Mitochondrial Metabolism Modulation: A New Therapeutic Approach for Parkinson's Disease

D.M. Arduíno[1], A.R. Esteves[1], C.R. Oliveira[1,2] and S.M. Cardoso[*,1,3]

[1]*Centro de Neurociências e Biologia Celular,* [2]*Instituto de Bioquímica,* [3]*Instituto de Biologia, Faculdade de Medicina, Universidade de Coimbra, 3004-517 Coimbra, Portugal*

Abstract: Mitochondrial metabolism is a highly orchestrated phenomenon in which many enzyme systems cooperate in a variety of pathways to dictate cellular fate. As well as its vital role in cellular energy metabolism (ATP production), mitochondria are powerful organelles that regulate reactive oxygen species production, NAD^+/NADH ratio and programmed cell death. In addition, mitochondrial abnormalities have been well-recognized to contribute to degenerative diseases, like Parkinson´s disease (PD). Particularly a deficiency in the mitochondrial respiratory chain complex I and cristae disruption have been consistently described in PD. Moreover, the products of PD-familial genes, including α-synuclein, Parkin, PINK1, DJ-1, LRRK2 and HTR2A, were shown to localize to the mitochondria under certain conditions. It seems that PD has a mitochondrial component so events that would modulate normal mitochondrial functions may compromise neuronal survival. However, it remains an open question whether alterations of these pathways lead to different aspects of PD or whether they converge at a point that is the common denominator of PD pathogenesis. In this review we will focus on mitochondrial metabolic control and its implications on sirtuins activation, microtubule dynamics and the autophagic-lysosomal pathway. We will address mitochondrial metabolism modulation as a new promising therapeutic tool for PD.

Keywords: Mitochondria, Parkinson's disease, autophagy, sirtuins, microtubules, mitochondrial metabolism, therapeutic targets, metabolic control.

1. THE INVOLVEMENT OF MITOCHONDRIA IN PARKINSON'S DISEASE

The classical view on mitochondria has stated that their prime role is to supply energy, in the form of ATP, to be utilized in cellular reactions. However, these organelles have turned out to play vital roles in calcium homeostasis, in formation of reactive oxygen species (ROS) and in the initiation of apoptosis and so they are intimately associated to cellular homeostasis. Therefore, cellular respiration and, consequently, mitochondrial metabolism has a critical role in normal brain function, where the impaired functioning of mitochondria has been implicated in several neurological disorders, like Parkinson's disease (PD) [1].

PD is a progressive, disabling neurodegenerative disease characterized clinically by the primary motor symptoms of resting tremor, rigidity and slowness. The neuropathological hallmark of PD includes not only the loss of dopaminergic neurons in the substantia nigra and other brainstem nuclei but also the presence of cytoplasmic inclusions (Lewy bodies, LBs) in surviving neurons (reviewed in [2]).

The most compelling evidence of mitochondrial dysfunction in PD emerged following the human accidental exposure to the synthetic meperidine analogue 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which induced a parkinsonian syndrome [3,4]. Mitochondrial association with idiopathic PD was first established when a complex-I (CXI) deficiency was identified in the substantia nigra (SN) of PD patients postmortem brain [5]. To address the potential causes of CXI defect, namely if it was due to an environmental toxin or to an alteration of mitochondrial DNA (mtDNA) or nuclear DNA, the cytoplasmic hybrid (cybrid) cell model was used. Such a model reproduces the respiratory chain defects present in diseased patients. These cybrid cells are created by the transfer of mtDNA to clonal neuronal-like cells which have been depleted of their endogenous mtDNA by the long-term application of a low concentration of ethidium bromide. Host cells are then polyethylene glycol fused with platelets, containing no nuclear DNA, from a control or diseased patient. This technique allows investigators to specifically investigate the role of mtDNA in cellular pathology. A stable decrease in CXI activity, increased ROS production and increased susceptibility to 1-methyl-4-phenylpyridinium ion (MPP^+) in PD cybrids, was described [6-9]. Moreover, the generation of fibrillar and vesicular inclusions in a cybrid model of sporadic PD, was reported. These inclusions replicate most antigenic and structural features of LBs, without the need for exogenous protein expression or inhibition of mitochondrial or proteasomal function [10].

Recently, another undeniable piece of evidence of mitochondrial dysfunction in PD has come from conditional knockout mice, termed "MitoPark" mice, the first animal model showing the slow progressive degeneration of dopamine neurons seen in PD. These mice have a disruption of the gene for mitochondrial transcription factor A (*Tfam*) in dopaminergic neurons. Moreover, this mouse model shows reduced mtDNA expression, reduced respiratory chain function in midbrain dopaminergic neurons which, in turn, leads to a parkinsonian phenotype, with adult onset of slowly progressive impairment of motor function associated to the formation of intraneuronal inclusions and dopamine nerve cell death [11]. All of these hallmarks are consistent with the involvement of respiratory chain dysfunction in PD pathogenesis. Further, there is evidence of reduced mitochondrial mass and size in mouse SN dopaminergic neurons as compared to non-dopaminergic neurons, suggesting selective vulnerability of dopaminergic neurons as a result of a mitochondrial dysfunction [12].

In addition to mtDNA mutations, pathogenic mutations in several genes, including α–synuclein, parkin, UCHL-1, DJ-1, PINK-1, LRRK-2, NURR- 1, tau, and HTRA2, also directly or indirectly implicate a role of mitochondrial dysfunction in familial PD pathogenesis [13-15]. α–Synuclein is a major component of LBs, and gene mutations are associated with autosomal dominant familial PD [14, 15]. Thus, even though the involvement of mitochondria in PD is quite well established, its exact role in the cellular degeneration cascade of events is still a debatable matter.

*Address correspondence to this author at the Faculdade de Medicina e Centro de Neurociências e Biologia Celular da Universidade de Coimbra, 3004-517 Coimbra, Portugal; Tel: +351-239-820190; Fax: +351-239-822776; E-mail: smacardoso@yahoo.com

1871-5273/10 $55.00+.00

In this review, we will address the role of mitochondria as a crucial organelle in the cellular fate in PD, focusing on mitochondrial metabolic control and its implications on sirtuins (SIRTs) activation, MT dynamics and intracellular trafficking.

2. MITOCHONDRIAL METABOLISM IN THE BRAIN

The human brain has a high-energy requirement. Even though it accounts for only 2% of the total body mass, the brain consumes almost 20% of the total oxygen, which constitutes the global resting metabolism. In an adult brain, glucose has been traditionally considered as the primary energy source for neurons, being fundamental to maintain its high metabolic rate. Effectively, the brain has no oxygen stores, and only small reserves of high-energy phosphate compounds and carbohydrates. These energy reserves are merely able to sustain ATP levels for about one minute in the absence of blood. Thus, a balance between a continual supply of nutrients from the blood and the energy demands for brain mitochondria is critical.

2.1. Mitochondria Structure and Function

Mitochondria are distinct and powerful organelles that have long been known to be the site of many central biochemical pathways, vital for normal cellular function, particularly cellular energy generation (in the form of ATP) *via* oxidative phosphorylation. Each mitochondrion comprises a matrix delimited by two membranes, the mitochondrial inner membrane (MIM) and the mitochondrial outer membrane. The convoluted and invaginated MIM includes multiple enzymes of oxidative phosphorylation, the cofactor coenzyme Q1 or ubiquinone, the F0-F1-ATP synthase, and some carrier proteins.

In the matrix, surrounded by the MIM, there are many enzymes for different metabolic pathways, including the citric acid cycle also known as the tricarboxylic acid cycle (TCA cycle), fatty acid oxidation, the urea cycle and also mtDNA, peptidases and chaperones. The mitochondrial respiratory chain, consisting of several enzyme complexes and cofactors (named complexes I–IV, the last enzyme is often referred to as complex V; I: NADH ubiquinone reductase, II: succinate ubiquinone reductase, III: ubiquinol cytochrome c reductase, IV: cytochrome c oxidase, V: F1F0-ATP synthase), is implanted in the inner membrane, arranged functionally according to the electrochemical hierarchy based on their redox potentials.

The TCA cycle maintains the coenzymes NADH and flavoproteins in a reduced state to supply reducing equivalents for the electron transport chain. Together with the latter, the transfer of electrons originating from the oxidation of $NADH_2$ (at complex I) or $FADH_2$ (at complex III) by ubiquinone to complex IV occurs where they react with molecular oxygen (O_2) to reduce it to H_2O.

Therapeutic intervention at the mitochondrial level will be discussed in section 5.1.

2.2. The Energetic Threshold Effect

Mitochondria are highly compartmentalized cellular organelles which have their own DNAs. mtDNA is a circular double-stranded molecule containing 37 genes (16,569 base pairs) within the mitochondrial matrix, encoding for 13 proteins (all of them part of the respiratory chain and oxidative phosphorylation system), 22 transfer RNAs, 12S and 16S ribosomal RNAs. The mitochondria divide mainly in response to the energy needs of the cell. When a cell needs high energy, the mitochondria grow and divide, and when the cell uses low energy, they are destroyed or become inactive. A single mitochondrion contains several copies of its genome (2–15 copies, termed "polyplasmia"). Notably, a peculiar characteristic to mtDNA is that it is inherited exclusively through the mother, and may exist in many different copies in the oocyte cytoplasm. This implies that no mtDNA recombination occurs at fertilization and only a sequential accumulation of mutations from the maternal lineage account for mtDNA variations. Moreover, mtDNA is particularly prone to mutation, being estimated as 10 times greater than nuclear DNA, due to the absence of protective proteins (such as histones) and of a high-efficiency repair system.

Thus, mutant and wild-type (normal) mtDNA can coexist within a cell in any proportion ("heteroplasmy"). Their proportion can be altered during ageing, and symptoms appear above a certain threshold ("threshold effect"). As consequence, there are at least four levels at which threshold effects occur in mitochondrial metabolism, with respect to their possible involvement in the pathogenesis of neurodegeneration. The first is the expression of the heteroplasmy of mtDNA at the level of a given enzymatic step, whereby mitochondria from patients might exhibit a particular ratio of defective DNA compared to normal DNA. The second is the threshold effect observed in mitochondrial metabolism as a result of a decrease in a given mitochondrial activity. The third may occur in the expression of defective mitochondria with respect to the whole cellular metabolism. The fourth is the fact that the metabolic control coefficient, which quantitatively expresses the fractional change in pathway flux of a metabolic network, under steady-state conditions, induced by a fractional change in the individual step under consideration, may vary depending on different types of mitochondria. This leads to the observation that the threshold value for a given complex of the electron transport chain can vary according to the threshold in the energy demand of different tissues. At each level the threshold effect will reinforce the others, as interpreted by the *Double threshold* hypothesis. This hypothesis associates the threshold in the expression of a deficit, to the threshold in the energy demand of different tissues, in order to describe various patterns of onset of mitochondrial diseases [16].

It is becoming increasingly clear that mutations at the mtDNA level may play an essential role in the pathogenesis of neurodegenerative diseases, and evidence for mitochondria being sites of damage in neurodegenerative disorders is based in part on observed decreases in the respiratory chain complex activities in PD [17]. Such defects in respiratory complex activities, possibly associated with oxidant/antioxidant imbalance, are thought to underlie defects in energy metabolism and induce cellular degeneration. The mitochondrial origin of these defects is also supported by the results of experiments with cybrid cells [18], which reproduce the respiratory chain defects present in diseased patients. Moreover, our previous results with PD cybrids allow us to propose that mitochondrial dysfunction could be the initial event in either sporadic or idiopathic PD [8, 9].

It is thus worth considering that molecules belonging to the fundamental mediators of diverse biological processes, including energy metabolism and mitochondrial functions, could be proposed as fundamental key players in an accurate modulation of the mitochondrial function, which is crucial to the cellular survival. Accordingly, it is tempting to suggest that NAD^+ and NADH, two classic molecules of intermediary metabolism, have remarkable roles in modulating key multiple factors in cell death and energy state, reductive biosynthesis and antioxidation, and as aging-influencing factors (for review see [19]). NAD^+ can mediate cytosolic energy metabolism through several pathways: NAD^+ mediates glycolysis by acting as the co-factors for the glycolytic enzyme GAPDH and also modulates other important energy metabolism-related cytosolic reactions, such as lactate dehydrogenase catalyzed lactate-pyruvate conversions. Besides, cytosolic NADH can also affect mitochondrial oxidative phosphorylation due to the NADH shuttling from cytosol to mitochondria. Concerning mitochondrial energy metabolism, NADH is one of the major electron donors for the electron transport chain and NAD^+ is the coenzyme for the three rate-limiting enzymes in the TCA cycle.

Studies of Belenky and colleagues [20] and Piper and colleagues [21] have shown that lifespan extension is dependent upon NAD^+ synthesis and that an efficient mitochondrial function was necessary for maximal longevity. In addition, mitochondrial uncoupling, which increases NADH oxidation, decreased telomere damage and delayed senescence in cultured human fibroblasts [22]. In agreement with the beneficial effects of NADH oxidation to regenerate NAD^+ by means of mitochondrial function, Easlon and colleagues [23] demonstrated that the malate-aspartate NADH shuttle played a major role in mediating lifespan extension under caloric restriction (CR) in yeast. The efficient regeneration of NAD^+ *via* effective mitochondria is also compatible with mitochondrial ageing theories which claim that mitochondrial dysfunction is the key to the onset of ageing.

Therapeutic intervention at the mitochondrial energetic level will be discussed in section 5.1.

3. MITOCHONDRIAL METABOLIC CONTROL AND SIRT REGULATION AND SORTING

Accumulating evidence suggests that NAD^+ could be a crucial factor in the ageing process by regulating SIRTs.

3.1. The Family of Mammalian SIRTs

Initially discovered in yeast, SIRTs are a family of mammalian proteins which mediate a deacetylation reaction that couples lysine deacetylation to NAD^+ hydrolysis. This hydrolysis yields O-acetyl-ADP-ribose, the deacetylated substrate, and nicotinamide (NAM) (reviewed in [24,25]).

Silent information regulator 2, the first gene discovered in this family, was primarily shown to be involved in transcriptional silencing at cell-mating type loci (a telomere in *Saccharomyces cerevisiae*), and in the suppression of recombination at yeast ribosomal DNA, through deacetylation of the epsilon-amino groups of lysines in the amino-terminal domains of histones.

