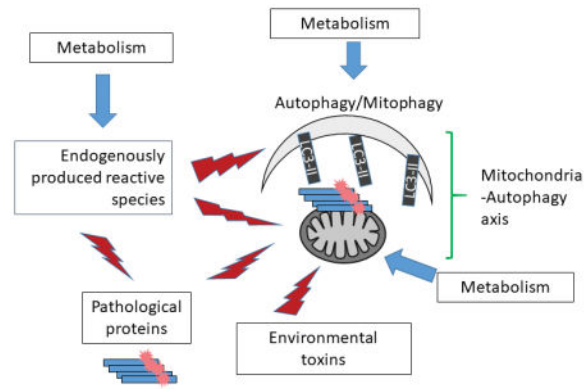
Parkinson's disease (PD) is a movement disorder with widespread neurodegeneration in the brain. Significant oxidative, reductive, metabolic, and proteotoxic alterations have been observed in PD postmortem brains. The alterations of mitochondrial function resulting in decreased bioenergetic health is important and needs to be further examined to help develop biomarkers for PD severity and prognosis. It is now becoming clear that multiple hits on metabolic and signaling pathways are likely to exacerbate PD pathogenesis. Indeed, data obtained from genetic and genome association studies have implicated interactive contributions of genes controlling protein quality control and metabolism. For example, loss of key proteins that are responsible for clearance of dysfunctional mitochondria through a process called mitophagy has been found to cause PD, and a significant proportion of genes associated with PD encode proteins involved in the autophagy-lysosomal pathway. In this review, we highlight the evidence for the targeting of mitochondria by proteotoxic, redox and metabolic stress and the role autophagic surveillance in maintenance of mitochondrial quality. Furthermore, we summarize the role of α -synuclein, LRRK2, and tau in modulating mitochondrial function and autophagy. Among the stressors that can overwhelm the mitochondrial quality control mechanisms, we will discuss 4-hydroxynonenal (HNE) and nitric oxide. The impact of autophagy is context depend and as such can have both beneficial and detrimental effects. Furthermore, we highlight the potential of targeting mitochondria and autophagic function as an integrated therapeutic strategy and the emerging contribution of the microbiome to PD susceptibility.

Graphical Abstract

This review provides highlights on recent observations regarding the multifacet impact of pathological proteins, endogenously produced reactive species, environmental toxins, and metabolism including glucose and fatty acid metabolism, on mitochondria - autophagy function, in the context of Parkinson's disease. The review also discuss future studies of designing targeted strategies to aid the treatment of Parkinson's Disease.

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Introduction

Parkinson's disease (PD) was described 200 years ago as a disease of shaking palsy (Obeso *et al.* 2017). We now know that PD is a condition with both motor and non-motor symptoms including cognitive impairment, due to neurodegeneration of dopaminergic and non-dopaminergic neurons, with prodromal symptoms with gastrointestinal, olfactory, and REM sleep disturbances. Approximately 1 in 100 Americans older than 60 years are affected by this disease and it is estimated that between 2010 and 2030, the number of individuals 65 years or older with PD will increase by 77% (Dorsey *et al.* 2013, Fritsch *et al.* 2012). The Parkinson's Disease Foundation has estimated that the combined annual direct and indirect cost of PD in the United States is \$25 billion. Worldwide, it affects 1–3% of the population of 50 years or older (Dehay *et al.* 2015).

A key pathological feature of PD is the appearance of Lewy bodies and Lewy Neurite in the brain. Protease resistant α -synuclein aggregates are a major component in the Lewy Bodies and Lewy Neurites. A 140 amino acid protein, α -synuclein has been found in the cytoplasm, mitochondria, and the nucleus, although its physiological function is still unclear other than it may be involved in synaptic function (Abeliovich *et al.* 2000). Pathologically α -synuclein may exert neurotoxicity by disrupting protein trafficking, or interfering with autophagy-lysosomal and mitochondrial function, thus contributing to neurodegeneration and PD pathogenesis and progression (Lee & Trojanowski 2006, Lashuel *et al.* 2013, Schmid *et al.* 2013). Cellular damage can also be spread from a donor cell to neighboring cells by inter-cellular transfer of α -synuclein (Luk *et al.* 2012a, Luk *et al.* 2012b, Volpicelli-Daley *et al.* 2011, Luk *et al.* 2009, Steiner *et al.* 2011).

There are currently no available pharmacological approaches for preventing or attenuating neuronal loss in PD; consequently, treatment options are limited to symptomatic management. Therefore, a better understanding of the fundamental processes that affect PD development and progression is of paramount importance and has been an area of intense

research. Because of the importance of mitochondria and the autophagy-lysosomal activities in neurons and PD, in this review, we highlight some of the recent studies that focused on oxidative, metabolic and proteotoxic stress and discuss how they affect mitochondrial and autophagic function. Finally, we will overview the progress in developing mitochondria and autophagy targeted therapeutic strategies (Fig. 1).

A. The mitochondrial-autophagy axis in Parkinson's disease

Accumulation of toxic proteins and damaged mitochondria have been noted as key features of PD (Goedert *et al.* 2017). Damaged mitochondria can be a source and further increase reactive species production (Murphy 2009). Removal of these toxic cellular components is therefore essential for neuronal health, and this is largely dependent on the lysosomal-mediated autophagic function. Both mitochondrial and autophagy-lysosomal dysfunction have been found in postmortem Parkinson's disease brains (Chu Y 2009, Schapira 2007, Ryan *et al.* 2015, Swerdlow 2009, Beal 2003, Parker & Swerdlow 1998, Schapira *et al.* 1990, Bindoff *et al.* 1989), as well as dopaminergic neurons derived from patients with idiopathic and familial PD (Burbulla *et al.* 2017, Wang *et al.* 2017). A recent study also demonstrated downregulation of mRNAs for key autophagy genes in the peripheral blood mononuclear cells of patients with PD (Miki *et al.* 2017). Here we will review the evidence of mitochondrial and autophagy dysfunction in PD. Understanding of the cause and consequences of this dysfunction is crucial to help find interventions for therapeutics for PD.

1. Mitochondria as targets for environmental toxins that contribute to

Parkinson's disease risk—The mitochondrion is a multifunctional organelle that plays an essential role not only in energy production, but also in thermogenesis, calcium homeostasis, generating and maintaining key cellular metabolites, and redox signaling (Hill *et al.* 2012). With age, mitochondrial function declines, while mtDNA damage/mutations increase, leading to increased risk for PD (Schapira & Gegg 2011, Reeve *et al.* 2013). In this section, we will discuss how environmental toxins contribute to proteotoxicity and the formation of Lewy bodies.

Mitochondria contain several hundred redox centers that have the potential to react with xenobiotics generating secondary reactive metabolites or can be secondary targets of ROS (reactive oxygen species) generated within the cell. Since these damaged redox proteins in mitochondria can exhibit a gain of function in which they amplify oxidative damage or disrupt normal redox signaling then it is essential that they are removed. If this does not occur, damaged respiratory proteins and mtDNA can impair the capacity of the organelle quality control mechanisms and thereby amplify the initial insult through a progressively increased metabolic dysfunction and oxidative stress (Hill *et al.* 2012, Murphy 2009). These patterns of responses have been shown experimentally in the models of PD, which have used xenobiotics that are mitochondrial toxins. For example, 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP), a by-product in the drug abused by a group of young adults in California was found to be responsible for causing *parkinsonism*, through a mechanism which was later found to be associated with mitochondrial complex I inhibition (Ramsay *et al.* 1987, Nicklas *et al.* 1987, Mizuno *et al.* 1987). Analogous data has been reported for the complex I inhibitor rotenone which is found in plants and used to control fish populations

(Clark 1933). There are possibly a range of environmental toxins which increase the susceptibility to PD including pesticides such as paraquat which directly or indirectly target mitochondrial function (Priyadarshi *et al.* 2001, Priyadarshi *et al.* 2000, van der *et al.* 2012, Tanner *et al.* 2011, Giordano *et al.* 2012, Giordano *et al.* 2014b) (Fig. 1).

