

# Function of $\alpha$ -synuclein and PINK1 in Lewy body dementia (Review)

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**Abstract.**  $\alpha$ -synuclein ( $\alpha$ -syn) is the major protein component of Lewy bodies, a key pathological characteristic of the degenerating brain. The misfolding and aggregation of  $\alpha$ -syn is associated with both the idiopathic and familial forms of Parkinson's disease (PD) and Lewy body dementia (LBD). However, the function of  $\alpha$ -syn is poorly understood, as it shows both neurotoxic and neuroprotective activities. Mutations in phosphatase and tensin homologue-induced putative kinase 1 (PINK1) also cause recessively inherited PD. Studies support the notion of neuroprotective roles for PINK1, as it protects cells from damage-induced mitochondrial dysfunction, oxidative stress and cell apoptosis. PINK1 plays an essential role in mitochondrial quality control and its homeostasis is maintained through mitochondrial stabilization. The  $\alpha$ -syn aggregation is linked to various aspects of mitochondrial dysfunction and PINK1-related mitophagy. Determination of the molecular pathways that lead to  $\alpha$ -syn oligomerization and further aggregation may be the basis for the successful design and development of treatments for these neurodegenerative diseases. The present review summarizes the function of PINK1 underlying  $\alpha$ -syn aggregation and the mechanisms through which mitochondrial dysfunction plays a role in this process.

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Abbreviations:  $\alpha$ -syn,  $\alpha$ -synuclein; AD, Alzheimer's disease; DHA, docosahexaenoic acid; LB, Lewy bodies; LBD, Lewy body dementia; NAC, non-amyloid component; PD, Parkinson's disease; PINK1, phosphatase and tensin homologue-induced putative kinase 1; PTEN, phosphatase and tensin homolog; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; TRAP1, tumor necrosis factor receptor-associated protein-1

**Key words:**  $\alpha$ -synuclein, phosphatase and tensin homologue-induced putative kinase 1, Parkin, mitochondria, Lewy body dementia

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## 1. Introduction

Lewy body disease is a heterogeneous group of neurodegenerative disorders characterized by  $\alpha$ -synuclein ( $\alpha$ -syn) accumulation that includes Lewy body dementia (LBD) and Parkinson's disease (PD) (1-3). While the progressive accumulation of amyloid  $\beta$  oligomers has been identified as one of the important toxic events in Alzheimer's disease (AD) leading to synaptic dysfunction, the accumulation of  $\alpha$ -syn has been linked to the pathogenesis of PD and LBD. Amyloid  $\beta$  also promotes  $\alpha$ -syn aggregation and toxicity in LBD (4,5). Aggregated  $\alpha$ -syn is a major component of inclusions in PD and other brains affected by synucleinopathy, indicating that  $\alpha$ -syn aggregation is associated with the key pathogenesis of neurodegenerative disorders, such as LBD (Fig. 1). The impairment of lysosomal pathways, including autophagy involved in  $\alpha$ -syn clearance may play an important role in LBD. Whereas in patients with LBD the clinical presentation is of dementia followed by parkinsonism, in patients with PD dementia the initial signs are of parkinsonism followed by dementia (6,7). Although the mechanisms underlying  $\alpha$ -syn aggregation and toxicity are not fully elucidated, it is clear that  $\alpha$ -syn undergoes post-translational modifications, and interacts with numerous proteins, hormones, neurotransmitters and metals that can modulate its aggregation propensity (8,9).

Oxidized forms of  $\alpha$ -syn found in sporadic PD and LBD have been shown to block autophagy/mitophagy (10,11). The term mitophagy has been created to describe the selective removal of mitochondria by autophagy. Mitophagy is constitutively active in healthy neurons. Evidence indicates that alterations in mitophagy may participate in the mechanisms of  $\alpha$ -syn-mediated neurodegeneration (synucleinopathy) (12,13). Modifications in the rate of aggregation and clearance of  $\alpha$ -syn may be responsible for the formation of toxic amyloid  $\beta$  and