It was subsequently recognized that a growing number of non-histone proteins are also deacetylated by SIRTs. These non-histone SIRT substrates comprise several transcriptional regulators, such as the nuclear factor-kB, forkhead box type O transcription factors (FOXO), and the peroxisome proliferator-activated receptor gamma, coactivator-1 alpha (PGC1-alpha), as well as enzymes such as acetyl coenzyme A synthetase 2 (AceCS2), and structural proteins, such as alpha-tubulin [26]. Seven human homologues of SIRTs (SIRT1-7), which share the catalytic domain with SIR2 enzyme in yeast, have been characterized [27,28]. SIRTs occupy several cellular compartments, such as the nucleus (SIRT1, 2, 3, 6, and 7), cytoplasm (SIRT1 and 2), and mitochondria (SIRT3, 4, and 5) [29-35].

Given the subcellular sorting of SIRTs and their dependence on NAD^+ and the cellular NAD^+/NADH ratio, the absolute levels of NAD^+, NADH, or NAM, or a combination of these variables, SIRTs may be considered extremely versatile energy sensors for cellular metabolic status [36-40]. Moreover, the fact that several of the SIRT protein substrates, such as AceCS2 and PGC-1alpha are involved in metabolism also suggests a metabolic role for this protein family.

3.2. Metabolic Functions of Mammalian SIRTs

SIRT1 seems to have a significant role in mammalian metabolic control as it may regulate many physiological processes known to be affected during aging and which are altered by CR. A study of Bordone and colleagues showed that SIRT1 transgenic mice display phenotypes resembling mice subjected to CR [41]. In fact, SIRT1 deacetylates a large number of substrates, including p53, Ku70, nuclear factor-kB to affect stress resistance in cells [42-47], which may be related to the observed stress resistance conferred by CR [48].

Possibly the most relevant target of SIRT1 in the metabolic field is the cofactor PGC1-alpha, a major regulator of mitochondrial biogenesis. Indeed, subtle evidence from studies of the polyphenol resveratrol in mice shows that SIRT1 activation may improve mitochondrial functions. Resveratrol affects the activity of SIRT1 *in vitro* [49], although its effects seem to depend on the nature of the substrate for deacetylation [24,50,51]. However, *in vivo*, resveratrol exerts its effects dependent on SIRT orthologs – extension of lifespan in yeast, *C. elegans* and *Drosophila*, and metabolic effects on mammalian cells [48,49,52]. The opposing actions of resveratrol have given rise to some controversy with respect to its beneficial action. This issue will be fully discussed in the section Therapeutic intervention at SIRTs level.

Recent studies have demonstrated that PGC1-alpha when activated by SIRT-1-mediated deacetylation promotes mitochondrial function in skeletal muscle and brown adipose tissue, leading to protection against associated metabolic dysfunction [53,54]. These studies also highlighted the role of SIRT1 acting upstream of PGC1-alpha as a fundamental regulator of mitochondrial activity. Thus, changes in cellular NAD^+ that affect SIRT1 deacetylase activity seem to signal PGC1-alpha concerning the cellular energy status. PGC1-alpha can further adapt cellular energy production through its powerful role on mitochondrial biogenesis and function.

SIRT1 may also have a role in neuronal cell survival. SIRT1 can act as an anti-apoptotic factor for cultured neurons, perhaps through the down-regulation of proapoptotic factors such as p53 [42, 43] and FOXO. More interestingly, a crucial role for SIRT1 in disease of the central nervous system has also been introduced in studies with animal models. In the Wallerian slow degeneration (Wlds) mouse model, SIRT1 activation protects axons against neuronal injury. This Wlds mouse carry a dominant mutation producing an overexpressed chimeric Wlds protein composed of the ubiquitin assembly protein Ufd2a and the NAD^+ salvage pathway enzyme nicotinamide mononucleotide adenyltransferase. Araki and colleagues [55] showed that a decreased SIRT1 activity reduced the axonal protection originally observed, whereas SIRT1 activation by resveratrol decreased axonal degeneration after neuronal injury. This suggests that the neuroprotective effect of nicotinamide mononucleotide adenyltransferase in the Wlds mouse model could be mediated by an increase of the neuronal NAD^+ reserve and/or SIRT1 activity [55].

In addition, a recent study has suggested that SIRT1-related signaling pathways are involved in the regulation of autophagy in mammals. Lee and colleagues [56] demonstrated that SIRT1 is an activator of autophagy, both in cultured cells and *in vivo* in transgenic mice. Some characteristics in the phenotype of $Sirt1^{-/-}$ knockout mice resemble those of $Atg5^{-/-}$ knockout mice. For example, there is an accumulation of damaged organelles, which might be evidence for inhibition of autophagy. Furthermore, SIRT1 interacted with and deacetylated several components in the complexes of forming autophagosomes, such as Atg5, Atg7 and Atg8 proteins [56]. These observations demonstrate that protein acetylation regulates macroautophagic processes. It is known that acetyltransferases and deacetylases can modulate protein stability, thereby altering the turnover of these target proteins [57]. The results of Lee and colleagues [56] are particularly interesting, as they link the Sir2 longevity factor with autophagic degradation impairment, which is closely associated with aging and age-related pathologies, like PD.

SIRT2 is predominantly a cytoplasmic protein [32,58,59] which colocalizes with tubulin, and can deacetylate a number of substrates *in vitro*, including alpha-tubulin [59]. Compared to SIRT1, the role of SIRT2 in neurodegenerative diseases are less well-established. In a recent study, SIRT2 was recognized as an inhibitor of

oligodendroglial cell differentiation through deacetylation of the MT cytoskeleton [60]. This could protect oligodendrocytes for the purposes of remyelination or myelinogenesis processes. However, in some circumstances, the effects of SIRT2 appear to be injurious to neuronal cell survival. For example, SIRT2 could lower MT stability, probably by reducing tubulin polymerization, increasing depolymerization, or altering the binding of tubulin to associated proteins. SIRT2-mediated tubulin deacetylation abolished degeneration resistance in the Wlds mouse model subjected to axonal injury induced by MT-depolymerizing drugs, suggesting that tubulin deacetylation promotes axonal MT destabilization [61].

Outeiro and colleagues [62] have identified potent inhibitors of SIRT2 such as NAM, *O*-acetyl-ADP-ribose, or the small molecule inhibitors AGK2 and AK-1. These authors demonstrated that inhibition of SIRT2 prevented α-synuclein cytotoxicity and modulated its aggregation in cultured cells; ameliorated mutant α-synuclein neurotoxicity in rat primary dopamine-positive neurons; rescued degeneration of dopaminergic neurons from α-synuclein toxicity in a *Drosophila* animal PD model; and also that the acetylation state of tubulin increased in a dose-dependent manner upon treatment with AGK2 in HeLa cells. The authors suggested that modulation of the α-synuclein aggregation pathway could be one of the SIRT2 neuroprotective mechanisms [62].

At first glance, there appears to be a paradox when comparing the protective action of SIRT1 and SITR2 in neurodegenerative diseases. Nevertheless, SIRT2 has also been implicated in a variety of other cellular processes. In addition to tubulin, SIRT2 deacetylates p53 [63], FOXO3a [64], and histones [65]. These interesting data point to a potentially broad regulatory role for SIRT2, particularly in mitosis/cell cycle regulation [58,66], apoptosis [63], and aging by oxidative stress [64]. So, depending on its localization in the cell and on the cellular redox status, SIRT2 appears to have a dual role concerning its putative targets of deacetylation.

Proteins capable of responding to metabolic changes in mitochondria that alter the NAD^+/NADH ratio include the three SIRTs located in that cellular compartment (SIRT3, 4, and 5). SIRT3 was originally described to be a mitochondrial protein, but afterward it was demonstrated that this SIRT translocates from its usual nuclear location to the mitochondria upon cellular stress [35,67,68]. SIRT3 expression is particularly in mitochondria-rich tissues [32,69]. Interestingly, SIRT3 expression in both white and brown adipose tissue is increased in response to CR and decreased in diabetic/obese ob/ob mice [69]. Recent experiments indicate that mice lacking both SIRT3 alleles exhibit a remarkable hyperacetylation of mitochondrial proteins. Given that about 20% of the mitochondrial proteins are acetylated [70], which is greater relative to cytosolic and nuclear proteins, it implies that SIRT3 could be one of the major deacetylases in mitochondria that regulate global mitochondrial lysine acetylation [71]. However, SIRT3-deficient mice are metabolically unaffected under basal conditions [71].

Analogous to SIRT1, the constitutive expression of SIRT3 promotes the expression of PGC-1alpha, uncoupling protein-1, and other genes involved in mitochondrial functions. One mitochondrial activity of SIRT3 is the deacetylation and activation of the mitochondrial form of AceCS2 [72, 73], an enzyme that catalyzes the formation of acetyl CoA from acetate. Deacetylation of AceCS2 consequently increases the conversion of acetate into acetyl CoA, an intermediate of the TCA cycle. As SIRT3 facilitates the metabolic use of acetate, it may be particularly important to guarantee energy production under conditions when ATP pools are limited [72-74].

Aside from AceCS2 and glutamate dehydrogenase, other interacting partners of SIRT3 have been reported. SIRT3 physically binds to and deacetylates Ku70, and this promotes its interaction with the proapoptotic protein Bax. Thus, increased expression of SIRT3 protects from stress stimuli, in part by hindering the translocation of Bax to mitochondria [75]. Moreover, a very recent study by Law and colleagues [76] demonstrated that ATP5A, a subunit of respiratory chain complex V, is one of the potential targets of human SIRT3. In addition, these authors showed that HSP70, a recognized molecular chaperone that assists in the folding and transport of proteins, and which plays protective roles against diverse stressful conditions, is one of the targets associated with both SIRT1 and SIRT3 [76]. These studies emphasize the metabolic and anti-aging function of SIRT1 and SIRT3 by regulating the acetylation status and other major cellular activities, recognizing them as cell-protective molecules and potential regulators of aging. Actually, mutations in the *sirt3* gene enhancer, which up-regulates its expression, were increased in long-lived individuals [77].

SIRT4 is another mitochondrial SIRT protein involved in regulating energy metabolism. In contrast to SIRT3, SIRT4 lacks detectable deacetylase activity [31,48,78]; however, it exhibits mono-ADP-ribosyltransferase activity *in vitro* [78]. Recently, SIRT4 was shown to interact with glutamate dehydrogenase, mediating ADP-ribosylation of this enzyme, leading to the inhibition of its enzymatic activity [31]. Moreover, SIRT4 activity appears to be down-regulated in cells during CR [31], which is in contrast to SIRT1 and SIRT3, whose expression is enhanced during CR. So, it seems that an orchestrated mechanism of regulation of SIRTs based on reversible activation/inhibition cycles which could be translated into feed-forward loops, determining both the redox and nutritional status of the cell. SIRT4 also interacts with two other mitochondrial proteins, the adenine nucleotide transporters ANT2 and 3, and a mitochondrial protease called insulin-degrading enzyme [78]. However, the physiological importance of these interactions has not yet been proved.

SIRT5, was initially described as a mitochondrial protein [32,71], with low-level deacetylase activity on chemically acetylated histone peptides *in vitro* and absence of ADP-ribosyltransferase activity [31, 59]. Recently, Pfister and colleagues (2008) demonstrated that although SIRT5 is strictly mitochondrial in many cells types, in cerebellar granule neurons it is generally present in the cytoplasm and nucleus, with only a small proportion of cells displaying selective mitochondrial localization. They found that when localized to the mitochondria the protective activity of SIRT5 is replaced by a pro-apoptotic effect [79]. However, no target or biological function has been described to SIRT5, making it an important topic for future studies.

Therapeutic intervention at SIRTs level will be discussed in section 5.2.

4. MITOCHONDRIAL METABOLIC CONTROL INVOLVEMENT ON THE MT DYNAMICS AND AUTOPHAGIC-LYSOSOMAL PATHWAY-RELATED VESICULAR TRAFFICKING

Mitochondria are extremely dynamic organelles that undergo constant changes in shape and cellular distribution in order to execute their role at the proper time and location. Therefore, mitochondria accumulate in subcellular regions with high metabolic energy demands and/or where calcium buffering is required [80] and reallocate in response to changes in the local energy state [81,82]. The main mechanism for delivering cellular components to their site of action is long-distance MT-based transport. In turn, fast, long-distance, axonal transport of mitochondria is accomplished through the MT network. MTs are one of the main components of the cytoskeleton, consisting of parallel polymers of α- and β-tubulin dimers. Another important feature of microtubule structure is polarity. Tubulin polymerizes end-to-end with the α-subunit of one tubulin dimer contacting the β-subunit of the next. Therefore, in a protofilament, one end will have the α-subunit exposed while

the other end will have the β-subunit exposed. These ends are designated the slow growing "minus" end and the fast growing "plus" end, respectively [83,84]. This polarity is utilized by MT-associated motor proteins that move "cargo" to the minus or plus ends of cellular MTs. Essential to the function of MTs is their rapid and time-sensitive growth and shortening dynamics (dynamic instability). This stochastic switch from growth to shrinking involves the binding and hydrolysis of GTP by tubulin. Each tubulin monomer binds one molecule of GTP.

Also implicated in mitochondrial transport and distribution are motor proteins of the kinesin superfamily for anterograde organelle transport, and those of the dynein family for retrograde transport [82]. For short-range transport, mitochondria can also move along actin filaments which serve as tracks to areas where the MTs do not reach [85-89]. Kinesins and dyneins are tyical molecular motors as they convert the energy of ATP in their work. The ATP/ADP and GTP/GDP recycling and bioavailability through functionally active mitochondria may control their proper motility and cellular localization. In fact, mitochondrial movements depend on respiration [90] and stop if respiration is inhibited or under extreme energetic conditions, *i.e.*, in the presence of high ATP and high ADP concentrations in the cytoplasm. Inhibition of mitochondrial migration by ADP has been considered to be the mechanism by which mitochondria become trapped at sites with high energy demand [90,91]. Part of the mechanism underlying this trapping could be a lack of ATP for binding and activity of the motor molecules driving the organelles along cytoskeletal structures. Inhibition of motility by high cytoplasmic ATP is supposed to follow similar principles. Motor molecules lose their connection to the cytoskeletal fibrils at increased ATP concentrations, as shown for dynein-MT interaction [92]. Efficient mitochondrial trafficking is particularly important in neurons, where mitochondria are obliged to travel considerable distances along axons to supply synaptic endings with the energy needed for neurotransmitter release and recycling [90].

Accumulating data indicate that abnormal mitochondrial dynamics can contribute to the pathogenesis of late-onset neurodegenerative conditions such as PD [93]. Interestingly, MPP^+ and rotenone, two mitochondrial complex I inhibitors that induce a parkinsonism syndrome *in vivo*, affect MT dynamics [94,95]. Moreover, MPP^+ decreased anterograde and increased retrograde transport of both mitochondria and vesicles, probably due to a reduction of ATP supply to molecular motors. Inhibition of anterograde mitochondrial transport leads to an increase in retrograde transport, which results in depletion of mitochondria from axons and subsequent accumulation in cell bodies [96].

