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α-Synuclein and Dopamine Metabolism

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Abstract

α-Synuclein (α-Syn), a 140-amino-acid protein richly expressed in presynaptic terminals in the central nervous system, has been shown to play a central role in the pathogenesis of Parkinson's disease. Although the normal functions of α-Syn remain elusive, accumulating evidence shows that the molecule is involved in multiple steps related to dopamine metabolism, including dopamine synthesis, storage, release, and uptake. The regulatory effect of α-Syn on dopamine metabolism is likely to tone down the amount of cytoplasmic dopamine at nerve terminals, thereby limiting its conversion to highly reactive oxidative molecules. Formation of α-Syn protofibrils triggered by factors such as gene mutations and environmental toxins can make the molecule lose its normal functions, leading to disrupted homeostasis of dopamine metabolism, increased cytoplasmic dopamine levels, and enhanced oxidative stress in dopaminergic neurons. The enhanced oxidative stress will, in turn, exacerbate the formation of α-Syn protofibrils and drive the neurons into a vicious cycle, which will finally result in the selective degeneration of the dopaminergic neurons associated with Parkinson's disease.

Index Entries: Parkinson's disease; α-synuclein; dopamine; neuron; oxidative stress.

Introduction

α-Synuclein (α-Syn), a 140-amino-acid protein richly expressed in presynaptic terminals in the central nervous system, has been strongly implicated in the pathogenesis of Parkinson's disease (PD) *(1)*. The first evidence linking α-Syn to PD is the findings that missense mutations (A30P and A53T) in the α-Syn gene are associated with early-onset, autosomal dominant forms of PD *(2,3)*. Although there is no α-Syn gene mutation identified in idiopathic PD *(4,5)*, aggregated α-Syn has been found to be the major component of Lewy bodies and Lewy neurites, the pathological hallmarks of

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PD *(6–11).* The implication of α-Syn in PD pathogenesis is further supported by observations of α-Syn transgenic mice and fruit flies, both of which exhibit the major pathological changes (Lewy-body-like intracellular inclusions, loss of dopaminergic [DA] neurons in flies, degeneration of DA terminals in mice) and age-dependent locomotor deficiency reminiscent of human PD *(12,13).* However, how α-Syn gets involved in the pathophysiological process of PD is far from obvious. A body of evidence shows that a prerequisite of α-Syn neuropathy is its aggregation into soluble protofibrils *(14–16).* Several experimental conditions, including overexpression of the protein, presence of the A30P or A53T mutations, exposure to neurotoxins, and oxidative factors, induce or accelerate α-Syn aggregation both in vivo and in vitro through procedures that remain poorly understood *(17–25).* Two opposite mechanisms could engage in the pathophysiological role of the aggregated α-Syn in PD. On one hand, aggregation of α-Syn could diminish the bioavailability of α-Syn and compromise its physiological functions. On the other hand, aggregated α-Syn could gain a toxic function, which could result in degeneration of DA neurons.

It is still an enigma why DA neurons in the substantia nigra are preferentially and selectively degenerated in PD and how the formation of α-Syn protofibrils might link to the selective degeneration of the DA neuron. Overexpression of α-Syn has been shown to be toxic only to DA neurons but not to non-DA neurons *(26,27).* Therefore, there must be some other factors responsible for the specific vulnerability of the DA neurons. Because the functional characteristic of DA neurons is to synthesize and release dopamine (DA) neurotransmitters, the prime candidate for the specific vulnerability is that DA itself and related processes that deal with DA synthesis, storage, release, and uptake. Indeed, in the DA neurons of the substantia nigra, DA is metabolized via enzymatic deamination by monoamine oxidases (MAO), with production of the nontoxic 3,4-dihydroxyphenylacetic acid (DOPAC) and H_2O_2 *(28).* In turn, H_2O_2 can be converted to highly toxic hydroxyl radicals in a reaction catalyzed by iron transition metals, which are found at high levels associated with neuromelanin in the substantia nigra pars compacta *(29).* DA can also undergo spontaneous autoxidation, at normal intracellular pH and in the presence of molecular oxygen, into toxic and reactive DA quinones, superoxide free radicals, and hydrogen peroxide *(30).* Moreover, superoxide can be either converted to H_2O_2 by superoxide dismutases or into labile, but very reactive and cytotoxic, peroxinitrite radicals in the presence of nitric oxide. Therefore, any factors that affect DA synthesis, storage, release, and reuptake will have the possibility to increase the concentration of cytoplasmic DA and the level of oxidative stress in DA neurons.

In this review, we will present evidence showing how α-Syn could help maintain the homeostasis of DA metabolism in normal conditions in DA neurons, keeping the neurons in a healthy state, and how loss of its normal functions could cause the disruption of the homeostasis, leading to selective degeneration of DA neurons in disease conditions.