$\alpha$ -syn oligomers in LBD. The autophagic pathway is the major pathway involved in the degradation of long-term proteins and organelles, cellular remodeling and survival, which has been linked to cell death, and is markedly activated in degenerative disorders (14,15). Alterations in the mitophagy pathway in LBD/PD support the possibility that modulators of the autophagic pathway may have potential therapeutic effects. For example, activating autophagy by rapamycin treatment has been shown to improve  $\alpha$ -syn accumulation, related neuropathology and the development of neurodegeneration (16). LBD represents the most common form of dementia and movement disorders in the aging population following AD, and displays widespread cortical and subcortical pathology (17). A better understanding of the mechanisms of  $\alpha$ -syn dysfunction may help to elucidate the molecular mechanisms of neurodegeneration and may be the basis for the development of novel therapeutic strategies. The present review discusses potential alterations in components of mitophagy in LBD. We also provide an overview on the cellular functions of the mitochondrial kinase phosphatase and tensin homologue-induced putative kinase 1 (PINK1), with particular emphasis on the mitochondrial damage response pathway and mitochondrial quality control involved in LBD synucleinopathy.

## 2. Association between LBD and mitophagy

LBD is characterized by the presence of intra-neuronal cell inclusions termed Lewy bodies with  $\alpha$ -syn as their chief component (18). Alterations of  $\alpha$ -syn expression and impairment of its degradation can lead to the formation of intra-cellular deposits of this protein.  $\alpha$ -syn aggregation is now accepted as a key step preceding the formation of Lewy bodies. Whereas LBD is characterized by the general neuronal loss of the dopaminergic system, a high percentage of surviving neurons contain inclusions in the form of Lewy bodies (19). Widespread distribution of Lewy bodies through almost all brain areas is a characteristic feature in LBD, while these are found mainly within the brainstem in PD (20,21) (Fig. 1). Oligomerization and accumulation of fibrillar  $\alpha$ -syn aggregates are the molecular processes involved in the pathophysiology of PD and LBD (22), in which the affectedness in PD brainstem causes parkinsonian symptoms and the additional cortical affectedness in LBD. The missense mutations in  $\alpha$ -syn also promote the aggregation process of this protein (23). Overexpressed or misfolded  $\alpha$ -syn can be secreted to the extracellular space (24). Although the precise sequence of events responsible for  $\alpha$ -syn fibrillation remains unknown, aggregated  $\alpha$ -syn species with altered solubility of  $\alpha$ -syn species with various molecular weights are found particularly in grey matter (25,26). The mechanisms underlying cellular  $\alpha$ -syn aggregation are crucial to the understanding of the pathogenic process of the diseases.

Protein misfolding and aggregation is a shared feature of a number of neurodegenerative diseases, which can be suppressed and promoted by several factors, such as protein degradation systems, molecular chaperones and free radical reactions. Several of these factors are under the control of normal mitochondrial function. Thus, mitochondrial dysfunction may cause the accumulation of protein aggregates, including the  $\alpha$ -syn protein. The toxic effects of  $\alpha$ -syn have been linked to the aggregated forms rather than the mono-

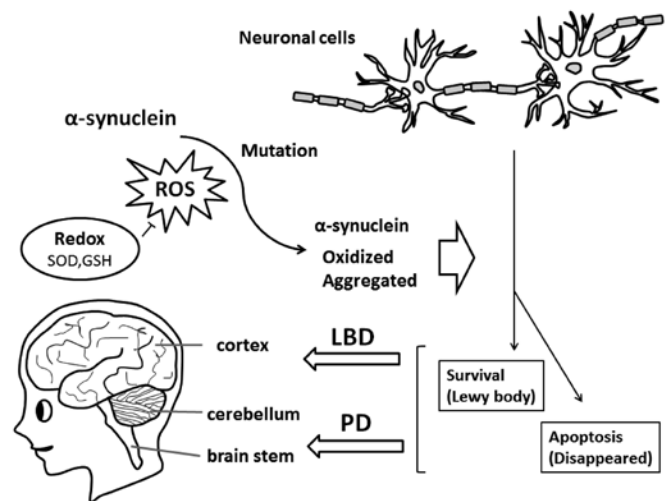


Figure 1. Implication of  $\alpha$ -synuclein modulation caused by reactive oxygen species (ROS) and mutations for neuronal cell death and Lewy body formation. Abnormality of  $\alpha$ -synuclein may be a causative factor in the development of Lewy body dementia (LBD) and Parkinson's disease (PD). Note that some critical molecules have been omitted for clarity. SOD, superoxide dismutase; GSH, glutathione.