The targets involved in the environmental xenobiotics are concordant with the biological pathways that are thought to be critical for the development of PD. For example, it has been shown that mitochondrial dysfunction in dopaminergic neurons leads to *parkinsonian* phenotypes (Ekstrand *et al.* 2007). Further, the protein α -synuclein associated with PD pathogenesis, targets the mitochondria and decreases mitochondrial function (Devi *et al.* 2008, Liu *et al.* 2009, Loeb *et al.* 2010b, Elkon *et al.* 2002, Ryan *et al.* 2015, Martinez & Greenamyre 2012, Swerdlow 2009, Schapira 2007, Beal 2003, Parker & Swerdlow 1998, Schapira *et al.* 1990, Bindoff *et al.* 1989). If these mechanisms act together, exposure to a xenobiotic, such as rotenone, can exacerbate an underlying synucleinopathy since the cell will be less capable of meeting the energetic requirements for protection against proteotoxicity. In this paradigm, sub-threshold levels of proteotoxicity or metabolic impairment can synergize to promote disease progression. It is likely that these effects are not solely confined to the central nervous system and can be expressed in other tissues and organs. This raises the possibility that circulating peripheral blood mononuclear cells or platelets can serve as biomarkers of deteriorating mitochondrial quality control (Kramer *et al.* 2014). This is now amenable to testing in human populations. Cellular Bioenergetics can be determined using the mitochondrial stress test which is based on the sequential addition of well-characterized inhibitors of oxidative phosphorylation to cells while measuring their rate of oxygen consumption and extracellular acidification (Hill *et al.* 2012). We have proposed that the resulting bioenergetic profile in cells isolated from normal compared to patients can be used to determine a Bioenergetic Health Index (BHI) (Chacko *et al.* 2014). Cellular bioenergetics health can then be compared to mitochondrial function in a parallel experiment using the same cells, by assessing the activity of protein complexes involved in oxidative phosphorylation including complex I and IV (Salabei *et al.* 2014). This new concept may help in developing a modifiable biomarker for PD development, prognosis and response to therapy as well as lead to a better understanding of mitochondrial damage and quality control in the disease.

2. Autophagy and mitophagy in neurodegeneration—Since different mechanisms converge to promote proteotoxicity and disrupt metabolic function, enhancing repair pathways would be beneficial irrespective of these diverse mechanisms of cell impairment. The removal of damaged proteins and mitochondria rely on the highly regulated lysosomal-mediated autophagy pathway (Zhang 2013). Autophagy integrates signals ranging from nutrient availability to cellular stress and, as shown recently, oxidative protein damage including oxidized lipids (Levonen *et al.* 2014, de Duve & Wattiaux 1966, Takeshige *et al.* 1992, Mizushima *et al.* 2008, Zhang 2015). Since its discovery in the 1960s and the identification of the genes encoding the autophagic machinery in the 1990s (de Duve & Wattiaux 1966, Takeshige *et al.* 1992), autophagy has been found to play an important role in health and disease (Mizushima *et al.* 2008). More than 38 autophagy related proteins and many lysosomal proteins are involved in the process (Zhang 2015).

At a technical level, the extension of the autophagy field to encompass a broad range of pathologies has been enabled by straightforward experimental strategies (Klionsky *et al.* 2016, Liang *et al.* 2013, Mitchell *et al.* 2013). For example, the formation and degradation by the lysosomes of the double membraned vesicles, which contain lipidated microtubule associated protein 1A/1B light chain 3 (LC3-II), has been used for assessment of autophagic activities (Klionsky *et al.* 2016, Liang *et al.* 2013, Mitchell *et al.* 2013). Accumulation of p62 protein in the absence of its transcription activation, and accumulated ubiquitinated proteins have been used as additional markers for autophagy inhibition (Jaber *et al.* 2012a, Jaber *et al.* 2012b, Parekh *et al.* 2013, Parekh *et al.* 2017). It is now technically possible to monitor autophagy and mitophagy in live cells. For example, RFP-GFP-LC3, MitoKeima and Mito-QC mice have been generated to facilitate autophagy/mitophagy flux analyses based on sensitivity of green fluorescent protein to the acidic environment of the lysosome. Using these approaches translocation of autophagosomes and mitochondria to the lysosomes for degradation can be monitored over a broad range of conditions (Li *et al.* 2014, Sun *et al.* 2015, Williams *et al.* 2017) (Fig. 2).

Key findings from these studies supporting the pathological importance of the autophagy pathway have come from a number of different model systems. For example, deficits in key autophagy genes, *Atg5* or *Atg7*, in knockout mice, leads to accumulation of ubiquitinated proteins and neurodegeneration (Hara *et al.* 2006, Komatsu *et al.* 2006). Mitophagy plays a key role in mitochondrial quality control (Kim & Lemasters 2011, Lemasters 2005) and proteins that regulate mitophagy such as Parkin, PINK1 and DJ-1, are associated with familial PD (Redmann *et al.* 2016, Lubbe & Morris 2014, Redmann *et al.* 2014). Recent studies demonstrate that the most frequently mutated protein for familial PD, LRRK2, also plays a role in regulating autophagy, and its mutation may be synergistic with α -synuclein in impairing autophagic flux (Saha *et al.* 2015). With more studies and a better understanding of mitophagy regulation (Kim *et al.* 2016, Williams *et al.* 2017), there is hope that new effective therapeutic targets may be developed to focus on improving mitochondrial quality control and cellular metabolism.

3. The importance of the lysosome—In the previous sections, we focused on mitochondria as both an initiator and target of proteotoxicity. Of course dysfunctional protein responses can have broad affects in the cell and in this regard as a key player in the autophagy pathway, lysosomes play a central role in integrating autophagic and endosomal activities and in degrading damaged lipids, proteins, and organelles. Fifty hydrolases perform degradative activities in the lysosomes. Nonetheless, many of the hydrolases are essential for lysosomal activities and organismal health. A single enzyme deficiency could produce lysosomal storage diseases (LSD) and neuronal ceroid lipofuscinosis (NCL) (Schneider & Zhang 2010). A significant proportion of the loci associated with PD are involved in or disrupt vesicular trafficking and the autophagy-lysosome pathway, including LRRK2 and GBA (Gan-Or *et al.* 2015, Diana Chang 2017, Schneider & Zhang 2010, Benitez *et al.* 2016, Chiasserini *et al.* 2015, Mata *et al.* 2015), suggesting a significant impact of lysosomal perturbation in the disease. Among the studies worth noting, a recent study further demonstrated deficient lysosomal enzyme alpha-Galactosidase A in sporadic PD postmortem brains (Nelson *et al.* 2018). LRRK2 has been shown to impact endolysosomes

and autophagy, and may have both autophagy promoting and autophagy inhibitory roles depending on the time/location/cellular context (Manzoni 2017).

Insufficient lysosomal turnover of the pathologic α -synuclein can result in α -synuclein accumulation. This is most evident from studies with the cathepsins that are lysosomal proteases responsible for autophagic degradation. For example, it has been shown that cathepsin D levels are decreased in substantia nigra neurons of PD patients compared to age-matched controls (Chu Y 2009). Furthermore, overexpression of cathepsin D, but not cathepsin B, in *C. elegans*, decreases α -synuclein toxicity (Qiao *et al.* 2008). Interestingly, cathepsin B is implicated in genome-wide association studies to be associated with PD (Diana Chang 2017). It has been shown that microglial secreted cathepsin B induces neuronal cell death (Kingham & Pocock 2001) and that cathepsin B enhances aggregate formation and production of reactive oxygen species induced by exogenous α -synuclein fibrils (Tsujimura *et al.* 2014, Freeman *et al.* 2013).