Misfolded and aggregated proteins are also transported to, and deposited in the pericentriolar region *via* the MT system [97]. These MT-dependent deposits of aggregates are called aggresomes and may explain the biogenesis of inclusion bodies found in neurodegenerative disorders such as PD. MT impairment is being increasingly associated with abnormal accumulation of α-synuclein, one of the major constituents of LB [98]. Although the mechanism by which α-synuclein accumulates in LB is not fully understood, evidence suggests that defective axonal transport of α-synuclein itself may contribute to the process. Saha and colleagues [99] found that mutant forms of α-synuclein exhibit reduced axonal transport in transfected cultured neurons. Moreover, transfection of mutant A30P α-synuclein, but not the *wild-type* protein results in its accumulation proximal to the cell body [99]. Thus, blocking α-synuclein transport may induce its accumulation in LB in both sporadic and some cases of familial PD. On the other hand, Lee and co-workers [98] showed that overexpression of α-synuclein caused disruption of the MT network and impairment of MT-dependent trafficking. Additionally, Kim and co-workers [100] showed that tubulin can stimulate α-synuclein fibrillization in yeast. Moreover, exposing cells to a MT assembly inhibitor or deleting genes involved in MT biogenesis increased α-synuclein aggregation and toxicity [100]. Thus, there are two possible mechanisms underlying the cross-talk between α-synuclein and MT function and, interestingly, they seem to be not mutually exclusive.

Another interesting finding was the fact that α-synuclein is a functional MT-associated protein. Alim and co-workers demonstrated for the first time the MT-polymerizing activity of α-synuclein [101,102], and showed that α-synuclein induces polymerization of purified tubulin into MTs whereas mutant forms of α-synuclein lose this potential We demonstrated that cells harboring mitochondrial deficits (PD cybrids) have an increase in free tubulin and in α-synuclein oligomer content. We proved that mitochondrial-dependent ATP depletion and ROS generation induced an increase in free/polymerized tubulin ratio that was responsible for the increased formation of α-synuclein oligomers [8]. This accumulation of functionally impaired mitochondria and MT network destabilization can be counteracted by the formation of new mitochondria, degradation of non-functional organelles and/or aggregated proteins within a cell, substituting the impaired functions. We believe these processes require a dynamic state of mitochondria at several levels of organization and interaction: (i) the distribution of mitochondria within a cell, (ii) changing overall morphology, (iii) the dynamic interaction with vesicular trafficking, and (iv) turnover mechanisms and rearrangements of proteins.

Macroautophagy is a cellular process that not only degrades long-lived proteins to produce the amino acids required for ATP synthesis when nutrients are limited (for instance, during starvation), but also eliminates functionally superfluous or damaged intracellular structures such as mitochondria and endoplasmic reticulum. Degradation of MTs or organelles by macroautophagy requires the formation of double-membrane vesicles called autophagosomes and subsequent fusion of autophagosomes with lysosomes. This fusion produces an autophagolysosome within which the cargo is degraded by acidic lysosomal hydrolases [96]. Thus, the cytoskeleton is fundamental in maintaining the spatial organization of the autophagic-lysosomal pathway (ALP) by conducting the trafficking of organelles involved in different interactions during this process. Autophagic flux is MT-dependent because depolymerization of MTs with nocodazole inhibits the fusion of autophagosomes with lysosomes [103,104]. Depolymerization of MTs results in a decrease in the clearance of autophagy substrates [105]. Within neurons, autophagosomes and endosomes are actively formed in synapses and along neurites. Efficient clearance of these compartments involves their retrograde transport towards the neuronal cell body, where lysosomes are concentrated. When the dynamic instability and, hence the functional integrity of MTs is compromised, autophagosome maturation [106,107] and autophagosome–lysosomal fusion are impaired, as autophagosomes are unable to move from the cell periphery to the MT organization center *via* MTs. Moreover, recently it was shown that the knockdown of dynein leads to similar effects [108], confirming that dyneins are the key motor proteins mediating transport of autophagosomes along MTs towards lysosomes [109].

Growing evidence suggests ALP failure in PD pathogenesis. Ultrastructural examination revealed autophagic vacuoles in myelinized neurons of the substantia nigra in PD patients. Moreover, increased autophagosomes have been observed in human PD nigral neurons [110], but not in nigral neurons during normal aging. More recently, autophagic vacuoles have been found to engulf mitochondria in PD substantia nigra [111]. ALP is also implicated in PD since its inhibition leads to *wild-type* α-synuclein accumulation, suggesting that this lysosomal pathway is also involved in normal α-synuclein turnover [112]. Moreover, wild-type α-synuclein is selectively translocated into lysosomes for degradation by the chaperone-mediated autophagy pathway [113]. The fact that mutant α-synucleins and dopamine-modified α-synuclein inhibit ALP functioning by tightly binding to the Lamp-2 receptor on the lysosomal membrane for autophagy pathway

[113,114] further supports the view that impairment of the ALP may be related to the development of PD.

Our preliminary results show that cells having an increase in α-synuclein oligomer content due to mitochondrial dysfunction have enhanced autophagic vacuole formation. We have also observed that inhibition of macroautophagy in MPP^+ treated cells prompts apoptosis. These results indicate that ALP is an important pathway for normal α-synuclein degradation in neurons and underlies the importance of autophagy as a degradation mechanism in the nervous system. It is therefore quite reasonable that α-synuclein accumulation in sporadic PD reflects a dysfunction in degradation mechanisms, although other processes such as mitochondrial alterations and impairment of axonal transport could also be involved. On the other hand, mutant proteins such as PINK and Parkin have been linked to the early onset Parkinsonism. These proteins are associated in a common pathway involved in the protection of mitochondrial integrity and function. PINK1 has a role in cellular protection against oxidative stress [115] and affects mitochondrial dynamics and morphology in cooperation with mitochondrial fusion/ fission proteins [116]. Others, like UCH-L1, a component of the ubiquitin-proteosomal system and ATP13A2, a lysosomal ATPase, support a role for alterations in the intracellular protein degradation systems in PD pathogenesis. It thus seems that mitochondrial and proteolytic mechanisms are intimately related and mutually influence each other.

Therapeutic intervention at the MT network and ALP levels will be discussed in sections 5.3 and 5.4, respectively.

5. THERAPEUTIC APPROACHES FOR PD TREATMENT

Because mitochondria have a crucial role in the neurodegenerative process in PD, a new area, called mitochondrial medicine, hascome into being [117], where mitochondria are viewed as potential therapeutic targets. Along with an increased understanding of the mechanisms underlying mitochondrial dysfunction, therapeutic strategies were developed based mainly on the administration of antioxidants, provision of nutrients, and cofactor replacement (reviewed in [2]). However, the lack of effective treatment for PD has stimulated great interest in the development of new neuroprotective approaches to prevent or treat progressive loss of neural function leading to serious impairment and death (Table **1**).

5.1. Therapeutic Intervention at Mitochondria Level

The development of new therapeutic agents that interact with mitochondrial function is actively under pursuit, including selective monoamine oxidase (MAO) inhibitors. MAO, an integral enzyme of the mitochondrial outer membrane, is present in peripheral organs and neuronal cells. Of the two isoforms, MAO-A is found predominantly in non-neuronal tissue, while MAO-B is the major isoform in the brain. MAO-B is abundant in the striatum, and is involved in dopamine metabolism. Selective MAO inhibitors are therefore of great interest for improving PD motor symptoms by augmenting striatal dopamine [118,119]. Beginning in the mid-1970s, selegiline (deprenyl), a selective and irreversible propargylamine drug, was the main selective MAO- B inhibitor used in the clinic. More recently, rasagiline [N-propargyl-l(R)-aminoindan], a second-generation propargylamine pharmacophore that selectively and irreversibly inhibits brain MAO-B, was specifically designed for the treatment of PD. Rasagiline, in contrast to selegiline, is not metabolized to potentially toxic amphetamines and its major metabolite 1-R-aminoindan has demonstrated therapeutic effects in neuronal cultures [120] and animal models of PD [121]. In addition, a large body of evidence indicates that selegiline and rasagiline are neuroprotective in a variety of pharmacological models *in vitro* and *in vivo* [122]. For example, selegiline and rasagiline (10 mg/kg body weight) markedly attenuated the neurotoxic effect of MPTP in a non-human primate PD model at the behavioral, histological, and biochemical levels, in parallel to significant inhibition of MAO activity [123]. Paradoxally, the anti-apoptotic and pro-survival properties of rasagiline appear to be independent of MAO-B inhibition but mediated by the propargyl moiety (as reviewed in [124]. The pharmacological mechanism of rasagiline-mediated neuroprotection was studied in neuronal cell cultures using different types of oxidative and trophic withdrawal stress models. The anti-apoptotic effects of rasagiline are proposed to be mediated by activation of anti-apoptotic proteins as a result of drug binding to the flavin adenine dinucleotide binding site in GAPDH and other anti-apoptotic proteins [122]. Based on the proof-of-concept of rasagiline's neuroprotective effects in *in vitro* and *in vivo* pharmacological models, the potential neuroprotective effect of rasagiline was also considered in clinical trials.

Dopamine agonists also represent a valid therapeutic option in PD. Their effect on non-motor domains like mood or cognition is now acknowledged as a key factor in fully addressing patients' needs. Pramipexole is a well-established dopamine agonist currently being investigated for its potential as mitochondrial neuroprotectant. Several studies have described an antioxidative action of the drug and/or a preservation of mitochondrial function [125-128]. In addition, Shapira and co-workers [129] showed that the neuroprotective effects of pramipexole against cell death induced by MPP^+ and rotenone *in vitro* were partly independent of its dopaminergic agonism. More recently, a study by Sayeed and colleagues [130] suggested that pramipexole could exert part of its neuroprotective effect by inhibition of the mitochondrial permeability transition pore, thus probably blocking the mitochondrial pathway of the apoptosis cascade [130].

The involvement of mitochondrial energy metabolism in neurodegenerative processes is also an attractive field. Several agents are currently under investigation for their potential neuroprotective effects, based on their capacity to modify mitochondrial dysfunction and improve mitochondrial bioenergetics. These include creatine, coenzyme Q10 (CoQ10), and NAM. Among them, creatine and CoQ10 are in clinical trials for PD. Creatine is a nitrogenous organic acid that occurs naturally in vertebrates and helps to supply energy to muscle and nerve cells. The creatine/phosphocreatine system, regulated by the mitochondrial creatine kinase, plays an important role in maintaining energy balance in the brain. The presence of this energy buffer system keeps the ATP/ADP ratio high at subcellular sites where ATP is needed, which ensures that the free energy of ATP remains high. This also minimizes the loss of adenosine nucleotides, which causes cellular dysfunction. Creatine supplementation conferred neuroprotective effects for PD in a series of *in vitro* and *in vivo* studies [131]. Chronic administration of creatine significantly increased survival, tyrosine hydroxylase immunoreactive fiber density, and soma size of dopaminergic neurons in mesencephalic culture by protecting against neurotoxic insults induced by serum and glucose deprivation, MPP^+, and 6-hydroxydopamine [132,133]. In aged mice, creatine improves health and survival [134]. Beal and co-workers [135] also found that creatine protected against dopamine loss and attenuated neuron loss in the SN of mice treated with MPTP [135]. The authors also showed that creatine significantly improves survival and motor performance, slows the development of brain atrophy, increases brain ATP levels, and delays atrophy of striatal neurons and the formation of huntingtin-positive aggregates in the R6/2 and N-171–82Q transgenic mouse models of Hungtinton's disease [136]. The success of creatine in experimental studies led to clinical trials in neurodegenerative diseases, including PD.

CoQ10 is an essential co-factor of the electron transport chain (accepts electrons from complexes I and II) and possesses antioxidant properties. CoQ10 levels were found to be significantly lower in mitochondria from PD patients as compared with age-matched controls [137]. The recognized beneficial effects of CoQ10 were evaluated in skin fibroblasts cultured from PD patients. The

Table 1. Compounds with Potential Neuroprotective Actions, Their Subcellular Targets/Mechanism Effects

Compound Type	Examples	Target/Mechanism	Potential Neuroprotective Effects
Mitochondria-targeted drugs	Rasagiline	FAD binding site in GAPDH; anti-apoptotic proteins	Activation of anti-apoptotic proteins
	Coenzyme Q10	Cofactor of complex I, II, III and MOMP inhibition	Antioxidants;
	Pramipexole MitoQ Szeto-Schiller (SS-31) peptide	Inhibition of MPT and MOMP	Prevention of lipid peroxidation, oxidative stress and damage; Reduction of ROS formation; Prevention of mitochondrial damage; Prevention of apoptosis, necrosis, oxidative stress
	SkQs	Inhibition of MIM disruption	
	Creatine	PC system; Inhibition of MPT	Increase of brain ATP levels
	Nicotinamide	Precursor of NADH; inhibition of poly-ADP-ribose polymerase	Induction of NAD^+ metabolism
Modulators of SIRTs activity	NAM NmR *O*-AA-ribose AGK2 AK-1	Inhibition of SIRT2 activity	Inhibition of SIRT2-mediated deacetylation of MTs
	Resveratrol	Induction of PGC-1alpha and SIRT1 activity	Antioxidant; Induction of mitochondrial activity
Microtubules inhibitors	Taxol (paclitaxel) Paclitaxel C-10 carbamate derivative	Stabilization of MTs (beta-tubulin binding)	Maintenance of the MT network structure
Autophagy- inducing compounds	Rapamycin	Inhibition mammalian target of rapamycin (mTOR)	Improvement of the clearance of aggregate- prone proteins
	Trehalose SMERs	Unknown, mTOR-independent	Inhibition of protein aggregation
	Lithium Carbamazepine Sodium valproate	Inhibition of inositol monophosphatase, and decrease of inositol and IP3 levels	

in vitro administration of CoQ10 partially ameliorated respiratory chain complexes I and IV activity [138]. Beal and co-workers [139] reported that the administration of CoQ10 to 24-month-old mice treated with MPTP significantly prevented the loss of tyrosine hydroxylase and neuronal dopamine depletion. Muller and collaborators [140] tested the symptomatic response of daily oral application of CoQ10 in 28 treated and stable PD patients, and observed a significant mild symptomatic benefit and a better improvement of performance compared to placebo. Mitochondria-targeted antioxidants/peptides that selectively block mitochondrial oxidative damage and prevent some types of cell death have also been developed. These compounds contain antioxidant moieties, such as MitoQ (an analogue of CoQ10), tocopherol, or nitroxide, which are targeted to mitochondria by covalent attachment to a lipophilic triphenylphosphonium cation. For example, MitoQ was shown to play an important role in modulating ROS-induced mitochondrial permeability transition and cell death and to be protective in several *in vitro* and *in vivo* models of neurodegeneration [141]. MitoQ reduces ROS formation and preserves mitochondrial function after glutathione depletion, even in cells lacking mitochondrial DNA [142]. MitoQ is currently in a phase II clinical trial for PD. Similarly, a novel antioxidant peptide

Szeto-Schiller (SS-31) targeted to the MIM prevents apoptosis, necrosis, oxidative stress, and inhibition of the mitochondrial electron transport chain [143].