mers (27). It has been demonstrated that oxidative stress may play a key role in the spread of  $\alpha$ -syn pathology (28,29), therefore,  $\alpha$ -syn-evoked protein oxidation leads to disturbances in synaptic transmission and neuronal cell death. The interaction of secreted  $\alpha$ -syn with other amyloidogenic proteins, such as amyloid  $\beta$  and its involvement in irreversible mitochondrial dysfunction with oxidative stress has been previously reported (30), which is an important factor involved in LBD neuronal cell loss. It is well known that some of the genes that cause recessive PD are associated with mitochondrial function, although recessive PD phenotypically differs from LBD.  $\alpha$ -syn accumulation is also caused by the inhibition of the proteasome and autophagy-lysosome (31). In general,  $\alpha$ -syn predominantly associates with the inner mitochondrial membrane, where it can apparently interact with complex I, resulting in reduced mitochondrial complex I activity and increased free radical production (32). The failure of the mitochondrial control eventually contributes to cell death. PINK1 may be a sensor of mitochondrial damage and marker of mitophagy. The presence of LB in the brainstem and nigral neuronal cells has been described in brains from PD carriers with heterozygous *PINK1* mutation (33).

## 3. Function and characteristics of $\alpha$ -synuclein and PINK1 in mitophagy

The  $\alpha$ -syn cDNA encodes a 140-amino-acid protein, which is predominantly expressed in the hippocampus, neocortex, thalamus, cerebellum, olfactory bulb and substantia nigra. In these neuronal tissue cells,  $\alpha$ -syn is highly expressed in the mitochondria, where the expression of cytosolic  $\alpha$ -syn is also high (34).  $\alpha$ -syn is a 19-kDa protein from which a 35-amino acid peptide [non-amyloid component (NAC)] is derived. The schematic structure of human  $\alpha$ -syn is shown in Fig. 2. NAC is the second component, after amyloid  $\beta$ , identified in the AD

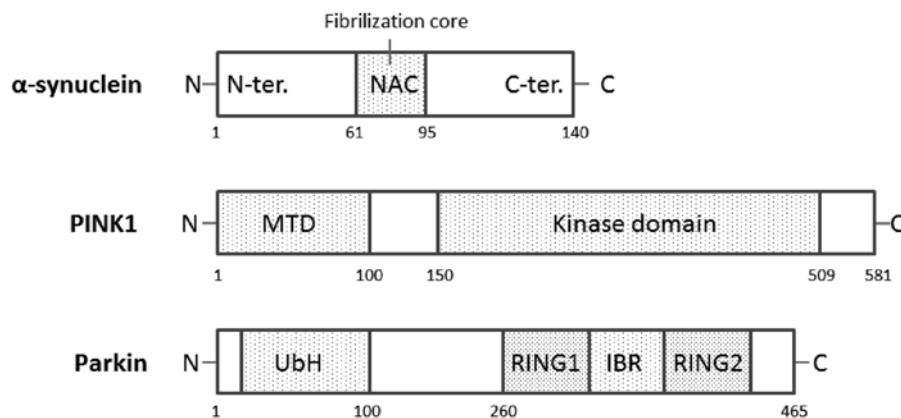


Figure 2. Schematic diagram indicating the domain structures of  $\alpha$ -synuclein, phosphatase and tensin homologue-induced putative kinase 1 (PINK1) and Parkin proteins. The predicted consensual important domain structures for each protein are depicted. NAC, non-amyloid component; MTD, mitochondrial-targeting domain; UbH, ubiquitin homology domain; RING1, RING2, RING finger domain; IBR, in between RING fingers.