B. Proteotoxic signaling regulates mitochondrial function and autophagy

As introduced above the proteotoxic potential of α -synuclein is an important aspect of the molecular mechanisms of cell dysfunction in neurodegenerative disease. The pathologies of PD are known to involve Lewy bodies and Lewy neurites, while the major component of these structures was identified as α -synuclein. Among others, α -synuclein, LRRK2 and PARK7 gene mutations have been found to be responsible for a subset of familial PD (Obeso *et al.* 2017). α -synuclein is a 140 amino acid protein expressed in neurons, while its accumulation occurs in PD, Alzheimer's disease, and Dementia with Lewy Bodies brains. Because α -synuclein is associated with pathology, the appearance of phosphorylated or oligomerized forms of α -synuclein in the cerebral spinal fluid are being explored as potential biomarkers with more improved and specific antibodies (Majbour *et al.* 2016). LRRK2 is a large protein of 2,537 amino acids encompassing both kinase and GTPase domains, its mutations are the most frequently found among familial PD (Obeso *et al.* 2017). Parkin, PINK1 and PARK7/DJ-1 are recessive PD, whose loss of function contribute to PD pathogenesis and all of these three genes are implicated in regulating mitophagy (Obeso *et al.* 2017).

1. α -synuclein—Although exact mechanisms are unclear, α -synuclein is normally involved in synaptic function (Abeliovich *et al.* 2000), and misfolding, mutation, or post-translational modification leading to its accumulation is potentially pathogenic (Lashuel *et al.* 2013, Goedert *et al.* 2017). Deficiencies in lysosomal-dependent α -synuclein protein degradation lead to α -synuclein accumulation and neurodegeneration in animal models (Qiao *et al.* 2008, Cullen *et al.* 2009). Lysosomal perturbation has been implicated in PD or *parkinsonism* genetic mutations or polymorphisms, including *LRRK2*, *SNCA*, *LAMP3*, *GBA*, and *ATP13A2* (Gan-Or *et al.* 2015). Pathological α -synuclein can disrupt protein trafficking, induce neuroinflammation, and interfere with the autophagy-lysosomal pathway and mitochondrial function (Lee & Trojanowski 2006, Lashuel *et al.* 2013, Schmid *et al.* 2013, Zhou *et al.* 2016), toxicity is not only intracellular, but also can be intercellular due to the fact that α -synuclein can spread from a donor cell to neighboring cells and thus propagate cellular damage (Luk *et al.* 2012a, Luk *et al.* 2012b, Volpicelli-Daley *et al.* 2011,

Luk et al. 2009, Steiner et al. 2011, Rutherford *et al.* 2015). Endocytosis, direct penetration, trans-synaptic transmission, membrane receptors, and Parkin regulation of lipid rafts have all been suggested to mediate α -synuclein spreading (Lashuel et al. 2013, Cha *et al.* 2015). Autophagic failure has been shown to promote the intercellular transfer of α -synuclein (Desplats *et al.* 2009, Lee *et al.* 2013, Ejlerskov *et al.* 2013).

Not surprisingly, α -synuclein interacts with key redox regulated pathways and proteins in the cell. In the cytosol, α -synuclein interacts with Cu, Zn superoxide dismutase and affects its dimerization without affecting its activity (Helferich *et al.* 2016). α -synuclein targets mitochondria and perturbs mitochondrial function in cell culture and transgenic mouse studies (Elkon et al. 2002, Devi et al. 2008, Shavali *et al.* 2008, Parihar *et al.* 2009, Liu et al. 2009, Loeb *et al.* 2010a, Chinta *et al.* 2010, Loeb et al. 2010b, Zhu *et al.* 2011, Imaizumi *et al.* 2012, Braidy *et al.* 2013, Perfeito *et al.* 2014, Chen *et al.* 2015a, Di Maio *et al.* 2016). A recent study using a novel protein oxidation detection method also demonstrated α -synuclein radical formation after exposure to the pesticides Maneb and paraquat and caused cytochrome *c* release to the cytosol (Kumar *et al.* 2016). In total, one of the key mediators of the proteotoxicity associated with PD has clear mechanistic links to metabolism and redox biology.

2. Other neurotoxic proteins—In addition to α -synuclein, a few other proteins are also either prone to aggregation or connected to autophagy/mitophagy regulation in PD. Mechanisms of their actions may involve their interactions with α -synuclein or each other. The microtubule associated protein tau has been shown to co-localize with α -synuclein in dopaminergic neurons (Wills *et al.* 2010). The co-localization may play a role in sequestering tau from its normal function as α -synuclein fibrils have been shown to promote tau aggregation, resulting in decreased microtubule polymerization (Oikawa *et al.* 2016). The ability of LRRK2 to interact with tau has been demonstrated in HEK-293 cells, where LRRK2 overexpression can increase tau protein, its formation of oligomers/aggregates in these cells, and its secretion with the underlying mechanism involving proteasomal but not autophagy impairment (Guerreiro *et al.* 2016). LRRK2 G2019S mutant induced dendrite degeneration in *Drosophila* in a tau dependent manner (Lin *et al.* 2010). Interestingly, as Tau, LRRK2 has also been shown to interact with cytoskeleton proteins, and may play a role in microtubule dynamics, and microtubule-mediated vesicular transport (Blanca Ramirez *et al.* 2017).

The impact of LRRK2 on autophagy and protein trafficking pathway is complicated, and observations vary depending on the cell type and on whether LRRK2 mutation, silencing, or kinase inhibition was used (Manzoni 2017). Among potential mechanisms of LRRK2 regulation of autophagy, in SH-SY5Y cells and in rat cortical neurons, overexpression of wild type and G2019S mutant LRRK2 induces mitochondrial fragmentation, and this appears to be dependent on Drp1 in SH-SY5Y cells (Wang *et al.* 2012). In HEK294 cells, it has been shown that LRRK2 overexpression can induce autophagy by a calcium-dependent mechanism mediated by CaMKK- β /AMPK pathway, LRRK2 has been found to phosphorylate NSF at T645 in the ATP binding pocket and thereby increases SNARE disassembly (Belluzzi *et al.* 2016), which in turn modulates autophagosome-lysosome fusion (Zhang 2015, Yu *et al.* 2017). Although autophagy can be neuroprotective in clearance of

damaged proteins and organelles, excessive autophagy in mouse cortical dendrites promoted by overexpression of LRRK2 mutant protein can be detrimental and this is also associated with calcium imbalance, and may be mediated by a pathological increase in mitochondrial calcium uptake (Verma *et al.* 2017) (Cherra *et al.* 2013).

PD recessive genes, Parkin, PINK1 and PARK7/DJ-1, have also been shown to play a role in mitochondrial quality control and development of approaches targeting these proteins as therapeutic strategies will likely be important (Goedert *et al.* 2017, Obeso *et al.* 2017, Zhang *et al.* 2017b, Barodia *et al.* 2016, Scott *et al.* 2017). As α -synuclein, PARK7/DJ-1 accumulates in PD brains (Choi *et al.* 2006), and can be a substrate of chaperone-mediated autophagy (Cuervo *et al.* 2004, Wang *et al.* 2016). In a mouse midbrain dopamine neuron progenitor cell line, MPP⁺ exposure increased DJ-1 carbonyl formation and degradation (Wang *et al.* 2016). Overexpression of LAMP2A, the lysosomal receptor of chaperone-mediated autophagy, enhances DJ-1 degradation and cell survival in response to MPP⁺ (Wang *et al.* 2016). Interestingly, recent studies also demonstrated a role of DJ-1 in microglia in autophagy and α -synuclein uptake and clearance (Nash *et al.* 2017).