Skulachev and co-workers [144,145] recently developed highly effective, mitochondrial-targeted, rechargeable antioxidants composed of plastoquinone, hydrocarbon linker, and a penetrating cation (SkQs). These compounds have been tested in a wide range of systems: from model membranes, isolated mitochondria, cell cultures, *ex vivo* organs, to living organis. Utilizing the fungus *Podospora*, invertebrates *Ceriodaphnia* and *Drosophila*, and mice, SkQ1 was found to increase lifespan at much lower concentrations than previously described for mitochondrial-targeted peptides. This effect is accompanied by the disappearance or retardation of many crucial traits of senescence and an increase in lifespan in mice. The authors suggested that SkQ1 is competent in switching off a senescence program responsible for a concerted decline with age of key physiological functions. This small molecule thus shows promise as an emergent drug for prolonging youth.

NAM is a precursor of NAD^+. NAM was first demonstrated to prevent MPTP-induced neurodegeneration in mice [146]. Araki and colleagues [55] later showed that preservation of NAD^+ levels by NAM supplementation protects neurons from axonal degeneration. Recently, it was confirmed that NAD^+ metabolism can protect neurons from excitotoxic degeneration [147]. Of note, Mattson and collaborators [147] showed that adding NAM to neuronal cultures preserved the total cellular NAD^+ level. Cellular SIRT1 and poly(ADP)ribose polymerase-1 expression levels were also preserved and excitotoxic neuronal death was attenuated [147]. Other studies have also raised the potential therapeutic role of other NAD^+ precursors. Sasaki and colleagues [148] found that exogenous application of NAD^+ precursors, such as, nicotinic acid mononucleotide, nicotinamide mononucleotide, and nicotinamide riboside protected against axonal degeneration after axotomy. Furthermore, they also showed that the Wlds effect is mediated *via* alteration of the NAD^+ biosynthetic pathway [148]. Another study by Belenky and colleagues reported that nicotinamide riboside can promote Sir2-dependent gene silencing and markedly extend the replicative lifespan of yeast without CR [20]. It was further confirmed that the beneficial effects of nicotinamide riboside are mediated by its capacity to increase NAD^+ synthesis. A very recent study demonstrated that intracellular assimilation of endogenous nicotinamide riboside is essential for CR-mediated life span extension in S*accharomyces cerevisiae* [149]. These studies suggest that maintenance of cellular bioenergetic homeostasis and NAD^+ levels are crucial to support the NAD^+-dependent enzymes, such as enhancing SIRT1 activities, and for protection against excitotoxicity.

5.2. Therapeutic Intervention at SIRTs Level

As previously discussed, SIRT2 inhibition rescued a-synuclein toxicity and modified inclusion morphology in several cellular models of PD, and promoted the acetylation state of tubulin in a concentration-dependent manner upon treatment with AGK2 in HeLa cells. The results suggested that the compounds may function by promoting formation of enlarged inclusion bodies, which are suggested to provide a cell survival advantage [62]. Many other compounds were described to inhibit SIRT2 activity. For example, phloroglucinol [150], indoles [151] and suramin derivatives [152] as well as adenosine mimetics [153] were shown to be as effective as AGK2 and AK-1, but with significantly lower selectivity for SIRT2. However, it is dubious whether compounds that inhibit SIRT2 deacetylation through competition for the adenosine moiety of NAD^+ or the acetyl-lysine binding site would provide protection in PD models. Moreover, the apparent paradox regarding the neuroprotective properties of SIRT1 and SIRT2 remains a contentious topic, since activation of SIRT1 but inhibition of SIRT2 deacetylase activity seem to promote neuroprotection. We suggest that the contrasting effects of these two SIRTs on neuronal survival are dictated by their subcellular localization. In fact, SIRT2 is generally cytoplasmic but it also translocates to the nucleus during mitosis [65]. Thus, factors or mechanisms that determine its sorting in those cellular compartments may influence effects on neuronal health.

Resveratrol is a polyphenolic compound produced by several plants when under attack by pathogens, and is mainly found in the skin of grapes as well as in red wine and other dietary products. It is well-known for its phytoestrogenic and antioxidant properties [51] and has been reported to provide some beneficial influences on neuronal injury. A study by Gelinas and colleagues in neuronal PC12 cells first demonstrated the protective role of resveratrol against MPP^+ neurotoxicity [154]. Resveratrol also potently induces mitochondrial activity by activating PGC-1alpha and SIRT1 activity [54]. More recently, studies on dopaminergic neurons in organotypic midbrain slice culture revealed that resveratrol, together with another SIRT-activating compound, quercetin, prevented the loss of dopaminergic neurons induced by a dopaminergic neurotoxin [155]. These authors suggested the involvement of antioxidant properties of resveratrol in its neuroprotective effect rather than SIRT1 activation in this model, since other SIRT inhibitors like sirtinol or NAM did not attenuate the protective resveratrol effects. However, resveratrol, as well as the SIRT activator NAD^+, inhibited dopaminergic neurotoxicity of a DNA alkylating agent, N-methyl-N'-nitro-N-nitrosoguanidine [155]. However, the neuroprotective action of resveratrol remains controversial. Suzuki and Koike [156] verified that resveratrol decreased MT acetylation in Wlds granule cells, and provided evidence that resveratrol promotes tubulin deacetylation by acting directly on SIRT2.

Care must thus be exercised regarding the neuroprotective role of resveratrol, since this compound differentially affects the degeneration status by modulating both SIRT1 activity and SIRT2-mediated tubulin deacetylation.

5.3. Therapeutic Intervention at MT Network Level

The additional findings that disruption of axonal transport and impairment of MT-dependent trafficking are present in PD and that these MT alterations might potentiate α-synuclein aggregation lead us to suggest that a MT-stabilization approach could be a potential therapeutic strategy to halt or even prevent the pathogenesis of PD and other LB diseases. Among the various classes of MT-stabilizing natural products, taxanes have been the most intensely studied. Indeed, paclitaxel (taxol), the first chemotherapeutic MT-stabilizing agent to receive US Food and Drug Administration approval for the treatment of cancer, was found to be a potential clinical candidate for other disorders, including neurodegenerative diseases [157,158]. We believe that non-toxic taxol concentrations promote MT stability, and may preserve the structure of the MT network in the presence of α-synuclein aggregates or due to a mitochondrial bioenergetic failure. In fact, a study by Karbowski and colleagues [159] showed that MT stabilization induced by taxol does not affect the intracellular trafficking and distribution of mitochondria. The authors also suggested that MT stabilization or destabilization may play a specific role in the regulation of intracellular trafficking, indicating that MTs polymerized by taxol exert essentially the same cellular functions as normal MTs. On the contrary MT depolymerization, induced by colchicine, affects the intracellular transport of mitochondria as well as their contacts with other organelles. Notably, in a previous study using *in vitro* models, prolonged treatment of cells with taxol caused a complete stabilization of MTs concomitant with acetylation of α-tubulin [160]. The acetylation of α-tubulin could regulate the presence of MTs in specific intracellular spaces by selective stabilization, promoting the pathways necessary for an efficient mobilization of cargos within the cell. A major limiting factor for these drugs is permeation across the blood-brain barrier, whereby, active efflux back into the circulation by overexpression of the multidrug-

resistant gene product 1 or P-glycoprotein P-gp is a major factor. Recently, a paclitaxel C-10 carbamate derivative was shown to be devoid of P-glycoprotein interactions, in an *in situ* mouse brain perfusion model, in comparison with 14C-paclitaxel and revealed a higher permeability relatively to paclitaxel [161,162]. Further studies, however, are needed to evaluate the therapeutic potential of these compounds for the treatment of central nervous system diseases.

5.4. Therapeutic Intervention at ALP Level

Autophagy and its fundamental function in cellular health and homeostasis have also emerged as an important field of research in the recent years, with implications in PD. Since protein oligomerization and aggregation and associated neuronal disturbances, such as MT destabilization, which can cause autophagic vacuole transport failure and loss of synaptic integrity, the induction of ALP and subsequent lysosomal function may also be a possible therapeutic for PD. The discovery of the mammalian target of rapamycin (mTOR) as a key negative regulator of autophagy induction in eukaryotes [163] has allowed researchers to exploit rapamycin as a way to induce autophagy. Pharmacological induction of autophagy by rapamycin enhanced the clearance of aggregate-prone proteins and reduced the number of aggregates [164]. Furthermore, autophagy induction also ameliorated neurodegenerative symptoms in both a *Drosophila* Huntington's disease (HD) model and in transgenic HD mice [165]. These findings were also seen in other proteinopathy models. For instance, rapamycin reduced toxicity and enhanced clearance of aggregate-prone proteins, e.g. polyalanine expansion proteins and tau (associated with front-temporal dementia or tauopathy). Notably, the effects of rapamycin in fly models of these diseases appear to be autophagy-dependent, as rapamycin had no effects on proteinopathy toxicity in flies expressing these mutant proteins on a background of reduced activity of different autophagy genes [166,167].

These findings suggest that mTOR inhibitors could be seriously considered as drugs for disease treatment. One obvious advantage is that several of them have been approved for human treatment or are at different stages of clinical trials. Severe neurological problems due to use of mTOR inhibitors have up till now not been reported. However, as mTOR is crucial for neuronal survival by regulating a range of pathways (such as translation of certain proteins, cell division, etc.) and proper synaptic plasticity, one must consider that prolonged mTOR inhibition may also impair these processes. Moreover, how mTOR regulates downstream mammalian autophagy is still not clear. Combination therapies utilizing rapamycin plus a drug acting on an mTOR-independent pathway may provide more effective treatments for neurodegeneration through additive effect on autophagy induction, simultaneously reducing the adverse side-effects of rapamycin treatment alone [168].

The recent search for drugs, which positively regulate autophagy without affecting the mTOR pathway [169], may constitute an example of such a strategy. Trehalose, a disaccharide found in many non-mammalian species, including bacteria, yeast, fungi, insects, invertebrates and plants has been shown to induce mTOR-independent autophagy. Trehalose enhanced the clearance A53T and A30P mutants forms of α-synuclein and mutant huntingtin, thereby reducing mutant protein aggregation/toxicity [170]. Trehalose treatment also increased autophagic flux in a variety of mammalian cells, an effect that was was mediated by intracellular trehalose [170,171]. The dual protective properties of trehalose ('autophagy inducer' for enhancing clearance of aggregate-prone proteins and 'chemical chaperone' for inhibiting aggregation), coupled with its lack of toxicity, make it a possible candidate for the treatment of neurodegenerative disorders.

Recently, an mTOR-independent pathway for autophagy induction has been discovered: inhibition of inositol monophosphatase reduces free inositol and myoinositol-1,4,5-triphosphate levels which lead to an upregulation of autophagy. Lithium, carbemazepine and valproate, drugs used to treat a range of neurological and psychiatric conditions, induce autophagy *via* this pathway. Like rapamycin, these drugs increase the clearance of aggregate-prone proteins like mutant huntingtin and have beneficial effects in fly models of HD [172-174]. Rubinsztein and co-workers [175] also described a chemical screening approach for identifying small molecule enhancers of mammalian autophagy, termed SMERs. Such a screen revealed that SMERs induced mTOR-independent autophagy, reduced mutant huntingtin aggregates/toxicity in HD cell models, and showed additive protective effects with rapamycin. These SMERs also protected against mutant huntingtin fragment toxicity in *Drosophila* [175]. A further study showed that treatment of transgenic G93A amyotrophic lateral sclerosis mice with lithium carbonate delayed death and onset of disease progression. Tissue collected from these mice demonstrated an increased autophagic function and a general decrease in disease phenotypes such as ubiquitin and α-synuclein accumulation [176].

Axonal transport involves members of the Ras superfamily that typically act as regulators of diverse cellular processes by cycling between biologically active GTP- and inactive GDP-bound conformations – GTPases. Rab GTPases constitute one of the branches the largest family of small GTPases and are known to regulate specific membrane transport events, in particular the docking/fusion stages. Rab7 GTPase has been postulated to control the aggregation and fusion of late endosomes/lysosomes, and is essential for maintenance of the perinuclear lysosomal compartment [177]. More recently, Rab7 was shown to be required for autophagic vacuole maturation [178] and for the normal progression of autophagy in mammalian cells, probably by increasing autophagosome-lysosome fusion. We suggest that Rab7 is needed for the transport of degradation products out of late autophagic vacuole since its inhibition resembles MT inhibitors, which completely stop all MT-dependent transport, causing accumulation of autophagic vacuoles [178]. SoThus, MTs and proteins involved in the ALP, like Rab7, are two possible molecular targets which may provide avenues for therapeutic intervention in PD.

6. CONCLUDING REMARKS

Age-related neurodegenerative disorders are an enormous burden to the so called "western" societies. As life expectancy increases, these diseases will assume even more serious proportions. Thus, understanding the molecular mechanisms involved in neurodegeneration is urgent so that we, as a society, can develop novel strategies for therapeutic intervention. These efforts must involve translational approaches that accelerate the bench to bedside movement of knowledge.

This review focuses on our current knowledge of molecular and cellular mechanisms in PD and allows us to propose new therapeutic thoroughfares based on the understanding of the key role of mitochondrial metabolism in this disorder. In fact, the emergent knowledge recognizing the possible role of SIRTs as regulators of lifespan of diverse organisms and their coupling to NAD^+ metabolism has stimulated interest in the inherent potential of targeting mitochondria and NAD^+ metabolism for therapeutic purposes. Moreover, recent research developments in the field of autophagy has raised our understanding of this process as a means of survival during starvation and lifespan extension, which point to the crucial role of autophagy and its biological modulation in the pharmacological treatment of neurodegeneration. In addition, beneficial effects in neurodegenerative diseases also highlighted MTs as significant therapeutic targets and that possible addressable MT stabilization sites may have greater potential to intervention.