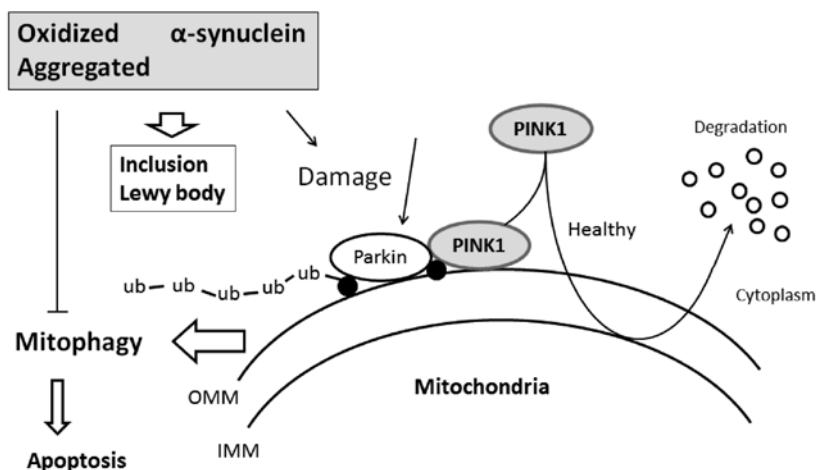


Figure 3. Hypothetical schematic representation of  $\alpha$ -synuclein and phosphatase and tensin homologue-induced putative kinase 1 (PINK1) regulatory pathway and Parkin-mediated mitophagy. In a healthy and steady state, PINK1 is degraded within the mitochondria. This may be inhibited by mitochondrial damage, resulting in PINK1 and Parkin accumulation in the outer membrane of the mitochondria. Parkin is presumed to ubiquitinate unidentified substrate (black circle), resulting in the induction of mitophagy. Oxidized and aggregated  $\alpha$ -synuclein may inhibit mitophagy, which can induce cell apoptosis. Interactions are shown as arrows indicating activation, while hammerheads indicate inhibition. Note that some critical pathways have been omitted for clarity. OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane.

amyloid preparation (35,36). Structure predictions indicate that the NAC peptide sequence has a tendency to form  $\beta$  structures consistent with its association with amyloid protein.  $\alpha$ -syn has also been shown to be linked to various aspects of mitochondrial dysfunction (37). The accumulation of  $\alpha$ -syn in the mitochondria of dopaminergic neurons leads to increased production of reactive oxygen species (ROS) (38,39) that can be involved in a number of pro-survival pathways, including the regulation of mitophagy via the removal of defective mitochondria (40). The overexpression and/or mutation of  $\alpha$ -syn are associated with protein aggregation and inhibit a number of cellular processes, including mitochondrial function.  $\alpha$ -syn and  $\beta$ -synuclein are homologous proteins implicated in synucleinopathies (4). While  $\alpha$ -syn is neurotoxic and its aggregation and deposition are related to neurodegeneration,  $\beta$ -synuclein is considered as a potent inhibitor of  $\alpha$ -syn aggregation and toxicity (41).

The *PINK1* gene consists of 8 exons, encoding a 581 amino acid protein with a predicted molecular mass of 62.8 kDa that is ubiquitously expressed in tissues (42). PINK1 has a C-terminal autoregulatory region, a conserved putative serine-threonine kinase domain, and an N-terminal mitochondrial targeting signal domain necessary for mitochondrial introduction (Fig. 2). In the brain, the higher neuronal expression of PINK1 is observed in the hippocampus, substantia nigra and cerebellar Purkinje cells (43). The protein can be found on the outer and inner mitochondrial membrane (Fig. 3) (44,45). PINK1 can be processed into at least 2 shorter forms, which are distributed in both mitochondrial and cytosolic compartments. More than 50 mutations of the *PINK1* gene have been mapped throughout the kinase and carboxyl-terminal regulatory domains of PINK1 with several effects on protein stability (46). It has been shown to phosphorylate the mitochondrial heat shock protein 75 kDa [tumor necrosis factor receptor-associated protein-1 (TRAP1)],

increasing neuronal survival against heat shock or oxidative stress by preventing the release of cytochrome *c* (47). PINK1 may also interact with Beclin1, an important pro-autophagic protein implicated in the pathogenesis of AD (48). In addition, PINK1 recruits Parkin to the mitochondria from the cytosol to initiate mitophagy (49). The importance of PINK1 in mechanisms underlying neurodegeneration is reflected by the neuroprotective properties of Parkin (49). The overexpression of wild-type PINK1 in neuronal cells stabilizes respiring mitochondrial networks through maintaining mitochondrial membrane potential (50), suppressing mitophagy. In healthy mitochondria, PINK1 is rapidly degraded in a process involving both mitochondrial proteases and the proteasome system (Fig. 3). Under conditions of PINK1 deficiency, it compromises the mitochondrial quality control (51).