C. Redox stress in the regulation of mitochondrial function and autophagy

1. Redox stress—The redox regulation of cellular environments is now emerging as an important area of research integrating metabolic and redox-dependent signaling. Interestingly, maintaining a stable intracellular redox environment is critical in both preventing proteotoxicity, and removing by autophagy the resulting damage that occurs before the toxic proteins are removed. It is now abundantly clear that both oxidative and reductive stress can arise if at a given cellular location and time the redox tone is not maintained within physiological boundaries. The regulation of these redox specific intracellular domains is controlled by the Keap1/Nrf2 system (Dodson *et al.* 2015). Interestingly, Nrf2 over activation increases the expression of pro-reductant pathways leading to a reductive stress and proteotoxicity in the heart (Dodson *et al.* 2015, Badia *et al.* 2013, Ralser & Benjamin 2008, Rajasekaran *et al.* 2008, Russell *et al.* 1999). Whether this also occurs in the brain is not well understood. In the context of the “redox signaling paradigm”, reactive oxygen species are produced by controlled by specific pathways and dysfunction arises not from non-specific oxidation of macromolecules but the loss of domain controlled signaling (Dodson *et al.* 2013a). In this context although it is well known that mitochondria can produce reactive oxygen species including superoxide and hydrogen peroxide it is less well appreciated that these can be tightly controlled through and play a role in cell signaling (Murphy 2009, Lee *et al.* 2012a, Dodson *et al.* 2013a, Quinlan *et al.* 2013, Wong *et al.* 2017, Brand 2016). In addition, NADPH oxidase, dual oxidases, monoamine oxidase, cytochrome p450, and xanthine oxidase also generate hydrogen peroxide and superoxide as their normal cellular function (Halliwell & Gutteridge 2015). Excessive reactive oxygen species are dynamically reduced by superoxide dismutases and catalase. Without these enzymes, these reactive species, together with nitric oxide generated by nitric oxide synthases can damage lipid and proteins, and resulting lipid peroxidation product such as HNE can cause further damage to cellular constituents (Dodson *et al.* 2013a). Oxidative post-translational modification of DNA, lipid, and protein is associated with a number of age related pathologies including PD (Halliwell 2012, Kalyanaraman

2013, Sies 2015). Interestingly, reactive lipid species also have a signaling function so the interface between pathological and physiological oxidative lipid signaling is critical to understand (Levonen et al. 2014, Higdon *et al.* 2012b). Since redox protein and lipid modifications are essential for normal physiology, the emerging concept is that pathology arises from loss of control of non-physiological redox modifications and the toxic gain of function of oxidized proteins and lipids (Wende *et al.* 2016b).

2. Oxidative stress and dopaminergic neurodegeneration—In neurons, there are some specific mechanism that can promote oxidative protein modification and result in toxicity. For example, a robust literature has reported an increased in oxidative stress markers in Parkinson's postmortem brains, including elevated lipid peroxidation-protein adducts and protein carbonyls, oxidized complex I subunits, nitration of α -synuclein, increased thiol-DOPA conjugates, and increased glutathione and Coenzyme Q10 oxidation (Giordano *et al.* 2014a, Selley 1998, Yoritaka *et al.* 1996). Oxidation of proteins can contribute to proteotoxicity by blocking the sites needed for proteasomal degradation, or by promoting the formation of insoluble aggregates. An example of a lipid peroxidation-dependent modification is the lipid aldehyde 4-hydroxynonenal (HNE) which forms stable adducts which change protein function by modification of histidine, arginine, lysine and cysteine residues (Higdon *et al.* 2012a). Once formed, the protein adducts can only be removed by controlled degradation of the protein. Their accumulation can result in a toxic gain of function through promotion of protein aggregation or, in the case of redox proteins, such as those found in the mitochondrion, increased levels of reactive oxygen species. Because of the physiological role of oxidized lipids in signaling, it is thus important to distinguish what constitutes a pathological level of these molecules which may vary according to cell type and age (Higdon *et al.* 2012c). As will be discussed further below α -synuclein can be modified by HNE and the modification promotes its aggregation and toxicity (Xiang *et al.* 2015). Aldehyde dehydrogenase 1A1 (ALDH1A1), an enzyme important for metabolizing nonenal as well as dopamine metabolites, is decreased in PD brains, and ALDH1A1 and ALDH2 double knockout mice exhibited increased HNE and 3,4-dihydroxyphenylacetaldehyde (DOPAL) and significant dopaminergic neurodegeneration (Grunblatt & Riederer 2016).

3. Oxidative stress and mitochondria—Since the reactive lipid species or neuronal specific reactive species discussed above can target mitochondria impaired detoxification can lead to the slow accumulation of adducted proteins and a greater demand on the autophagy pathway. In support of this concept experimental evidence has established that the mitochondria are a nexus for both the physiological and pathological effects of reactive lipid species and as such are an important target for potential dysfunction in neurodegenerative disease (Higdon et al. 2012b). Indeed, mitochondrial dysfunction occurs in the brains of PD patients (Ryan et al. 2015, Swerdlow 2009, Schapira 2007, Beal 2003, Parker & Swerdlow 1998, Schapira et al. 1990, Bindoff et al. 1989). Neurotoxins have been shown to target mitochondria in Parkinsonism patients and in animal models (Martinez & Greenamyre 2012, Bove *et al.* 2005). Specifically, mitochondria are a target for HNE modification and this can influence bioenergetic function in a variety of cell types (Dranka *et al.* 2011a, Dranka *et al.* 2011b, Ravi *et al.* 2015). HNE adducts with mitochondrial respiratory chain subunits have

been reported and are associated with enhanced reactive species production and a decrease in the mitochondrial bioenergetic reserve capacity, all of which contribute to a decreased ability of the cell to combat metabolic and proteotoxic stress (Reed *et al.* 2008, Lee *et al.* 2012b, Breitzig *et al.* 2016, Galam *et al.* 2016, Harris *et al.* 2015, Fritz *et al.* 2011, Singh *et al.* 2015). If these damaged mitochondria, which are capable of generating secondary reactive oxygen species and amplifying the initial oxidative insult, are not removed, the threshold for pathology is lowered (Hill *et al.* 2012, Lee *et al.* 2012a, Redmann *et al.* 2014).

4. Oxidative stress and autophagy—The importance of thresholds for toxicity in neuronal cells depends on age, genetics and other factors and can be assessed *in vitro*. For example, in primary neurons, HNE inhibits mitochondrial function and although autophagy is initially protective, it can be overwhelmed as HNE concentrations increase in neurons (Dodson *et al.* 2017, Zhang *et al.* 2017a). It is now possible to identify the HNE modified proteins using sensitive tagging techniques that can help relate specific targets to changes in metabolism and autophagy (Dodson *et al.* 2017, Dodson *et al.* 2013a, Dodson *et al.* 2015, Dodson *et al.* 2013b). Protein thiols targeted by HNE and other oxidants have been demonstrated for autophagy-lysosomal pathway proteins including ATG4, LC3, PARK7/DJ-1, and cathepsins that in the cysteine proteinases family (Lee *et al.* 2012a, Dodson *et al.* 2015, Dodson *et al.* 2013a, Wani *et al.* 2015). ATG4 thiol modification at Cys81 decreases its activity by suppressing LC3 delipidation (Scherz-Shouval *et al.* 2007). The lipidation mechanism is a possible link to inflammation since a 12/15-lipoxygenase-derived oxidized phospholipid, 12-hydroxyeicosatetraenoic acid-phosphatidylethanolamine, was found to be a preferred substrate compared to native phosphatidylethanolamine for yeast Atg8 lipidation (Morgan *et al.* 2015). Sulfenic acid formation at Cys106 of PARK7/DJ-1, a protein encoded by a recessive PD gene, is important for its function in maintaining mitochondrial connectivity and in cell protection when exposed to the mitochondrial toxin rotenone (Blackinton *et al.* 2009). A recent study demonstrated that wildtype but not C106 mutant PARK7/DJ-1 can convert methylglyoxal (pyruvaldehyde)-adducted glutathione to free glutathione and lactate suggesting that it is potentially involved in aldehyde detoxification (Matsuda *et al.* 2017).