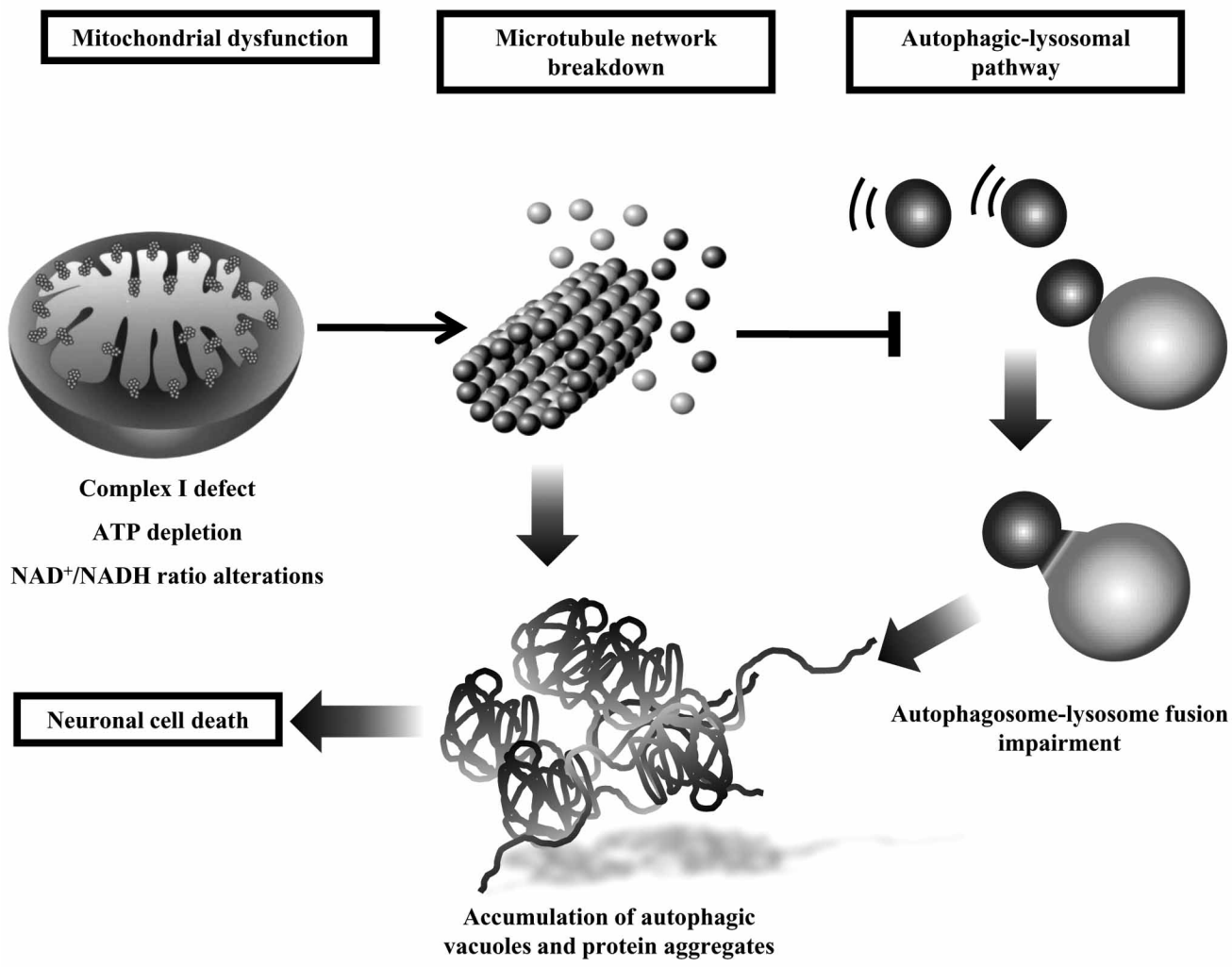

Fig. (1). Rationale for the contribution of mitochondrial bioenergetics to microtubule network integrity and autophagic-lysosomal pathway function in sporadic PD. Mitochondrial dysfunction mediated by complex I defect leads to alterations in mitochondria-dependent metabolism (reduced ATP levels and alterations in the NAD^+/NADH ratio). This bioenergetic failure seems to be responsible for microtubule network breakdown. Subsequently, when the dynamic instability and functional integrity of microtubules are compromised vesicular trafficking, autophagosome maturation and autophagosome–lysosomal fusion are impaired, resulting in a deficient clearance of autophagy substrates (e.g. protein aggregates). Microtubule network disruption may lead also to an increase in free tubulin that could interact with aggregate-prone proteins, inducing its oligomerization. Our model implies that mitochondrial dependent-energy failure may be the initial event in sporadic PD.

We are currently developing new therapeutic approaches involving intracellular trafficking, by improving cellular highways (stabilizing MTs) and by promoting an efficient degradation of damaged organelles or toxic protein aggregates (potentiating ALP) (Fig. **1**).

Due to chronic and progressive nature of PD and because there are no available treatments to delay or halt disease progression, we believe that this knowledge can then be translated into new prophylactic and more effective therapeutic strategies.

ABBREVIATIONS

AceCS2	=	Coenzyme A synthetase 2
ALP	=	Autophagic-lysosomal pathway
CoQ10	=	Coenzyme Q10
CR	=	Caloric restriction
CX1	=	Complex I
FOXO	=	Forkhead box type O transcription factors
HD	=	Huntington's disease
LB	=	Lewy body
MAO	=	Monoamine oxidase
MIM	=	Mitochondrial inner membrane
MPP^+	=	1-methyl-4-phenylpyridinium ion
MPTP	=	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mtDNA	=	Mitochondrial DNA
mTOR	=	Mammalian target of rapamycin
NAM	=	Nicotinamide
PD	=	Parkinson's disease
PGC1-alpha	=	Coactivator-1 alpha
ROS	=	Reactive oxygen species
SIRT	=	sirtuin
SMERs	=	Small molecule enhancers of mammalian autophagy

SN = Substantia nigra

TCA = Tricarboxylic acid cycle

Wlds = Wallerian slow degeneration

REFERENCES

[1] Schapira, A.H. Mitochondria in the aetiology and pathogenesis of Parkinson's disease. *Lancet Neurol.,* **2008**, *7*(1), 97-109.

[2] Cardoso, S.M.; Moreira, P.I.; Agostinho, P.; Pereira, C.; Oliveira, C.R. Neurodegenerative pathways in Parkinson's disease: therapeutic strategies. *Curr. Drug Targets CNS Neurol. Disord.,* **2005**, *4*(4), 405-419.

[3] Langston, J.W.; Ballard, P.; Tetrud, J.W.; Irwin, I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science,* **1983**, *219*(4587), 979-980.

[4] Burns, R.S.; LeWitt, P.A.; Ebert, M.H.; Pakkenberg, H.; Kopin, I.J. The clinical syndrome of striatal dopamine deficiency. Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *N. Engl. J. Med.,* **1985**, *312*(22), 1418-1421.

[5] Schapira, A.H.; Cooper, J.M.; Dexter, D.; Jenner, P.; Clark, J.B.; Marsden, C.D. Mitochondrial complex I deficiency in Parkinson's disease. *Lancet,* **1989**, *1*(8649), 1269.

[6] Swerdlow, R.H.; Parks, J.K.; Miller, S.W.; Tuttle, J.B.; Trimmer, P.A.; Sheehan, J.P.; Bennett, J.P., Jr.; Davis, R.E.; Parker, W.D., Jr. Origin and functional consequences of the complex I defect in Parkinson's disease. *Ann. Neurol.,* **1996**, *40*(4), 663-671.

[7] Cassarino, D.S.; Fall, C.P.; Swerdlow, R.H.; Smith, T.S.; Halvorsen, E.M.; Miller, S.W.; Parks, J.P.; Parker, W.D., Jr.; Bennett, J.P., Jr. Elevated reactive oxygen species and antioxidant enzyme activities in animal and cellular models of Parkinson's disease. *Biochim. Biophys. Acta,* **1997**, *1362*(1), 77-86.

[8] Esteves, A.R.; Arduino, D.M.; Swerdlow, R.H.; Oliveira, C.R.; Cardoso, S.M. Oxidative Stress involvement in alpha-synuclein oligomerization in Parkinsons disease cybrids. *Antioxid. Redox. Signal.,* **2009**, *11*(3), 439-448.

[9] Esteves, A.R.; Domingues, A.F.; Ferreira, I.L.; Januario, C.; Swerdlow, R.H.; Oliveira, C.R.; Cardoso, S.M. Mitochondrial function in Parkinson's disease cybrids containing an nt2 neuron-like nuclear background. *Mitochondrion,* **2008**, *8*(3), 219-228.

[10] Trimmer, P.A.; Borland, M.K.; Keeney, P.M.; Bennett, J.P., Jr.; Parker, W.D., Jr. Parkinson's disease transgenic mitochondrial cybrids generate Lewy inclusion bodies. *J. Neurochem.,* **2004**, *88*(4), 800-812.

[11] Ekstrand, M.I.; Terzioglu, M.; Galter, D.; Zhu, S.; Hofstetter, C.; Lindqvist, E.; Thams, S.; Bergstrand A.; Hansson F.S.; Trifunovic A.; Hoffer B.; Cullheim S.; Mohammed A.H.; Olson L.; Larsson N.G. Progressive parkinsonism in mice with respiratory-chain-deficient dopamine neurons. *Proc. Natl. Acad. Sci. USA,* **2007**, *104*(4), 1325-1330.

[12] Liang, C.L.; Wang, T.T.; Luby-Phelps, K.; German, D.C. Mitochondria mass is low in mouse substantia nigra dopamine neurons: implications for Parkinson's disease. *Exp. Neurol.,* **2007**, *203*(2), 370-380.

[13] Kwong, J.Q.; Beal, M.F.; Manfredi, G. The role of mitochondria in inherited neurodegenerative diseases. *J. Neurochem.,* **2006**, *97*(6), 1659-1675.

[14] Thomas, B.; Beal, M.F. Parkinson's disease. *Hum. Mol. Genet.,* **2007**, *16*(Spec No. 2), R183-R194.

[15] Dauer, W.; Przedborski, S. Parkinson's disease: mechanisms and models. *Neuron,* **2003**, 39, (6), 889-909.

[16] Letellier, T.; Malgat, M.; Rossignol, R.; Mazat, J.P. Metabolic control analysis and mitochondrial pathologies. *Mol. Cell Biochem.,* **1998**, *184*(1-2), 409-417.

[17] Calabrese, V.; Scapagnini, G.; Giuffrida Stella, A.M.; Bates, T.E.; Clark, J.B. Mitochondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disorders and longevity. *Neurochem. Res.,* **2001**, *26*(6), 739-764.

[18] Swerdlow, R.H.; Parks, J.K.; Cassarino, D.S.; Maguire, D.J.; Maguire, R.S.; Bennett, J.P., Jr.; Davis, R.E.; Parker, W.D., Jr. Cybrids in Alzheimer's disease: a cellular model of the disease? *Neurology,* **1997**, *49*(4), 918-925.

[19] Ying, W. NAD+/NADH and NADP+/NADPH in cellular functions and cell death: regulation and biological consequences. *Antioxid. Redox. Signal.,* **2008**, *10*(2), 179-206.

[20] Belenky, P.; Racette, F.G.; Bogan, K.L.; McClure, J.M.; Smith, J.S.; Brenner, C. Nicotinamide riboside promotes Sir2 silencing and extends lifespan *via* Nrk and Urh1/Pnp1/Meu1 pathways to NAD+. *Cell,* **2007**, *129*(3), 473-484.

[21] Piper, P.W.; Harris, N.L.; MacLean, M. Preadaptation to efficient respiratory maintenance is essential both for maximal longevity and the retention of replicative potential in chronologically ageing yeast. *Mech. Ageing Dev.,* **2006**, *127*(9), 733-740.

[22] Passos, J.F.; Saretzki, G.; Ahmed, S.; Nelson, G.; Richter, T.; Peters, H.; Wappler, I.; Birket, M.J.; Harold, G.; Schaeuble, K.; Birch-Machin, M.A.; Kirkwood, T.B.; von Zglinicki, T. Mitochondrial dysfunction accounts for the stochastic heterogeneity in telomere-dependent senescence. *PLoS Biol.,* **2007**, *5*(5), 1138-1151.

[23] Easlon, E.; Tsang, F.; Skinner, C.; Wang, C.; Lin, S.J. The malate-aspartate NADH shuttle components are novel metabolic longevity regulators required for calorie restriction-mediated life span extension in yeast. *Genes Dev.,* **2008**, *22*(7), 931-944.

[24] Denu, J.M. The Sir 2 family of protein deacetylases. *Curr. Opin. Chem. Biol.,* **2005**, *9*(5), 431-440.

[25] Sauve, A.A.; Wolberger, C.; Schramm, V.L.; Boeke, J.D. The biochemistry of sirtuins. *Annu. Rev. Biochem.,* **2006**, *75*, 435-465.

[26] Yamamoto, H.; Schoonjans, K.; Auwerx, J. Sirtuin functions in health and disease. *Mol. Endocrinol.,* **2007**, *21*(8), 1745-1755.

[27] Frye, R.A. Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. *Biochem. Biophys. Res. Commun.,* **1999**, *260*(1), 273-279.

[28] Frye, R.A. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. *Biochem. Biophys. Res. Commun.,* **2000**, *273*(2), 793-798.

[29] Blander, G.; Guarente, L. The Sir2 family of protein deacetylases. *Annu. Rev. Biochem.,* **2004**, *73,* 417-435.

[30] Chen, I.Y.; Lypowy, J.; Pain, J.; Sayed, D.; Grinberg, S.; Alcendor, R.R.; Sadoshima, J.; Abdellatif, M. Histone H2A.z is essential for cardiac myocyte hypertrophy but opposed by silent information regulator 2alpha. *J. Biol. Chem.,* **2006**, *281*(28), 19369-19377.

[31] Haigis, M.C.; Mostoslavsky, R.; Haigis, K.M.; Fahie, K.; Christodoulou, D.C.; Murphy, A.J.; Valenzuela, D.M.; Yancopoulos, G.D.; Karow, M.; Blander, G.; Wolberger, C.; Prolla, T.A.; Weindruch, R.; Alt, F.W.; Guarente, L. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. *Cell,* **2006**, *126*(5), 941-954.

[32] Michishita, E.; Park, J.Y.; Burneskis, J.M.; Barrett, J.C.; Horikawa, I. Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. *Mol. Biol. Cell.,* **2005**, *16*(10), 4623-4635.

[33] North, B.J.; Verdin, E. Sirtuins: Sir2-related NAD-dependent protein deacetylases. *Genome Biol.,* **2004**, *5*(5), 224.