Increased mitophagy activity observed in cells expressing  $\alpha$ -syn is an important phenomenon linked to  $\alpha$ -syn-induced toxicity. When mitochondrial import is compromised by depolarization, PINK1 accumulates on the mitochondrial surface, which in turn mediates mitophagic destruction (52), which decreases  $\alpha$ -syn toxicity and promotes inclusion body formation. Therefore, PINK1 can protect neuronal cells against possibly harmful proteins, such as  $\alpha$ -syn that would result in cellular stress (53). Consequently, PINK1 is involved in  $\alpha$ -synucleinopathy (54). The mitochondrial chaperone protein TRAP1 which has been shown to be phosphorylated by PINK1 also mitigates  $\alpha$ -syn toxicity (55). However, the neuroprotective role for the  $\alpha$ -syn has been shown in attenuating manganese (Mn)-induced toxicity in the background of PD (56). Evidence suggests that  $\alpha$ -syn has an effect on protein quality control systems, such as the ubiquitin-proteasome system and autophagy, suggesting that increased mitophagy activity is an important phenomenon linked to  $\alpha$ -syn toxicity during aging (57). PINK1 overexpression rescues the  $\alpha$ -syn-induced PD phenotype in *D. melanogaster*, apparently through targeting of  $\alpha$ -syn for degradation (58). A simultaneous increase in  $\alpha$ -syn and PINK1 may have a synergistic effect for cell protection, which seems to be a result of the upregulation of pro-survival mechanisms in response to an increase in ROS signaling due to the effect of  $\alpha$ -syn overexpression (59).

#### 4. Effect of diet on $\alpha$ -syn and mitophagy pathway and its contribution to neuroprotection in LBD

Therapeutic strategies exploit the observation that defects in key processes required for cellular homeostasis produce an alternative metabolic situation or diseases.  $\omega$ -3 polyunsaturated fatty acid (PUFA) induces autophagy. In general, several foods can affect the cognitive processes in the central nervous system neurons by  $\omega$ -3 PUFA. It has been known that dietary  $\omega$ -3 PUFA improves memory and learning processes, and also affects gene expression in neurons (60). A long-term diet rich in  $\omega$ -3 PUFA and/or docosahexaenoic acid (DHA) leads to lower protein oxidative damage with no modifications in the number of cortical astrocytes and microglial cells and with no effects in  $\alpha$ -syn expression. However,  $\alpha$ -syn oligomerization is markedly enhanced by  $\omega$ -3 PUFA, while  $\beta$ -synuclein oligomerization is not affected (41). PUFA-enriched diets significantly alter the mRNA expression levels of several genes in central nervous system neurons, and these effects may be related to the balance of polyenoic fatty acids [(n-3)/(n-6)] in the cell membrane. Diets

enriched in saturated fatty acids and simple carbohydrates are often deficient in  $\omega$ -3 PUFA (61). *Perilla frutescens* is a good source of  $\omega$ -3 PUFA (>50% total fatty acid) which are indispensable fatty acids that can be converted to DHA in the liver and the developing brain, and contains one of the highest contents of  $\omega$ -3 fatty acid among edible plant seeds (62). The perilla diet supplementation promotes neuronal signaling and alters synaptic plasticity for improved learning and memory (63). A diet with spirulina, a natural product from blue green algae, also provides neuroprotection, as demonstrated in an  $\alpha$ -syn model of PD (64). In other words, spirulina can protect against the neuronal loss induced by  $\alpha$ -syn.