Lysosomal cysteine proteases cathepsin B and L, but not aspartate proteases are inactivated when murine macrophage-like cells are exposed to protein hydroperoxides, consistent with the sensitivity of the active site thiol to oxidative stress (Rahmanto *et al.* 2010). Nonetheless, cathepsin D oxidative modification has been found in the brains of Down syndrome patients with a slight increase of enzyme activity, which could be viewed as a potential compensatory response to increased plaques and tangles (Di *et al.* 2016). We propose that autophagy is critical in maintaining cellular redox homeostasis and by removing damaged mitochondria and oxidized proteins it serves a cellular antioxidant function in removing toxic proteins and organelles (Giordano *et al.* 2014a). α -synuclein contains 1 histidine (H50) and 15 lysine residues that are targets for modification by electrophilic lipid peroxidation products such as HNE. Interestingly, it has been shown that HNE promotes α -synuclein toxicity (Xiang *et al.* 2013). This result is intriguing as H50Q is responsible for a subset of familial PD cases (Kiely *et al.* 2015). It turns out that although H50Q α -synuclein is less susceptible to HNE

modification than wildtype α -synuclein, it forms more aggregates and cells expressing H50Q are more sensitive to hydrogen peroxide-induced cell death (Xiang et al. 2015).

5. The impact of nitric oxide on mitochondrial function and autophagy in the context of Parkinson's disease

The previous discussion has focused on either endogenous xenobiotics or the products of lipid or protein oxidation as mediators of toxicity. However, it is important to note that the controlled production of reactive species is an important part of normal physiology. This in itself is potential vulnerability if control of these pathways is disrupted. An important example is nitric oxide (NO) has contrasting roles in neurodegeneration with evidence for both protective and toxic effects. As we have discussed previously, these are best understood in the concentration dependent engagement of distinct cellular targets in the cell (Hill *et al.* 2010). There are essentially two heme based targets; guanylate cyclase and cytochrome c oxidase. The effects on mitochondrial function are more complex. Nitric oxide can reversibly inhibit mitochondrial complex IV at high concentrations or when oxygen tension is low (Cleeter *et al.* 1994, Benavides *et al.* 2013). This can be protective by maintaining physiological oxygen gradients but if NO compromises ATP production then it will become detrimental (Shiva *et al.* 2005). When nitric oxide reacts with superoxide to generate peroxynitrite, the effects on the modified lipid and proteins can be irreversible and detrimental (Benavides *et al.* 2013, Radi *et al.* 1991a, Radi *et al.* 1991b, Ischiropoulos *et al.* 1992, Beckman & Crow 1993, Szabo *et al.* 2007). Interestingly, the mitochondria are thought to be one of the main targets of peroxynitrite in neuronal cells (Estevez *et al.* 2017). In the central nervous system, nitric oxide is generated in the neurons by nNOS, induced by NMDA and glutamate receptor activation, and in microglia by iNOS induced by inflammatory signals. nNOS variants have been found to be associated with PD in conjunction with organophosphate pesticides (Paul *et al.* 2016). Nitric oxide produced by either neurons or glia significantly impacts susceptibility of neurons to toxic insults, as evidence by that nNOS or iNOS gene deletion protects mice against MPTP induced dopaminergic neurodegeneration (Przedborski *et al.* 1996, Liberatore *et al.* 1999). As iNOS production in microglia can be induced by LPS and TNF α , it has been hypothesized that neuroinflammation may play a role in nitric oxide production and impact PD pathogenesis. The important role of microglia, a major source of nitric oxide, in PD is further demonstrated by the finding that a single nucleotide polymorphism (SNP) in the human leukocyte antigen (HLA-DRA) gene, which encodes HLA-DR antigens expressed in microglia, has been identified as a risk factor for PD (Wisseemann *et al.* 2013, Tansey & Goldberg 2010). It remains to be determined whether microglial expansion, activation or morphological changes are beneficial or detrimental factors in PD pathogenesis.

Although a direct hit of excessive nitric oxide to neurons appear to be detrimental to its mitochondrial function, the role of systemic nitric oxide is dependent on its source and cellular context. Nitric oxide generated from inorganic nitrite has been shown to play a protective role in variety of pathologies including cardiovascular diseases (Weitzberg *et al.* 2010, Rocha *et al.* 2016). Nitrite administration in zebrafish protected dopaminergic neurodegeneration induced by 1-methyl-4-phenylpyridinium (MPP $^{+}$), in rat protected dopaminergic neurodegeneration induced by rotenone or 6-hydroxydopamine, and in dermal fibroblasts from human PD patients with LRRK2 mutations (Chiara Milanese 2017). Since,

as mentioned above, nNOS appears to be detrimental in this context then an alternative mechanism of action for nitrite is implied. Indeed, in MPP⁺ treated SH-SY5Y cells, it appeared that the mechanisms of nitrite protection involve nitrosation of mitochondrial complex I as well as activation of transcription factor Nrf2 (Chiara Milanese 2017).

It has been shown that in a variety of cell types, nitric oxide can inhibit autophagy (Benavides et al. 2013, Sarkar *et al.* 2011), although how the regulation of autophagy by nitric oxide affects PD pathogenesis is still unclear. Transient autophagosome accumulation is induced by kainic acid, an agonist of glutamate receptors, and delayed increase of p-Akt and p-mTOR follows (Shacka *et al.* 2007). The regulation of autophagy by kainic acid may be mediated by nitric oxide as glutamate receptor activation has been shown to activate nNOS (Dawson & Dawson 1998). Inhibition of autophagy by nitric oxide during hypoxia-reoxygenation may contribute to bioenergetics impairment and promote cell death in neurons (Benavides et al. 2013).

D. Metabolic signaling regulates mitochondrial function and autophagy

1. Glucose metabolism—From the previous discussion it is evident that physiological metabolism utilizes a broad range of redox reactions and is essential for normal neuronal function. For this reason, attention has increasingly focused on cellular bioenergetics and metabolism in health and diseases (Holmes *et al.* 2008). Deficiencies of PD genes, Parkin and PINK1, affect mitochondrial homeostasis or quality control (Narendra *et al.* 2012). Parkinsonism gene Atg13a2 mutations have been shown to cause lysosomal (Ramirez *et al.* 2006, Usenovic *et al.* 2012), mitochondrial (Grunewald *et al.* 2012), and glycolytic dysfunction (Park *et al.* 2016). As introduced above, MPTP and pesticides that induce *Parkinsonism* or increase PD risk target mitochondrial complex I, thereby depressing mitochondrial function (Ramsay et al. 1987, Nicklas et al. 1987, Mizuno et al. 1987, Priyadarshi et al. 2001, Priyadarshi et al. 2000, van der et al. 2012, Tanner et al. 2011, Giordano et al. 2012, Giordano et al. 2014b). In addition to direct targeting of mitochondrial electron transport proteins, additional signaling mechanisms may impact PD pathogenesis, including modification of mitochondrial fission and fusion, and modification of mitochondrial movement within the neuron (Yan *et al.* 2013). Because complex and chronic diseases such as PD are affected by systemic as well as organ-specific metabolism, changes in metabolic activities may be monitored as biomarkers for progression of the diseases as well as therapeutic responses (Chacko et al. 2014).

While there is some emphasis on glycolysis to provide ATP in the brain, glucose metabolism is also essential to provide NADPH to maintain lipid oxidation products including HNE within the physiological range. This link between metabolism and redox signaling is an important part of normal physiology. In addition, it is known that glucose metabolism can regulate autophagy through several signaling mechanisms in a variety of cell types (Moruno *et al.* 2012). Key enzymes in the glucose metabolism pathway, hexokinase and GAPDH, have been shown to interact with mTOR or Rheb and decreases mTOR signaling when glucose is low (Eng & Abraham 2011, Roberts & Miyamoto 2015). The integration of glucose metabolism and autophagy has also been demonstrated by a recent study that autophagy proteins, ULK1 and ULK2, can also directly phosphorylate enzymes in the

glycolytic and gluconeogenic pathways to enhance glucose uptake and decrease gluconeogenesis in order to maintain cellular ATP production and redox homeostasis (Li *et al.* 2016). Whether similar regulatory mechanisms play a role in neurons and in the pathogenesis of PD is currently unclear. Assessment of glucose metabolism by 18-F-2-fluoro-deoxyglucose and positron emission tomography has shown a decline of glucose metabolism in aging (Zuendorf *et al.* 2003). This assessment has been used in Alzheimer's disease diagnosis (Nordberg *et al.* 2010). Furthermore, it has been shown that diabetes increases Alzheimer's disease risk (Holscher 2011). Despite of these findings, the association between glucose metabolism and non-demented PD has not been firmly established (Cereda *et al.* 2013, Hu *et al.* 2000).