[34] Tanno, M.; Sakamoto, J.; Miura, T.; Shimamoto, K.; Horio, Y. Nucleocytoplasmic shuttling of the NAD+-dependent histone deacetylase SIRT1. *J. Biol. Chem.,* **2007**, *282*(9), 6823-6832.

[35] Scher, M.B.; Vaquero, A.; Reinberg, D. SirT3 is a nuclear NAD+-dependent histone deacetylase that translocates to the mitochondria upon cellular stress. *Genes Dev.,* **2007**, *21*(8), 920-928.

[36] Anderson, R.M.; Bitterman, K.J.; Wood, J.G.; Medvedik, O.; Sinclair, D.A. Nicotinamide and PNC1 govern lifespan extension by calorie restriction in Saccharomyces cerevisiae. *Nature,* **2003**, *423*(6936), 181-185.

[37] Bitterman, K.J.; Anderson, R.M.; Cohen, H.Y.; Latorre-Esteves, M.; Sinclair, D.A. Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1. *J. Biol. Chem.,* **2002**, *277*(47), 45099-45107.

[38] Lin, J.; Wu, P.H.; Tarr, P.T.; Lindenberg, K.S.; St-Pierre, J.; Zhang, C.Y.; Mootha, V.K.; Jager, S.; Vianna, C.R.; Reznick, R.M.; Cui, L.; Manieri, M.; Donovan, M.X.; Wu, Z.; Cooper, M.P.; Fan, M.C.; Rohas, L.M.; Zavacki, A.M.; Cinti, S.; Shulman, G.I.; Lowell, B.B.; Krainc, D.; Spiegelman, B.M. Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. *Cell,* **2004**, *119*(1), 121-135.

[39] Lin, S.J.; Defossez, P.A.; Guarente, L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. *Science,* **2000**, *289*(5487), 2126-2128.

[40] Lin, S.J.; Kaeberlein, M.; Andalis, A.A.; Sturtz, L.A.; Defossez, P.A.; Culotta, V.C.; Fink, G.R.; Guarente, L. Calorie restriction

extends Saccharomyces cerevisiae lifespan by increasing respiration. *Nature,* **2002**, *418*(6895), 344-348.

[41] Bordone, L.; Cohen, D.; Robinson, A.; Motta, M.C.; van Veen, E.; Czopik, A.; Steele, A.D.; Crowe, H.; Marmor, S.; Luo, J.; Gu, W.; Guarente, L. SIRT1 transgenic mice show phenotypes resembling calorie restriction. *Aging Cell,* **2007**, *6*(6), 759-767.

[42] Luo, J.; Nikolaev, A.Y.; Imai, S.; Chen, D.; Su, F.; Shiloh, A.; Guarente, L.; Gu, W. Negative control of p53 by Sir2alpha promotes cell survival under stress. *Cell* **2001**, *107*(2), 137-148.

[43] Vaziri, H.; Dessain, S.K.; Ng Eaton, E.; Imai, S.I.; Frye, R.A.; Pandita, T.K.; Guarente, L.; Weinberg, R.A. hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. *Cell,* **2001**, *107*(2), 149-159.

[44] Brunet, A.; Sweeney, L.B.; Sturgill, J.F.; Chua, K.F.; Greer, P.L.; Lin, Y.; Tran, H.; Ross, S.E.; Mostoslavsky, R.; Cohen, H.Y.; Hu, L.S.; Cheng, H.L.; Jedrychowski, M.P.; Gygi, S.P.; Sinclair, D.A.; Alt, F.W.; Greenberg, M.E. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science,* **2004**, *303*(5666), 2011-2015.

[45] Cohen, H.Y.; Miller, C.; Bitterman, K.J.; Wall, N.R.; Hekking, B.; Kessler, B.; Howitz, K.T.; Gorospe, M.; de Cabo, R.; Sinclair, D.A. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science,* **2004**, *305*(5682), 390-392.

[46] Motta, M.C.; Divecha, N.; Lemieux, M.; Kamel, C.; Chen, D.; Gu, W.; Bultsma, Y.; McBurney, M.; Guarente, L. Mammalian SIRT1 represses forkhead transcription factors. *Cell,* **2004**, *116*(4), 551-563.

[47] Yeung, F.; Hoberg, J.E.; Ramsey, C.S.; Keller, M.D.; Jones, D.R.; Frye, R.A.; Mayo, M.W. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J.,* **2004**, *23*(12), 2369-2380.

[48] Haigis, M.C.; Guarente, L.P. Mammalian sirtuins--emerging roles in physiology, aging, and calorie restriction. *Genes Dev.,* **2006**, *20*(21), 2913-2921.

[49] Howitz, K.T.; Bitterman, K.J.; Cohen, H.Y.; Lamming, D.W.; Lavu, S.; Wood, J.G.; Zipkin, R.E.; Chung, P.; Kisielewski, A.; Zhang, L.L.; Scherer, B.; Sinclair, D.A. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. *Nature,* **2003**, *425*, (6954), 191-196.

[50] Kaeberlein, M.; McDonagh, T.; Heltweg, B.; Hixon, J.; Westman, E.A.; Caldwell, S.D.; Napper, A.; Curtis, R.; DiStefano, P.S.; Fields, S.; Bedalov, A.; Kennedy, B.K. Substrate-specific activation of sirtuins by resveratrol. *J. Biol. Chem.,* **2005**, *280*(17), 17038-17045.

[51] Baur, J.A.; Sinclair, D.A. Therapeutic potential of resveratrol: the *in vivo* evidence. *Nat. Rev. Drug Discov.,* **2006**, *5*(6), 493-506.

[52] Tissenbaum, H.A.; Guarente, L. Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. *Nature,* **2001**, *410*(6825), 227-230.

[53] Baur, J.A.; Pearson, K.J.; Price, N.L.; Jamieson, H.A.; Lerin, C.; Kalra, A.; Prabhu, V.V.; Allard, J.S.; Lopez-Lluch, G.; Lewis, K.; Pistell, P.J.; Poosala, S.; Becker, K.G.; Boss, O.; Gwinn, D.; Wang, M.; Ramaswamy, S.; Fishbein, K.W.; Spencer, R.G.; Lakatta, E.G.; Le Couteur, D.; Shaw, R.J.; Navas, P.; Puigserver, P.; Ingram, D.K.; de Cabo, R.; Sinclair, D.A. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature,* **2006**, *444*(7117), 337-342.

[54] Lagouge, M.; Argmann, C.; Gerhart-Hines, Z.; Meziane, H.; Lerin, C.; Daussin, F.; Messadeq, N.; Milne, J.; Lambert, P.; Elliott, P.; Geny, B.; Laakso, M.; Puigserver, P.; Auwerx, J. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell,* **2006**, *127*(6), 1109-1122.

[55] Araki, T.; Sasaki, Y.; Milbrandt, J. Increased nuclear NAD biosynthesis and SIRT1 activation prevent axonal degeneration. *Science,* **2004**, *305*(5686), 1010-1013.

[56] Lee, I.H.; Cao, L.; Mostoslavsky, R.; Lombard, D.B.; Liu, J.; Bruns, N.E.; Tsokos, M.; Alt, F.W.; Finkel, T. A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. *Proc. Natl. Acad. Sci. USA,* **2008**, *105*(9), 3374-3379.

[57] Sadoul, K.; Boyault, C.; Pabion, M.; Khochbin, S. Regulation of protein turnover by acetyltransferases and deacetylases. *Biochimie,* **2008**, *90*(2), 306-312.

[58] Dryden, S.C.; Nahhas, F.A.; Nowak, J.E.; Goustin, A.S.; Tainsky, M.A. Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle. *Mol. Cell Biol.,* **2003**, *23*(9), 3173-3185.

[59] North, B.J.; Marshall, B.L.; Borra, M.T.; Denu, J.M.; Verdin, E. The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase. *Mol. Cell,* **2003**, *11*(2), 437-444.

[60] Li, W.; Zhang, B.; Tang, J.; Cao, Q.; Wu, Y.; Wu, C.; Guo, J.; Ling, E.A.; Liang, F. Sirtuin 2, a mammalian homolog of yeast silent information regulator-2 longevity regulator, is an oligodendroglial protein that decelerates cell differentiation through deacetylating alpha-tubulin. *J. Neurosci.,* **2007**, *27*(10), 2606-2616.

[61] Suzuki, K.; Koike, T. Mammalian Sir2-related protein (SIRT) 2-mediated modulation of resistance to axonal degeneration in slow Wallerian degeneration mice: a crucial role of tubulin deacetylation. *Neuroscience,* **2007**, *147*(3), 599-612.

[62] Outeiro, T.F.; Kontopoulos, E.; Altmann, S.M.; Kufareva, I.; Strathearn, K.E.; Amore, A.M.; Volk, C.B.; Maxwell, M.M.; Rochet, J.C.; McLean, P.J.; Young, A.B.; Abagyan, R.; Feany, M.B.; Hyman, B.T.; Kazantsev, A.G. Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of Parkinson's disease. *Science,* **2007**, *317*(5837), 516-519.

[63] Heltweg, B.; Gatbonton, T.; Schuler, A.D.; Posakony, J.; Li, H.; Goehle, S.; Kollipara, R.; Depinho, R.A.; Gu, Y.; Simon, J.A.; Bedalov, A. Antitumor activity of a small-molecule inhibitor of human silent information regulator 2 enzymes. *Cancer Res.,* **2006**, *66*(8), 4368-4377.

[64] Wang, F.; Nguyen, M.; Qin, F.X.; Tong, Q. SIRT2 deacetylates FOXO3a in response to oxidative stress and caloric restriction. *Aging Cell,* **2007**, *6*(4), 505-514.

[65] Vaquero, A.; Scher, M.B.; Lee, D.H.; Sutton, A.; Cheng, H.L.; Alt, F.W.; Serrano, L.; Sternglanz, R.; Reinberg, D. SirT2 is a histone deacetylase with preference for histone H4 Lys 16 during mitosis. *Genes Dev.,* **2006**, *20*(10), 1256-1261.

[66] Inoue, T.; Hiratsuka, M.; Osaki, M.; Yamada, H.; Kishimoto, I.; Yamaguchi, S.; Nakano, S.; Katoh, M.; Ito, H.; Oshimura, M. SIRT2, a tubulin deacetylase, acts to block the entry to chromosome condensation in response to mitotic stress. *Oncogene,* **2007**, *26*(7), 945-957.

[67] Onyango, P.; Celic, I.; McCaffery, J.M.; Boeke, J.D.; Feinberg, A.P. SIRT3, a human SIR2 homologue, is an NAD-dependent deacetylase localized to mitochondria. *Proc. Natl. Acad. Sci. USA,* **2002**, *99*(21), 13653-13658.

[68] Schwer, B.; North, B.J.; Frye, R.A.; Ott, M.; Verdin, E. The human silent information regulator (Sir)2 homologue hSIRT3 is a mitochondrial nicotinamide adenine dinucleotide-dependent deacetylase. *J. Cell Biol.,* **2002**, *158*(4), 647-657.

[69] Shi, T.; Wang, F.; Stieren, E.; Tong, Q. SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. *J. Biol. Chem.* **2005**, *280*(14), 13560-13567.

[70] Kim, S.C.; Sprung, R.; Chen, Y.; Xu, Y.; Ball, H.; Pei, J.; Cheng, T.; Kho, Y.; Xiao, H.; Xiao, L.; Grishin, N.V.; White, M.; Yang, X.J.; Zhao, Y. Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. *Mol. Cell.,* **2006**, *23*(4), 607-618.

[71] Lombard, D.B.; Alt, F.W.; Cheng, H.L.; Bunkenborg, J.; Streeper, R.S.; Mostoslavsky, R.; Kim, J.; Yancopoulos, G.; Valenzuela, D.; Murphy, A.; Yang, Y.; Chen, Y.; Hirschey, M.D.; Bronson, R.T.; Haigis, M.; Guarente, L.P.; Farese, R.V., Jr.; Weissman, S.;Verdin, E.; Schwer, B. Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation. *Mol. Cell Biol.,* **2007**, *27*(24), 8807-8814.

[72] Hallows, W.C.; Lee, S.; Denu, J.M. Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases. *Proc. Natl. Acad. Sci. USA,* **2006**, *103*(27), 10230-10235.

[73] Schwer, B.; Bunkenborg, J.; Verdin, R.O.; Andersen, J.S.; Verdin, E. Reversible lysine acetylation controls the activity of the mitochondrial enzyme acetyl-CoA synthetase 2. *Proc. Natl. Acad. Sci. USA,* **2006**, *103*(27), 10224-10229.

[74] Fujino, T.; Kondo, J.; Ishikawa, M.; Morikawa, K.; Yamamoto, T.T. Acetyl-CoA synthetase 2, a mitochondrial matrix enzyme involved in the oxidation of acetate. *J. Biol. Chem.,* **2001**, *276*(14), 11420-11426.

[75] Sundaresan, N.R.; Samant, S.A.; Pillai, V.B.; Rajamohan, S.B.; Gupta, M.P. SIRT3 is a stress-responsive deacetylase in cardiomyocytes that protects cells from stress-mediated cell death

by deacetylation of Ku70. *Mol. Cell Biol.,* **2008**, *28*(20), 6384-6401.

[76] Law, I.K.; Liu, L.; Xu, A.; Lam, K.S.; Vanhoutte, P.M.; Che, C.M.; Leung, P.T.; Wang, Y. Identification and characterization of proteins interacting with SIRT1 and SIRT3: implications in the anti-aging and metabolic effects of sirtuins. *Proteomics,* **2009**, *9*(9), 2444-2456.

[77] Bellizzi, D.; Rose, G.; Cavalcante, P.; Covello, G.; Dato, S.; De Rango, F.; Greco, V.; Maggiolini, M.; Feraco, E.; Mari, V.; Franceschi, C.; Passarino, G.; De Benedictis, G. A novel VNTR enhancer within the SIRT3 gene, a human homologue of SIR2, is associated with survival at oldest ages. *Genomics,* **2005**, *85*(2), 258-263.

[78] Ahuja, N.; Schwer, B.; Carobbio, S.; Waltregny, D.; North, B.J.; Castronovo, V.; Maechler, P.; Verdin, E. Regulation of insulin secretion by SIRT4, a mitochondrial ADP-ribosyltransferase. *J. Biol. Chem.,* **2007**, *282*(46), 33583-33592.

[79] Pfister, J.A.; Ma, C.; Morrison, B.E.; D'Mello, S.R. Opposing effects of sirtuins on neuronal survival: SIRT1-mediated neuroprotection is independent of its deacetylase activity. *PLoS ONE,* **2008**, *3*(12), e4090.