Molecular Structure, Localization, and Function Characteristic of α-Syn

Syn belongs to a multigene family that consists of three distinct members: α-Syn, β-Syn, and γ-Syn *(31).* The first synuclein gene was identified in 1988 by Maroteaux et al. *(32),* who screened an expression library with an antiserum against the purified cholinergic vesicles from the electric organ of the Pacific electric ray *Torpedo califonica.* This initial complementary DNA (cDNA) clone encoding electric ray γ-Syn was then used to isolate another cDNA clone encoding rat α-Syn. Human α-Syn was originally identified by cDNA cloning of the precursor of non-β-amyloid component (or NAC) in Alzheimer's disease *(33).*

The α-Syn molecule consists of a highly conserved amino-terminal domain that is composed of 7 imperfect repeat sequences of 11 amino acids with the core consensus sequence motif KTKEGV *(31–34),* a hydrophobic NAC domain, and a less conserved carboxy-terminal domain that includes a preponderance of acidic residues. The 11-mer repeats make up a conserved apolipoprotein-like A2 helix, which mediates binding of synucleins to phospholipid membranes; lipid binding is accompanied by a large shift in protein secondary structure, from around 3% to more than 70% α-helix *(35).* The NAC domain is highly hydrophobic and easy to aggregate. The "GAV motif" (residues 66–74) in the NAC domain has been demonstrated to be responsible for aggregation or fibrillization of α-Syn *(36).*

α-Syn is highly enriched in presynaptic terminals *(32,37).* α-Syn immunoreactivity can be also found in the perinuclear area of the cytoplasm and, in some cases, the nucleus of brain neurons *(37,38).* α-Syn appears equally distributed between cytosolic and intracellular membrane fractions *(39,40)* and is widely expressed in the neocortex, hippocampus, dentate gyrus, olfactory bulb, thalamus, amygdala, nucleus accumben, and cerebellum *(37,41).* Somata of DA neurons of the substantia nigra pars compacta contain only low levels of α-Syn, which, in turn, might be accumulated inside the terminals of the corresponding projections in the striatum, but this is still a matter of debate *(38,42–44).*

The function of α-Syn remains unclear. One of the characteristics of α-Syn is its close association with cellular membrane structures. First, as mentioned earlier, α-Syn can be isolated from either cylosolic or intracellular membrane fraction *(37,40).* Second, its amphipathic α-helical domains of the 11-residue repeats are reminiscent of those in the class A2 apolipoproteins, which carry lipid molecules by reversibly binding to them *(35).* Third, α-Syn undergoes a major structural transition from random coil to α-helical structure upon binding to lipid vesicles *(45),* supporting the possible role of α-Syn in lipid binding and transport. Interestingly, the PD-linked point mutations (A30P and A53T) abolish the ability of α-Syn to bind to the lipid vesicles *(35),* emphasizing the possibility that lipid binding is a normal function of α-Syn. Fourth, α-Syn has been reported to be loosely associated with synaptic vesicles *(32,37,46),* further suggesting its relation with membrane structures. Except for its association with the membrane structures, α-Syn functions as a chaperone protein closely related to the function of 14-3-3-chaperone molecules *(47,48),* with which it shares considerable sequence homology, thus displaying pleiotropic effects in cells. Moreover, our recent observations (unpublished data), together with others, suggest that α-Syn might be involved in the regulation of gene expressions. Evidence for this function includes localization of the molecule in the nuclei of normal brain neurons, nuclear translocation in response to insults, and change in gene expressions in α-Syn-overexpressed cells *(38,49,50).*

The Normal Functions of α-Syn Maintain the Homeostasis of DA Metabolism

Synaptic Vesicle Recycling, DA Storage, and DA Release

Several observations support that α-Syn might regulate synaptic vesicle recycling, DA storage, and DA release at nerve terminals by maintenance of a subset of presynaptic vesicles in the "reserve" or "resting" pools. First, inhibition of α-Syn expression with antisense oligonucleotides in cultured hippocampal neurons decreased the number of synaptic vesicles, particularly in the reserve pool *(46).* Second, the α-Syn knockout mice exhibited significant impairments in synaptic response to a prolonged train of repetitive stimulation capable of depleting docked as well as reserve pool vesicles, and decreased expression levels of synapsin, an essential protein for synaptic vesicle recycling *(51,52).* Third, replenishment of the docked vesicles by reserve pool vesicles after depletion was slower in the mutant synapses *(51,52).*