A significant amount of glucose is metabolized through the hexosamine biosynthetic pathway, which produces UDP-GlcNAc. UDP-GlcNAc can be added to protein Ser/Thr residues and modify protein functions. This modification may be of importance to Alzheimer's and PD through direct modification of tau or α -synuclein (Yuzwa *et al.* 2008, Yuzwa *et al.* 2012, Yuzwa *et al.* 2014, Wani *et al.* 2016, Wani *et al.* 2017), and through regulation of mitochondrial function and autophagy (Hu *et al.* 2009, Makino *et al.* 2011, Pekkurnaz *et al.* 2014, Lozano *et al.* 2014, Dassanayaka *et al.* 2015, Trapannone *et al.* 2016, Wani *et al.* 2017, Guo *et al.* 2014, Roberts *et al.* 2014, Jo *et al.* 2016). The extent and biological impact of O-GlcNAc modification of proteins in the context of mitochondrial function, autophagy and PD still needs to be further defined. Other metabolites that have been shown to play an important role in regulating mitochondrial and autophagic activities include glutamine and acetyl CoA (Tan *et al.* 2017, Hjelmeland & Zhang 2016, Marino *et al.* 2014, Shi & Tu 2015). Although studies have been performed regarding changes of metabolites in toxin and transgenic mice, the involvement of particular metabolites in PD are currently uncertain (Chen *et al.* 2015b, Worth *et al.* 2014).

2. Fatty acid oxidation—Although glucose is the preferred fuel for the brain, fatty acid oxidation products are also important substrates under conditions of hypoglycemia (Romano *et al.* 2017). A higher uptake of hydrogenated and saturated fat in the brain has been associated with development of insulin resistance and Alzheimer's disease (Holscher 2011), while a ketogenic diet has been shown to be neuroprotective in a variety of conditions (Danial *et al.* 2013, Romano *et al.* 2017). The connection between lipid metabolism and autophagy has been further demonstrated by the finding that SREBP1 and SREBF1 can regulate autophagy and mitophagy (Ivatt *et al.* 2014, Jegga *et al.* 2011). As *SREBF1* is a risk locus for sporadic PD, this finding provides insight into the connection between fatty acid metabolism, mitochondria, and mitophagy in PD pathogenic mechanisms. This important link between metabolism and PD is further supported by the observations that gastrointestinal abnormalities and sleep perturbations are often symptoms preceding motor dysfunction in PD patients.

3. Systemic regulation of metabolism—Naturally, we have largely focused on the local effects of metabolism and protein quality control in the central nervous system. An important and emerging area relates to how the systemic effects of metabolism and redox regulation interact with the pathophysiology of neurodegenerative disease. α -synuclein

pathology has been shown to occur early in the enteric nervous system in PD patients (Del Tredici & Braak 2016). A recent study has shown benefit for motor function of a glucagon-like peptide-1 receptor agonist approved for treatment of type 2 diabetes on PD patients in a clinical trial (Athauda *et al.* 2017). Furthermore, systemic control of metabolism is particularly interesting with respect to the microbiome which has recently been shown to be a potential player in PD (Nair *et al.* 2018, Hill-Burns *et al.* 2017). Recent studies identified differences in microbiome in PD patients compared to controls (Scheperjans *et al.* 2015). In mice overexpressing α -synuclein, both germ-free condition and antibiotic treatment have shown to ameliorate motor deficits, α -synuclein accumulation, microglial activation and cytokine production (Sampson *et al.* 2016). Colonization with microbiota from PD patients, or oral administration of short-chain fatty acids, which are produced during viral infection by the gut microbiome and are decreased by germ-free condition or antibiotic treatment, exacerbates these phenotypes (Sampson *et al.* 2016). The interconnection of the gut environment, the metabolites generated, and PD pathogenesis warrants further investigation.

Circadian rhythms integrate metabolism with time-of-day demands for normal physiology, and disruption is associated with increased susceptibility to disease (Wende *et al.* 2016a, Abbott *et al.* 2018). Indeed, sleep perturbation is an important clinical feature of PD with as many as 64% PD patients reporting sleep problems. The mechanisms are still emerging but it has been shown that BMAL1, a clock regulatory protein, is decreased in the peripheral blood of PD patients and is no longer rhythmic (Breen *et al.* 2014). As circadian clock is a strong regulator of metabolism, and recent studies identified key autophagy genes under circadian regulation (Wende *et al.* 2016a), the clock-metabolism-autophagy connection may play a key role in PD pathogenesis and may be targeted for therapeutic purposes (Musiek & Holtzman 2016, Breen *et al.* 2014, Wende *et al.* 2016a).

Therapeutic strategies and future directions

In this review, we have highlighted the integrated nature of metabolism, the response to stress and how the limited capacity of repair systems contributes to the pathophysiology of PD. At the level of the proteotoxicity mechanisms, multiple strategies have been explored to target α -synuclein for the development of new therapies for PD (Olanow & Kordower 2017, Valera & Masliah 2016). Proteasomal and lysosomal pathways can degrade α -synuclein *in vivo* but these functions decline both with age and with the development of PD (Chu Y 2009). There is an urgent unmet need to investigate novel approaches to enhance the clearance of α -synuclein protein. Autophagy is an attractive therapeutic strategy because it has the potential to not only remove excessive or toxic α -synuclein species, but also degrade other damaged cellular constituents. In this regard, rapamycin and other autophagy activators have been shown to be protective in cell and animal models of neurodegenerative diseases (Hochfeld *et al.* 2013). However, rapamycin may promote a starvation response that degrades both healthy and unhealthy components of the cells. Furthermore, the potential benefits of inducing autophagy initiation are dependent on optimal lysosomal function, which appears to be inhibited in PD. Thus, it is important to find a strategy to restore the efficiency of clearing damaged cellular proteins and organelles in the lysosomes, without depleting those that are still functional. Studies have identified roles of TFEB as a transcription factor activating the expression of genes encoding lysosomal proteins

(Decressac *et al.* 2013, Settembre *et al.* 2011). Transcriptional activation of a wide range of autophagy proteins is effective in animal models (Decressac *et al.* 2013), although it may also lead to excessive autophagy which depletes cellular contents and is then detrimental. Thus, it is important to increase the efficiency of the clearance of damaged cellular proteins and organelles without affecting the undamaged cell constituents.

In this regard, lysosomal cathepsin D is a viable target for enhancement of autophagic clearance. As discussed early, cathepsin D is a major lysosomal aspartate protease, its levels are decreased to <50% in PD substantia nigra neurons with α -synuclein inclusions compared to age-matched controls (Chu Y 2009), and its deficiencies have pathological consequences. Cathepsin D inactivation in humans caused neuronal ceroid lipofuscinosis and early lethality (Siintola *et al.* 2006, Steinfeld *et al.* 2006). In humans, sheep and mice, cathepsin D mutations resulted in accumulation of α -synuclein (Qiao *et al.* 2008, Cullen *et al.* 2009). The therapeutic potential of overexpression of cathepsin D has been demonstrated in worms and mammalian cells to decrease α -synuclein aggregation and toxicity (Qiao *et al.* 2008), although its effectiveness in mammalian models *in vivo* has not been examined. Strategies need to be explored for enhancing cathepsin D activities *in vivo* by nutraceutical and pharmaceutical agents or by allosteric activators (Ambrosi *et al.* 2015, McNeill *et al.* 2014, Parker *et al.* 2017) or by better delivery strategies that allow BBB penetration (Spencer *et al.* 2015). In addition to lysosomal enhancement, in light of the special importance of mitochondrial quality control, specific mitophagy enhancement strategies may be effective in disease cases when mitochondrial damage occurs (Hertz *et al.* 2013, Zhang *et al.* 2017b).