[80] Morris, R.L.; Hollenbeck, P.J. The regulation of bidirectional mitochondrial transport is coordinated with axonal outgrowth. *J. Cell Sci.,* **1993**, *104*(Pt 3), 917-927.

[81] Wong-Riley, M.T.; Welt, C. Histochemical changes in cytochrome oxidase of cortical barrels after vibrissal removal in neonatal and adult mice. *Proc. Natl. Acad. Sci. USA,* **1980**, *77*(4), 2333-2337.

[82] Hollenbeck, P.J. The pattern and mechanism of mitochondrial transport in axons. *Front. Biosci.,* **1996**, *1*, d91-d102.

[83] Heidemann, S.R. Cytoplasmic mechanisms of axonal and dendritic growth in neurons. *Int. Rev. Cytol.,* **1996**, *165*, 235-296.

[84] Baas, P.W. Microtubule transport in the axon. *Int. Rev. Cytol.,* **2002**, *212*, 41-62.

[85] Kuznetsov, S.A.; Langford, G.M.; Weiss, D.G. Actin-dependent organelle movement in squid axoplasm. *Nature,* **1992**, *356*(6371), 722-725.

[86] Morris, R.L.; Hollenbeck, P.J. Axonal transport of mitochondria along microtubules and F-actin in living vertebrate neurons. *J. Cell Biol.,* **1995**, *131*(5), 1315-1326.

[87] Ligon, L.A.; Steward, O. Role of microtubules and actin filaments in the movement of mitochondria in the axons and dendrites of cultured hippocampal neurons. *J. Comp. Neurol.,* **2000**, *427*(3), 351-361.

[88] Langford, G.M. Myosin-V, a versatile motor for short-range vesicle transport. *Traffic,* **2002**, *3*(12), 859-865.

[89] Bridgman, P.C. Myosin-dependent transport in neurons. *J. Neurobiol.,* **2004**, *58*(2), 164-174.

[90] Katayama, M.; Zhong, Z.; Lai, L.; Sutovsky, P.; Prather, R.S.; Schatten, H. Mitochondrial distribution and microtubule organization in fertilized and cloned porcine embryos: implications for developmental potential. *Dev. Biol.,* **2006**, *299*(1), 206-220.

[91] Riva, A.; Tandler, B.; Lesnefsky, E.J.; Conti, G.; Loffredo, F.; Vazquez, E.; Hoppel, C.L. Structure of cristae in cardiac mitochondria of aged rat. *Mech. Ageing Dev.,* **2006**, *127*(12), 917-921.

[92] Johnson, K.A. The pathway of ATP hydrolysis by dynein. Kinetics of a presteady state phosphate burst. *J. Biol. Chem.,* **1983**, *258*(22), 13825-13832.

[93] Lin, M.T.; Beal, M.F. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature,* **2006**, *443*(7113), 787-795.

[94] Cappelletti, G.; Surrey, T.; Maci, R. The parkinsonism producing neurotoxin MPP+ affects microtubule dynamics by acting as a destabilising factor. *FEBS Lett.,* **2005**, *579*(21), 4781-4786.

[95] Ren, Y.; Feng, J. Rotenone selectively kills serotonergic neurons through a microtubule-dependent mechanism. *J. Neurochem.,* **2007**, *103*(1), 303-311.

[96] Cardoso, S.M.; Esteves, A.R.; Arduíno, D.M.; Domingues, A.F.; Oliveira, C.R. In: *Recent Research Developments in Neuroscience;* Signpost, R., Ed.; Kerala, **2009**, *Vol. 3*, pp.43-84

[97] Kopito, R.R. Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol.,* **2000**, *10*(12), 524-530.

[98] Lee, H.J.; Khoshaghideh, F.; Lee, S.; Lee, S.J. Impairment of microtubule-dependent trafficking by overexpression of alpha-synuclein. *Eur. J. Neurosci.,* **2006**, *24*(11), 3153-3162.

[99] Saha, A.R.; Hill, J.; Utton, M.A.; Asuni, A.A.; Ackerley, S.; Grierson, A.J.; Miller, C.C.; Davies, A.M.; Buchman, V.L.; Anderton, B.H.; Hanger, D.P. Parkinson's disease alpha-synuclein mutations exhibit defective axonal transport in cultured neurons. *J. Cell Sci.,* **2004**, *117*(Pt 7), 1017-1024.

[100] Kim, M.; Jung, W.; Lee, I.H.; Bhak, G.; Paik, S.R.; Hahn, J.S. Impairment of microtubule system increases alpha-synuclein aggregation and toxicity. *Biochem. Biophys. Res. Commun.,* **2008**, *365*(4), 628-635.

[101] Alim, M.A.; Ma, Q.L.; Takeda, K.; Aizawa, T.; Matsubara, M.; Nakamura, M.; Asada, A.; Saito, T.; Kaji, H.; Yoshii, M.; Hisanaga, S.; Ueda, K. Demonstration of a role for alpha-synuclein as a functional microtubule-associated protein. *J. Alzheimers Dis.,* **2004**, *6*(4), 435-442; discussion 443-449.

[102] Alim, M.A.; Hossain, M.S.; Arima, K.; Takeda, K.; Izumiyama, Y.; Nakamura, M.; Kaji, H.; Shinoda, T.; Hisanaga, S.; Ueda, K. Tubulin seeds alpha-synuclein fibril formation. *J. Biol. Chem.,* **2002**, *277*(3), 2112-2117.

[103] Aplin, A.; Jasionowski, T.; Tuttle, D.L.; Lenk, S.E.; Dunn, W.A., Jr. Cytoskeletal elements are required for the formation and maturation of autophagic vacuoles. *J. Cell Physiol.,* **1992**, *152*(3), 458-466.

[104] Webb, J.L.; Ravikumar, B.; Rubinsztein, D.C. Microtubule disruption inhibits autophagosome-lysosome fusion: implications for studying the roles of aggresomes in polyglutamine diseases. *Int. J. Biochem. Cell Biol.,* **2004**, *36*(12), 2541-2550.

[105] Ravikumar, B.; Acevedo-Arozena, A.; Imarisio, S.; Berger, Z.; Vacher, C.; O'Kane, C.J.; Brown, S.D.; Rubinsztein, D.C. Dynein mutations impair autophagic clearance of aggregate-prone proteins. *Nat. Genet.,* **2005**, *37*(7), 771-776.

[106] Kochl, R.; Hu, X.W.; Chan, E.Y.; Tooze, S.A. Microtubules facilitate autophagosome formation and fusion of autophagosomes with endosomes. *Traffic,* **2006**, *7*(2), 129-145.

[107] Fass, E.; Shvets, E.; Degani, I.; Hirschberg, K.; Elazar, Z. Microtubules support production of starvation-induced autophagosomes but not their targeting and fusion with lysosomes. *J. Biol. Chem.,* **2006**, *281*(47), 36303-36316.

[108] Jahreiss, L.; Menzies, F.M.; Rubinsztein, D.C. The itinerary of autophagosomes: from peripheral formation to kiss-and-run fusion with lysosomes. *Traffic,* **2008**, *9*(4), 574-587.

[109] Kimura, S.; Noda, T.; Yoshimori, T. Dynein-dependent movement of autophagosomes mediates efficient encounters with lysosomes. *Cell Struct. Funct.,* **2008**, *33*(1), 109-122.

[110] Anglade, P.; Vyas, S.; Javoy-Agid, F.; Herrero, M.T.; Michel, P.P.; Marquez, J.; Mouatt-Prigent, A.; Ruberg, M.; Hirsch, E.C.; Agid, Y. Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. *Histol. Histopathol.,* **1997**, *12*(1), 25-31.

[111] Zhu, J.H.; Guo, F.; Shelburne, J.; Watkins, S.; Chu, C.T. Localization of phosphorylated ERK/MAP kinases to mitochondria and autophagosomes in Lewy body diseases. *Brain Pathol.,* **2003**, *13*(4), 473-481.

[112] Vogiatzi, T.; Xilouri, M.; Vekrellis, K.; Stefanis, L. Wild type alpha-synuclein is degraded by chaperone-mediated autophagy and macroautophagy in neuronal cells. *J. Biol. Chem.,* **2008**, *283*(35), 23542-23556.

[113] Cuervo, A.M.; Stefanis, L.; Fredenburg, R.; Lansbury, P.T.; Sulzer, D. Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. *Science,* **2004**, *305*(5688), 1292-1295.

[114] Martinez-Vicente, M.; Talloczy, Z.; Kaushik, S.; Massey, A.C.; Mazzulli, J.; Mosharov, E.V.; Hodara, R.; Fredenburg, R.; Wu, D.C.; Follenzi, A.; Dauer, W.; Przedborski, S.; Ischiropoulos, H.; Lansbury, P.T.; Sulzer, D.; Cuervo, A.M. Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. *J. Clin. Invest.,* **2008**, *118*(2), 777-788.

[115] Pridgeon, J.W.; Olzmann, J.A.; Chin, L.S.; Li, L. PINK1 protects against oxidative stress by phosphorylating mitochondrial chaperone TRAP1. *PLoS Biol.,* **2007**, *5*(7), e172.

[116] Yang, Y.; Ouyang, Y.; Yang, L.; Beal, M.F.; McQuibban, A.; Vogel, H.; Lu, B. Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery. *Proc. Natl. Acad. Sci. USA,* **2008**, *105*(19), 7070-7075.

[117] Luft, R. The development of mitochondrial medicine. *Proc. Natl. Acad. Sci. USA,* **1994**, *91*(19), 8731-8.

[118] Riederer, P.; Lachenmayer, L.; Laux, G. Clinical applications of MAO-inhibitors. *Curr. Med. Chem.,* **2004**, *11*(15), 2033-2043.

[119] Youdim, M.B.; Riederer, P.F. A review of the mechanisms and role of monoamine oxidase inhibitors in Parkinson's disease. *Neurology,* **2004**, *63*(7 Suppl 2), S32-S35.

[120] Abu-Raya, S.; Tabakman, R.; Blaugrund, E.; Trembovler, V.; Lazarovici, P. Neuroprotective and neurotoxic effects of monoamine oxidase-B inhibitors and derived metabolites under ischemia in PC12 cells. *Eur. J. Pharmacol.,* **2002**, *434*(3), 109-116.

[121] Speiser, Z.; Levy, R.; Cohen, S. Effects of N-propargyl-1-(R)aminoindan (rasagiline) in models of motor and cognition disorders. *J. Neural Transm. Suppl.,* **1998**, *52*, 287-300.

[122] Tabakman, R.; Lecht, S.; Lazarovici, P. Neuroprotection by monoamine oxidase B inhibitors: a therapeutic strategy for Parkinson's disease? *Bioessays,* **2004**, *26*(1), 80-90.

[123] Kupsch, A.; Sautter, J.; Gotz, M.E.; Breithaupt, W.; Schwarz, J.; Youdim, M.B.; Riederer, P.; Gerlach, M.; Oertel, W.H. Monoamine oxidase-inhibition and MPTP-induced neurotoxicity in the non-human primate: comparison of rasagiline (TVP 1012) with selegiline. *J. Neural. Transm.,* **2001**, *108*(8-9), 985-1009.

[124] Lecht, S.; Haroutiunian, S.; Hoffman, A.; Lazarovici, P. Rasagiline - a novel MAO B inhibitor in Parkinson's disease therapy. *Ther. Clin. Risk Manag.,* **2007**, *3*(3), 467-474.

[125] Cassarino, D.S.; Fall, C.P.; Smith, T.S.; Bennett, J.P., Jr. Pramipexole reduces reactive oxygen species production *in vivo* and *in vitro* and inhibits the mitochondrial permeability transition produced by the parkinsonian neurotoxin methylpyridinium ion. *J. Neurochem.,* **1998**, *71*(1), 295-301.

[126] Kitamura, Y.; Kosaka, T.; Kakimura, J.I.; Matsuoka, Y.; Kohno, Y.; Nomura, Y.; Taniguchi, T. Protective effects of the antiparkinsonian drugs talipexole and pramipexole against 1-methyl-4-phenylpyridinium-induced apoptotic death in human neuroblastoma SH-SY5Y cells. *Mol. Pharmacol.,* **1998**, *54*(6), 1046-1054.

[127] Kakimura, J.; Kitamura, Y.; Takata, K.; Kohno, Y.; Nomura, Y.; Taniguchi, T. Release and aggregation of cytochrome c and alpha-synuclein are inhibited by the antiparkinsonian drugs, talipexole and pramipexole. *Eur. J. Pharmacol.,* **2001**, *417*(1-2), 59-67.

[128] Abramova, N.A.; Cassarino, D.S.; Khan, S.M.; Painter, T.W.; Bennett, J.P., Jr. Inhibition by R(+) or S(-) pramipexole of caspase activation and cell death induced by methylpyridinium ion or beta amyloid peptide in SH-SY5Y neuroblastoma. *J. Neurosci. Res.,* **2002**, *67*(4), 494-500.

[129] Gu, M.; Iravani, M.M.; Cooper, J.M.; King, D.; Jenner, P.; Schapira, A.H. Pramipexole protects against apoptotic cell death by non-dopaminergic mechanisms. *J. Neurochem.,* **2004**, *91*(5), 1075-1081.

[130] Sayeed, I.; Parvez, S.; Winkler-Stuck, K.; Seitz, G.; Trieu, I.; Wallesch, C.W.; Schonfeld, P.; Siemen, D. Patch clamp reveals powerful blockade of the mitochondrial permeability transition pore by the D2-receptor agonist pramipexole. *FASEB J.,* **2006**, *20*(3), 556-558.

[131] Beal, M.F. Bioenergetic approaches for neuroprotection in Parkinson's disease. *Ann. Neurol.,* **2003**, *53*(Suppl 3), S39-S47; discussion S47-S48.

[132] Andres, R.H.; Huber, A.W.; Schlattner, U.; Perez-Bouza, A.; Krebs, S.H.; Seiler, R.; Wallimann, T.; Widmer, H.R. Effects of creatine treatment on the survival of dopaminergic neurons in cultured fetal ventral mesencephalic tissue. *Neuroscience,* **2005**, *133*(3), 701-713.

[133] Andres, R.H.; Ducray, A.D.; Perez-Bouza, A.; Schlattner, U.; Huber, A.W.; Krebs, S.H.; Seiler, R.W.; Wallimann, T.; Widmer, H.R. Creatine supplementation improves dopaminergic cell survival and protects against MPP+ toxicity in an organotypic tissue culture system. *Cell Transplant.,* **2005**, *14*(8), 537-550.