Curcumin is a well-known polyphenol in commonly used in the preparation of Asian food, known as the ingredient turmeric, and has been shown to exhibit anti-inflammatory, anti-carcinogenic and anti-microbial activities (65). Studies have suggested the potential therapeutic role of curcumin in neurological disorders, including PD and LBD. Curcumin has been shown to inhibit  $\alpha$ -syn aggregation and attenuate  $\alpha$ -syn oligomer toxicity in neuronal cells (66). Curcumin binds to the preformed  $\alpha$ -syn aggregates, and strongly reduces their cellular toxicity by minimizing their hydrophobic surface exposure. In addition, curcumin accelerates  $\alpha$ -syn aggregation and reduces the population of soluble oligomers which are cytotoxic. Of note, curcumin does not bind to monomeric  $\alpha$ -syn but binds specifically to oligomeric intermediates (66). The degree of curcumin binding correlates with the extent of  $\alpha$ -syn oligomerization, suggesting that the oligomeric structure of  $\alpha$ -syn is required for effective curcumin binding. Curcumin also prevents aggregation of  $\alpha$ -syn by increasing the reconfiguration rate (67), which may decrease the population of toxic oligomeric intermediates of  $\alpha$ -syn.

It is known that caloric restriction and the polyphenolic antioxidant, resveratrol, may promote longevity. Recently, several studies have shown that Sirt3 along with FoxO3 in addition to Sirt1 are of importance in promoting the anti-aging function of resveratrol (68). Sirt3 in cooperation with Sirt1 activates FoxO3, and they contain the initial mitochondrial signaling response to activate PINK-1, thereby promoting mitophagy. By the way, PINK1 is overexpressed in the mitochondria of hepatocytes of ethanol-treated rats, in which ethanol treatment represents a possible protective mechanism through the stimulation of mitophagy (69). Antioxidant vitamins, such as vitamin E and the vitamin-like substance coenzyme Q10 have been used in the treatment of LBD with some efficacy (70). With the potent anti-fibrillogenic, as well as fibril-destabilizing activities of  $\alpha$ -syn, these vitamin compounds may prove to be useful in the prevention of LBD. Treatment with rotenone, a toxic isoflavonoid, can reproduce nigra-striatal cell loss and other features of PD in rodents (71). Rotenone treatment results in decreased spontaneous locomotor movement and increased cytoplasmic  $\alpha$ -syn expression. The mitochondrial PINK1 protein levels are also increased following treatment with rotenone.

#### 5. Perspectives

The aggregation of  $\alpha$ -syn may be the consequence of proteasome dysfunction (72). Cells with mutant *PINK1* or *PINK1* knockdown are accompanied by increased  $\alpha$ -syn aggregation, indicating that mitochondrial functional deficits are related to  $\alpha$ -syn aggregation (73). The physiological function of  $\alpha$ -syn

is largely unknown. However,  $\alpha$ -syn may have an inhibitory function in membrane fusion and may lead to mitochondrial fragmentation when  $\alpha$ -syn binds to the mitochondria (74). The mitochondrial fragmentation induced by the expression of  $\alpha$ -syn is rescued by the coexpression of PINK1 (74). Precisely, it is unclear which molecular components of the mitophagy pathway are dysregulated in the brains of patients with LBD/PD. Mitophagy is significant to maintain mitochondrial quality and ensure cellular homeostasis; however, excessive rates of mitophagy may prove to be harmful. Mitochondrial protein phosphorylation is involved in cell stress-induced programmed cell death, which also contributes to the regulation of mitochondrial dynamics and mitophagy. PINK1 may function in the first line of mitochondrial quality control, monitoring oxygen respiratory chain function and triggering the localized degradation of damaged mitochondrial proteins. The involvement of PINK1 and Parkin in the mitochondrial dysfunction has been extensively investigated in PD pathogenesis (75); however, these pathological mechanisms are not restricted to PD, but may be common characteristics of various neurodegenerative disorders, including LBD. LB is also found in a relatively high percentage of asymptomatic individuals (76,77), indicating that some individuals may have protective mechanisms against LBD. Enhancing pathways that promote selective mitophagy may delay the disease development by promoting a healthy pool of viable mitochondria in neuronal cells. Strategies to promote mitophagy may prove effective for multiple forms of neurodegenerative disease. Future experimental studies are required in order to elucidate the precise mitochondria protective roles.

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