Since oxidative stress has been associated with PD, various antioxidants have been tested and found to show efficacy in pre-clinical models of PD, although so far largely ineffective in clinical trials (Giordano *et al.* 2014a). An important and emerging aspect of redox biology is the recognition that the redox state of the cell must be retained at defined set points which vary within different regions of the cell. This finding in itself requires that redox modulatory therapeutics must be targeted to the intracellular compartment that is in a hyperoxidative or reductive state relative to the physiological set point. At the present time, we know little about how the redox status of the intracellular organelles varies in PD and this is an essential piece of information for the development of targeted therapeutics in this area. Nevertheless, testing natural products and anti-inflammatory reagents are still ongoing with majority of studies relying on pre-clinical models (Baranyi *et al.* 2016, Chen *et al.* 2015c, Zhou *et al.* 2016). A more mechanistic approach with an underlying understanding of the different phases of redox biology which occur during the life history of the disease is an urgent need as more potent and druggable targets emerge from the pre-clinical studies.

Because of the importance of mitochondria as an energy generating and signaling organelle and autophagy as the cellular quality control machinery, therapeutics targeting both mitochondria and the autophagy pathway are being actively considered. In this regard, several mito-centric approaches have been used. For example, toxin-resistant complex I subunit, enhancing complex II activities, mitochondrially targeted therapeutics, or stabilizing mitochondrial membrane potential, have all shown some degree of efficacy (Marella *et al.* 2008, Tieu *et al.* 2003, Reeves *et al.* 2007). A recent study also demonstrated that

administration of inorganic nitrite ameliorates pathology in animal models of PD by complex I S-nitrosation and activation of antioxidant Nrf2 pathway (Chiara Milanese 2017). Enhancement of mitochondrial biogenesis has been shown to be neuroprotective in human dopaminergic neurons (Makela *et al.* 2016). In further support for enhancing mitochondrial function as a therapeutic development, it has also been shown that an FDA approved anti-helminth drug nitazoxanide may induce mitochondrial uncoupling and protects against MPP + toxin-induced parkinsonism in mice (Amireddy *et al.* 2017). Mitochondrial targeted antioxidants show promises in some but not all clinical scenarios that involve oxidative stress, and thus more effective means to counter cellular damage need to be developed (Logan & Murphy 2017, Jin *et al.* 2014). Efforts have also been made to directly target potential antioxidant or modulatory molecules (catalase, SS-31 peptide or mitoQ) to the mitochondria (Basisty *et al.* 2016, Dai *et al.* 2016, Chaturvedi & Beal 2013, Reily *et al.* 2013, Dashdorj *et al.* 2013, Solesio *et al.* 2013, Gioscia-Ryan *et al.* 2014, Miquel *et al.* 2014, Dare *et al.* 2015, Stucki *et al.* 2016, Rehman *et al.* 2016, Logan & Murphy 2017). Effectiveness of these approaches on PD needs to be further investigated. Metabolomics studies have also help identify new targets for cardioprotection (Chouchani *et al.* 2014, Vinje *et al.* 2014). Similar strategies may also help develop new target for PD therapeutics. Last but not least, with better understanding of how circadian rhythm plays a role in regulating metabolism, mitochondrial function and autophagy, pharmacological regulation of circadian behavior and metabolic processes is an area which is as yet relatively unexplored (Breen *et al.* 2014, Solt *et al.* 2012) (Fig. 3).

Although progress has been made regarding mitochondrial and autophagy function regulated by redox, metabolic and proteotoxic signaling, there is still a great deal we do not know. Some key questions include the following:

- In susceptible neurons and glia, are there specific targets for specific redox signals, metabolites, and/or toxic protein species?
- Which steps are reversible or can be counteracted to attenuate disease progression?
- Can we design targeted and effective antioxidants, efficient and stress resistant mitochondria, and specific lysosomal enhancement strategies to attenuate Parkinson's disease?
- Can targeting the microbiome change the progression or susceptibility to neurodegenerative disease?

Research effort to address these questions will be informative in the next decades.

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Abbreviation List

| | |
|------------|-----------------|
| 3MA | 3-methyladenine |
|------------|-----------------|

| | |
|----------------|---|
| ADH1A1 | aldehyde dehydrogenase 1A1 |
| ADH2 | aldehyde dehydrogenase 2 |
| AMPK | adenosine monophosphate (AMP)-activated protein kinase |
| ATG5 | autophagy related 5 |
| ATG7 | autophagy related 7 |
| ATP13A2 | ATPase type 13A2 |
| BECN1 | beclin 1, autophagy related |
| BHI | bioenergetic health index |
| BMAL1 | brain and muscle Arnt-like protein 1 |
| CaMKK | calcium-dependent protein kinase kinase |
| CQ | chloroquine |
| Drp1 | dynamin 1-like |
| FCCP | carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazine |
| GAPDH | glyceraldehyde 3-phosphate dehydrogenase |
| GBA | glucosidase, β , acid |
| HBP | hexosamine biosynthetic pathway |
| HK | hexokinase |
| HNE | 4-hydroxynonenal |
| LRRK2 | leucine-rich repeat kinase 2 |
| LC3 | microtubule-associated protein 1 light chain 3 alpha/beta |
| MFN1 | mitofusin 1 |
| MFN2 | mitofusin 2 |
| mtDNA | mitochondrial DNA |
| mTOR | mechanistic target of rapamycin (serine/threonine kinase) |
| NO | nitric oxide |
| NRF2 | Nuclear factor (erythroid-derived 2)-like 2 |
| OCR | oxygen consumption rate |
| OPA1 | mitochondrial dynamin like GTPase |
| PD | Parkinson's Disease |

| | |
|--------------------------------|---|
| PINK1 | PTEN-induced putative kinase 1 |
| PGC1α | peroxisome proliferative activated receptor, gamma, coactivator 1 alpha |
| PPP | pentose phosphate pathways |
| REM | rapid eye movement |
| SNARE | Soluble NSF-Attachment protein Receptor |
| SOD2 | superoxide dismutase 2, mitochondrial |
| SQSTM1/p62 | sequestosome 1 |
| SREBF1 | sterol regulatory element binding transcription factor 1 |
| TCA | tricarboxylic acid |
| TFAM | transcription factor A, mitochondrial |
| TFEB | transcription factor EB |
| ULK | unc-51 Like Autophagy Activating Kinase |
| VDAC1 | voltage-dependent anion channel 1 |

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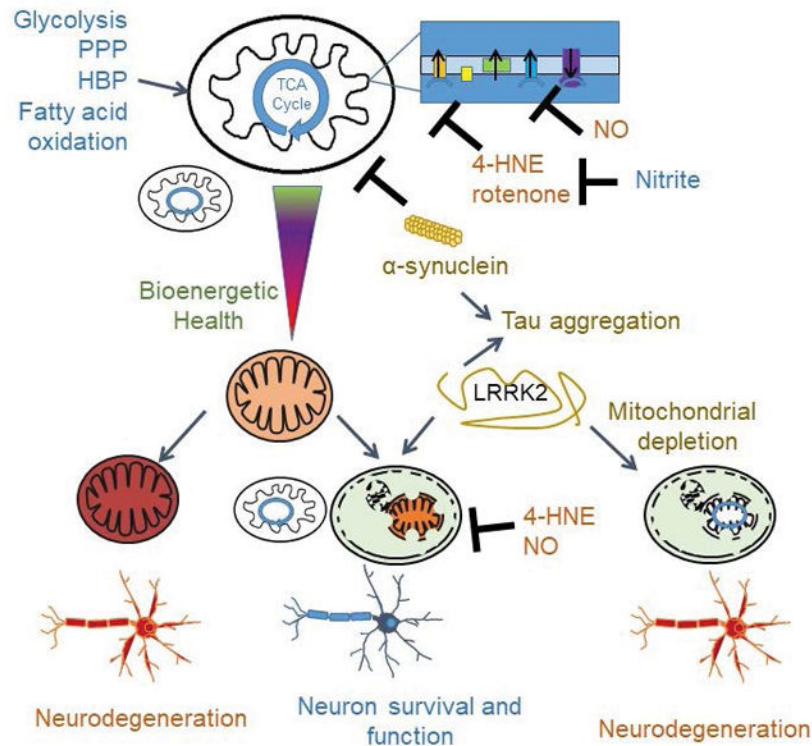