[134] Bender, A.; Beckers, J.; Schneider, I.; Holter, S.M.; Haack, T.; Ruthsatz, T.; Vogt-Weisenhorn D.M.; Becker, L.; Genius, J.; Rujescu, D.; Irmler, M.; Mijalski, T.; Mader, M.; Quintanilla-Martinez, L.; Fuchs, H.; Gailus-Durner, V.; de Angelis, M.H.; Wurst, W.; Schmidt, J.; Klopstock, T. Creatine improves health and survival of mice. *Neurobiol. Aging,* **2008**, *29*(9), 1404-1411.

[135] Klivenyi, P.; Gardian, G.; Calingasan, N.Y.; Yang, L.; Beal, M.F. Additive neuroprotective effects of creatine and a cyclooxygenase 2 inhibitor against dopamine depletion in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease. *J. Mol. Neurosci.,* **2003**, *21*(3), 191-198.

[136] Dedeoglu, A.; Kubilus, J.K.; Yang, L.; Ferrante, K.L.; Hersch, S.M.; Beal, M.F.; Ferrante, R.J. Creatine therapy provides neuroprotection after onset of clinical symptoms in Huntington's disease transgenic mice. *J. Neurochem.,* **2003**, *85*(6), 1359-1367.

[137] Shults, C.W.; Haas, R.H.; Passov, D.; Beal, M.F. Coenzyme Q10 levels correlate with the activities of complexes I and II/III in mitochondria from parkinsonian and nonparkinsonian subjects. *Ann. Neurol.,* **1997**, *42*(2), 261-264.

[138] Winkler-Stuck, K.; Wiedemann, F.R.; Wallesch, C.W.; Kunz, W.S. Effect of coenzyme Q10 on the mitochondrial function of skin fibroblasts from Parkinson patients. *J. Neurol. Sci.,* **2004**, *220*(1-2), 41-48.

[139] Beal, M.F.; Matthews, R.T.; Tieleman, A.; Shults, C.W. Coenzyme Q10 attenuates the 1-methyl-4-phenyl-1,2,3,tetrahydropyridine (MPTP) induced loss of striatal dopamine and dopaminergic axons in aged mice. *Brain Res.,* **1998**, *783*(1), 109-114.

[140] Muller, T.; Buttner, T.; Gholipour, A.F.; Kuhn, W. Coenzyme Q10 supplementation provides mild symptomatic benefit in patients with Parkinson's disease. *Neurosci. Lett.,* **2003**, *341*(3), 201-204.

[141] Armstrong, J.S. Mitochondria-directed therapeutics. *Antioxid. Redox. Signal.,* **2008**, *10*(3), 575-578.

[142] Lu, C.; Zhang, D.; Whiteman, M.; Armstrong, J.S. Is antioxidant potential of the mitochondrial targeted ubiquinone derivative MitoQ conserved in cells lacking mtDNA? *Antioxid. Redox. Signal.,* **2008**, *10*(3), 651-660.

[143] Szeto, H.H. Mitochondria-targeted cytoprotective peptides for ischemia-reperfusion injury. *Antioxid. Redox. Signal.,* **2008**, *10*(3), 601-619.

[144] Skulachev, V.P. Functions of mitochondria: from intracellular power stations to mediators of a senescence program. *Cell Mol. Life Sci.,* **2009**, *66*(11-12), 1785-1793.

[145] Skulachev, V.P.; Anisimov, V.N.; Antonenko, Y.N.; Bakeeva, L.E.; Chernyak, B.V.; Erichev, V.P.; Filenko, O.F.; Kalinina, N.I.; Kapelko, V.I.; Kolosova, N.G.; Kopnin, B.P.; Korshunova, G.A.; Lichinitser, M.R.; Obukhova, L.A.; Pasyukova, E.G.; Pisarenko, O.I.; Roginsky, V.A.; Ruuge, E.K.; Senin, I.I.; Severina, I.I.; Skulachev, M.V.; Spivak, I.M.; Tashlitsky, V.N.; Tkachuk, V.A.; Vyssokikh, M.Y.; Yaguzhinsky, L.S.; Zorov, D.B. An attempt to prevent senescence: a mitochondrial approach. *Biochim. Biophys. Acta,* **2009**, *1787*(5), 437-461.

[146] Schulz, J.B.; Henshaw, D.R.; Matthews, R.T.; Beal, M.F. Coenzyme Q10 and nicotinamide and a free radical spin trap protect against MPTP neurotoxicity. *Exp. Neurol.,* **1995**, *132*(2), 279-283.

[147] Liu, D.; Pitta, M.; Mattson, M.P. Preventing NAD(+) depletion protects neurons against excitotoxicity: bioenergetic effects of mild mitochondrial uncoupling and caloric restriction. *Ann. NY Acad. Sci.,* **2008**, *1147*, 275-282.

[148] Sasaki, Y.; Araki, T.; Milbrandt, J. Stimulation of nicotinamide adenine dinucleotide biosynthetic pathways delays axonal degeneration after axotomy. *J. Neurosci.,* **2006**, *26*(33), 8484-8491.

[149] Lu, S.P.; Kato, M.; Lin, S.J. Assimilation of endogenous nicotinamide riboside is essential for calorie restriction-mediated life span extension in Saccharomyces cerevisiae. *J. Biol. Chem.,* **2009**, *284*(25), 17110-17119.

[150] Gey, C.; Kyrylenko, S.; Hennig, L.; Nguyen, L.H.; Buttner, A.; Pham, H.D.; Giannis, A. Phloroglucinol derivatives guttiferone G, aristoforin, and hyperforin: inhibitors of human sirtuins SIRT1 and SIRT2. *Angew Chem. Int. Ed. Engl.,* **2007**, *46*(27), 5219-5222.

[151] Napper, A.D.; Hixon, J.; McDonagh, T.; Keavey, K.; Pons, J.F.; Barker, J.; Yau, W.T.; Amouzegh, P.; Flegg, A.; Hamelin, E.; Thomas, R.J.; Kates, M.; Jones, S.; Navia, M.A.; Saunders, J.O.; DiStefano, P.S.; Curtis, R. Discovery of indoles as potent and selective inhibitors of the deacetylase SIRT1. *J. Med. Chem.,* **2005**, *48*(25), 8045-8054.

[152] Trapp, J.; Meier, R.; Hongwiset, D.; Kassack, M.U.; Sippl, W.; Jung, M. Structure-activity studies on suramin analogues as inhibitors of NAD+-dependent histone deacetylases (sirtuins). *ChemMedChem,* **2007**, *2*(10), 1419-1431.

[153] Trapp, J.; Jochum, A.; Meier, R.; Saunders, L.; Marshall, B.; Kunick, C.; Verdin, E.; Goekjian, P.; Sippl, W.; Jung, M. Adenosine mimetics as inhibitors of NAD+-dependent histone deacetylases, from kinase to sirtuin inhibition. *J. Med. Chem.,* **2006**, *49*(25), 7307-7316.

[154] Gelinas, S.; Martinoli, M.G. Neuroprotective effect of estradiol and phytoestrogens on MPP+-induced cytotoxicity in neuronal PC12 cells. *J. Neurosci. Res.,* **2002**, *70*(1), 90-96.

[155] Okawara, M.; Katsuki, H.; Kurimoto, E.; Shibata, H.; Kume, T.; Akaike, A. Resveratrol protects dopaminergic neurons in midbrain slice culture from multiple insults. *Biochem. Pharmacol.,* **2007**, *73*(4), 550-560.

[156] Suzuki, K.; Koike, T. Resveratrol abolishes resistance to axonal degeneration in slow Wallerian degeneration (WldS) mice: activation of SIRT2, an NAD-dependent tubulin deacetylase. *Biochem. Biophys. Res. Commun.,* **2007**, *359*(3), 665-671.

[157] Zhang, B.; Maiti, A.; Shively, S.; Lakhani, F.; McDonald-Jones, G.; Bruce, J.; Lee, E.B.; Xie, S.X.; Joyce, S.; Li, C.; Toleikis, P.M.; Lee, V.M.; Trojanowski, J.Q. Microtubule-binding drugs offset tau sequestration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model. *Proc. Natl. Acad. Sci. USA,* **2005**, *102*(1), 227-231.

[158] Michaelis, M.L.; Ansar, S.; Chen, Y.; Reiff, E.R.; Seyb, K.I.; Himes, R.H.; Audus, K.L.; Georg, G.I. {beta}-Amyloid-induced neurodegeneration and protection by structurally diverse microtubule-stabilizing agents. *J. Pharmacol. Exp. Ther.,* **2005**, *312*(2), 659-668.

[159] Karbowski, M.; Spodnik, J.H.; Teranishi, M.; Wozniak, M.; Nishizawa, Y.; Usukura, J.; Wakabayashi, T. Opposite effects of microtubule-stabilizing and microtubule-destabilizing drugs on biogenesis of mitochondria in mammalian cells. *J. Cell Sci.,* **2001**, *114*(Pt 2), 281-291.

[160] Piperno, G.; LeDizet, M.; Chang, X.J. Microtubules containing acetylated alpha-tubulin in mammalian cells in culture. *J. Cell Biol.,* **1987**, *104*(2), 289-302.

[161] Ballatore, C.; Zhang, B.; Trojanowski, J.Q.; Lee, V.M.; Smith, A.B., 3rd. In situ blood-brain barrier permeability of a C-10 paclitaxel carbamate. *Bioorg. Med. Chem. Lett.,* **2008**, *18*(23), 6119-6121.

[162] Rice, A.; Michaelis, M.L.; Georg, G.; Liu, Y.; Turunen, B.; Audus, K.L. Overcoming the blood-brain barrier to taxane delivery for neurodegenerative diseases and brain tumors. *J. Mol. Neurosci.,* **2003**, *20*(3), 339-343.

[163] Noda, T.; Ohsumi, Y. Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. *J. Biol. Chem.,* **1998**, *273*(7), 3963-3966.

[164] Ravikumar, B.; Duden, R.; Rubinsztein, D.C. Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. *Hum. Mol. Genet.,* **2002**, *11*(9), 1107-1117.

[165] Ravikumar, B.; Vacher, C.; Berger, Z.; Davies, J.E.; Luo, S.; Oroz, L.G.; Scaravilli, F.; Easton, D.F.; Duden, R.; O'Kane, C.J.; Rubinsztein, D.C. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nat. Genet.,* **2004**, *36*(6), 585-595.

[166] Momeni, P.; Schymick, J.; Jain, S.; Cookson, M.R.; Cairns, N.J.; Greggio, E.; Greenway, M.J.; Berger, S.; Pickering-Brown, S.; Chio, A.; Fung, H.C.; Holtzman, D.M.; Huey, E.D.; Wassermann, E.M.; Adamson, J.; Hutton, M.L.; Rogaeva, E.; St George-Hyslop, P.; Rothstein, J.D.; Hardiman, O.; Grafman, J.; Singleton, A.; Hardy, J.; Traynor, B.J. Analysis of IFT74 as a candidate gene for chromosome 9p-linked ALS-FTD. *BMC Neurol.,* **2006**, *6*, 44.

[167] Taylor, J.P.; Tanaka, F.; Robitschek, J.; Sandoval, C.M.; Taye, A.; Markovic-Plese, S.; Fischbeck, K.H. Aggresomes protect cells by enhancing the degradation of toxic polyglutamine-containing protein. *Hum. Mol. Genet.,* **2003**, *12*(7), 749-757.

[168] Winslow, A.R.; Rubinsztein, D.C. Autophagy in neurodegeneration and development. *Biochim. Biophys. Acta,* **2008**, *1782*(12), 723-729.

[169] Rubinsztein, D.C.; Gestwicki, J.E.; Murphy, L.O.; Klionsky, D.J. Potential therapeutic applications of autophagy. *Nat. Rev. Drug Discov.,* **2007**, *6*(4), 304-312.

[170] Sarkar, S.; Davies, J.E.; Huang, Z.; Tunnacliffe, A.; Rubinsztein, D.C. Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and alpha-synuclein. *J. Biol. Chem.,* **2007**, *282*(8), 5641-5652.

[171] Davies, J.E.; Sarkar, S.; Rubinsztein, D.C. Trehalose reduces aggregate formation and delays pathology in a transgenic mouse model of oculopharyngeal muscular dystrophy. *Hum. Mol. Genet.,* **2006**, *15*(1), 23-31.

[172] Sarkar, S.; Floto, R.A.; Berger, Z.; Imarisio, S.; Cordenier, A.; Pasco, M.; Cook, L.J.; Rubinsztein, D.C. Lithium induces autophagy by inhibiting inositol monophosphatase. *J. Cell Biol.,* **2005**, *170*(7), 1101-1111.

[173] Berger, Z.; Ttofi, E.K.; Michel, C.H.; Pasco, M.Y.; Tenant, S.; Rubinsztein, D.C.; O'Kane, C.J. Lithium rescues toxicity of aggregate-prone proteins in Drosophila by perturbing Wnt pathway. *Hum. Mol. Genet.,* **2005**, *14*(20), 3003-3011.

[174] Sarkar, S.; Krishna, G.; Imarisio, S.; Saiki, S.; O'Kane, C.J.; Rubinsztein, D.C. A rational mechanism for combination treatment of Huntington's disease using lithium and rapamycin. *Hum. Mol. Genet.,* **2008**, *17*(2), 170-178.

[175] Sarkar, S.; Perlstein, E.O.; Imarisio, S.; Pineau, S.; Cordenier, A.; Maglathlin, R.L.; Webster, J.A.; Lewis, T.A.; O'Kane, C.J.; Schreiber, S.L.; Rubinsztein, D.C. Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. *Nat. Chem. Biol.,* **2007**, *3*(6), 331-338.

[176] Fornai, F.; Longone, P.; Cafaro, L.; Kastsiuchenka, O.; Ferrucci, M.; Manca, M.L.; Lazzeri, G.; Spalloni, A.; Bellio, N.; Lenzi, P.; Modugno, N.; Siciliano, G.; Isidoro, C.; Murri, L.; Ruggieri, S.; Paparelli, A. Lithium delays progression of amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. USA,* **2008**, *105*(6), 2052-2057.

[177] Bucci, C.; Thomsen, P.; Nicoziani, P.; McCarthy, J.; van Deurs, B. Rab7: a key to lysosome biogenesis. *Mol. Biol. Cell,* **2000**, *11*(2), 467-480.

[178] Jager, S.; Bucci, C.; Tanida, I.; Ueno, T.; Kominami, E.; Saftig, P.; Eskelinen, E.L. Role for Rab7 in maturation of late autophagic vacuoles. *J. Cell Sci.,* **2004**, *117*(Pt 20), 4837-4848.

Received: July 2, 2009 **Revised: August 2, 2009** **Accepted: November 5, 2009**