Figure 1. Mitochondrial function and autophagy: integrating redox, metabolic and proteotoxic stress in Parkinson's disease

Mitochondria and the autophagy machinery are essential for neuronal function and health. Mitochondria are not only the major source of cellular energy but also a signaling nexus for metabolites and modulating redox status and the bioenergetic health may be used as biomarkers for disease prognosis and response to therapeutics. Because of the high demand of cellular energy and the high content of fatty acids in neurons, reactive oxygen and nitrogen species can be generated intracellularly, as well as from neighboring glia cells, or even due to exposure to environmental toxins. Metabolic regulation of redox signaling, mitochondrial function and autophagy is of importance in the central nervous system via pathways including but not limited to glycolysis, hexoamine biosynthesis, and fatty acid oxidation. Furthermore, protein homeostasis, as represented by α -synuclein and tau homeostasis is important for Parkinson's disease. α -synuclein can target to and inhibit mitochondrial function, and itself is subject to HNE modification and nitration. Autophagy is a major pathway for α -synuclein degradation and the clearance of damaged mitochondria, but is also targeted by proteotoxic proteins including α -synuclein, tau and LRRK2. In this review, we highlight the evidence of how mitochondria are targeted by proteotoxic, redox and metabolic stress, while maintained by the autophagic surveillance. These areas present opportunities to better understand Parkinson's disease pathogenesis and potential for development of effective therapies.

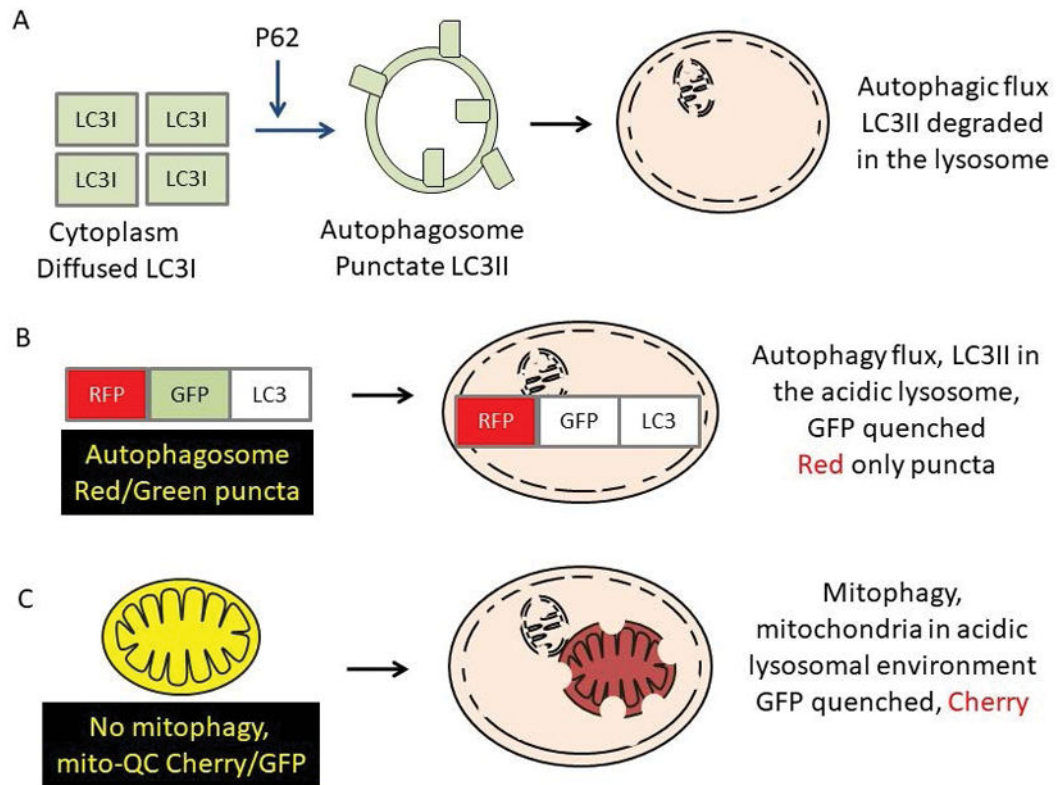


Figure 2. Assessment of autophagic and mitophagic flux

A. The amount of LC3II in the presence and absence of inhibitors that blocks lysosomal degradation of LC3II, and the p62 accumulation in the absence of enhanced p62 transcription activation is used to assess autophagic flux. **B.** Cells or animals expressing reporter gene RFP-GFP-LC3 can also be examined for autophagic flux based on the amount of LC3 that reaches the lysosomes due to the pH sensitivity of GFP. **C.** Cells or animals expressing reporter gene mitochondrial matrix targeted Cherry-GFP-LC3 can be examined for mitophagic flux based on the amount of LC3 that reaches the lysosomes due to the pH sensitivity of GFP.

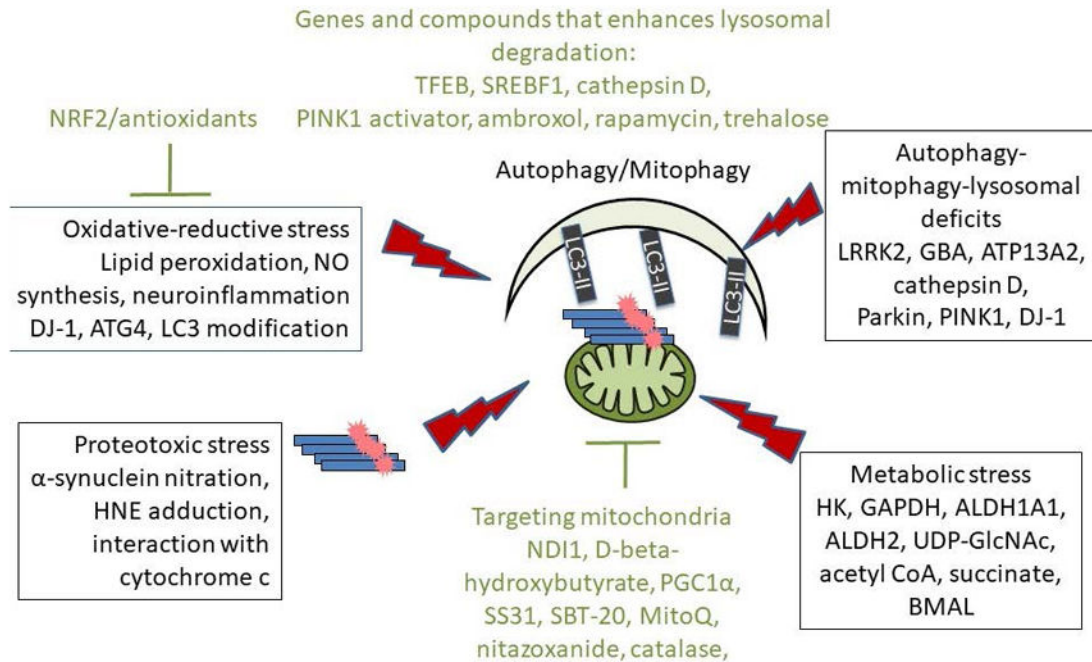


Figure 3. Potential targets and therapeutic strategies for treating Parkinson's disease based on autophagy-lysosomal pathway, bioenergetics and metabolism, as well as redox signaling and oxidative-reductive stress

Major efforts have been made in recent years to develop broad spectrum and/or organelle targeted antioxidant strategies, and enhanced autophagy, mitophagy, and lysosomal activities. Highlighted in this figure are some of the key events of metabolic, oxidative-reductive, and proteotoxic stress (in boxes), as well as molecules that have been tested in *in vitro* and animal models (in green) that target metabolism and autophagy/mitophagy/lysosomal pathways.