The molecular mechanisms for the action of α-Syn on the maintenance of presynaptic vesicles could involve regulation of key components of the metabolic pathways generating phospholipids of the cellular membrane. Evidence shows that phospholipase D2 (PLD2) might play an important role in vesicle formation and recycling at or near the plasma membrane or endosomal compartments in response to external stimuli *(53,54).* α-Syn has been demonstrated to interact with and inhibit the activity of PLD2 through its amino-terminal repeat region *(55,56).* Therefore, by modulating PLD2 activity, a putative key function of α-Syn might be to regulate synaptic vesicular recycling. This putative physiological function of α-Syn might be tightly regulated by various intracellular seryl/threonyl protein kinases or tyrosyl protein kinases that phosphorylate α-Syn at various sites *(57–61).* For example, phosphorylation of α-Syn by G protein-coupled receptor kinases lowers the ability of α-Syn to inhibit PLD2 activity and reduces binding of α-Syn to phospholipids *(59).* Thus, through reduction of its tonic inhibition of PLD2, phosphorylated α-Syn might promote vesicle recycling during periods of high neuronal activity and favor synaptic plasticity. Except through inhibition of PLD2 activity, α-Syn might also modulate vesicle recycling by its fatty acid binding protein (FABP) properties *(40).* The amphipathic amino-terminal region of α-Syn bears significant homology to the lipid binding class A apolipoproteins A2, which is implicated in lipid transport *(19).* α-Syn might, therefore, transfer fatty acids to sites of synaptic vesicle formation (i.e., early endosomes) and/or regulate, as a lipid chaperone, the turnover or local organization of polyunsaturated fatty acid acyl groups that have been implicated in clathrin-mediated endocytosis *(62)* and, therefore, in vesicle recycling *(63).* Moreover, α-Syn has been suggested to interfere with axonal transport of synaptic vesicles *(64)* by interacting with several proteins that either bind to or are part of the cytoskeleton, such as tubulin *(65),* τ *(58),* microtubule-associated protein 1B (MAP1B) (66), MAP2 *(67),* synphilin-1 *(68),* and torsin A *(69).*

Because synaptic vesicles are important places for storage of DA transmitters in DA neurons, regulation of the number of the vesicles by α-Syn will affect DA storage and release. This has been demonstrated by the evidence that depletion of α-Syn significantly impairs the synaptic response to prolonged train of repetitive stimulation and loss of α-Syn function by A53T mutation increases the cytoplasmic DA levels *(51,52).*

DA Transporter Activity and DA Reuptake

α-Syn has been shown to downregulate the activity of human DA transporter (hDAT) at the nerve terminals of DA neurons. α-Syn decreases hDAT activity by reducing the uptake velocity of DA but does not affect the affinity of hDAT to DA *(70).* Only the wild-type and A30P α-Syn mutant rather than the A53T mutant have the regulatory effect on hDAT activity *(71,72).* It seems that the action of α-Syn on hDAT is mediated by its direct interaction with the transporter, because coimmunoprecipitation studies show that α-Syn can form a stable complex with hDAT *(70).* Consistent with the findings that only the wild type and A30P mutant of α-Syn can downregulate the activity of hDAT, complex formation only occurs between hDAT and wild-type or A30P mutant of α-Syn but not the A53T mutant *(71).* In addition, a recent study showed that trypsin completely reversed the attenuation of hDAT function mediated by wild-type and the A30P mutant *(72).* In A53T and hDAT-coexpressing cells, where hDAT activity is not downregulated, trypsinization did not induce any changes. Confocal microscopy and biotinylation studies showed that in cells expressing the wild-type or A30P variants, but not the A53T mutant, hDAT was sequestered away from the plasma membrane into the cytoplasm, an effect that was reversed by trypsin *(72).* These results suggest that α-Syn modulates hDAT function through trafficking of the transporter in a process that can be disrupted by trypsin.

Dopamine transporter plays a critical role in maintaining DA homeostasis and synaptic

strength. It is by this transporter that released DA neurotransmitters are rapidly removed from the synaptic cleft. Increases or decreases in DAT function will concomitantly decrease or increase synaptic DA concentrations. Thus, regulation of the activity of the transporter will directly affect the levels of intracellular DA and reactive oxidative stress *(70).* However, in α-Syn- and hDAT cotransfected Ltk(–) cells, α-Syn attenuated the reuptake of DA by hDAT in a manner dependent on the expression levels of α-Syn *(73).* In cells transfected with A53T mutant α-Syn, which neither form a strong complex with hDAT nor modulates DA uptake by hDAT, hDAT-dependent cytotoxicity is higher than with either wild-type or the A30P variant of α-Syn, but not significantly different from that of cells expressing hDAT alone *(71).* Moreover, DA-mediated oxidative stress and cell death were reduced in α-Syn and DAT cotransfected cells compared with only DAT-transfected cells *(74).*

DA Biosynthesis

Dopamine synthesis depends on a rate-limiting enzyme, tyrosine hydroxylase (TH), which catalyzes the conversion of tyrosine into L-DOPA, the latter is then converted into DA by the rather ubiquitous aromatic amino acid decarboxylase. In both α-Syn-transgenic mice *(12)* and α-Syn-transfected DA cells *(75),* TH activity was significantly reduced. In α-Syn-transfected DA cells, overexpression of wild-type or A53T mutant α-Syn significantly reduced TH activity and cellular DA levels *(75).* These results indicate that α-Syn might inhibit TH activity. To confirm this, Perez et al. *(75)* used a cell-free system to examine whether TH activity could be directly inhibited by α-Syn. They found that the activity of isolated TH from the adrenal gland was also inhibited by recombinant human α-Syn in a dose-dependent manner. In our laboratory, we used a reaction system containing TH, tyrosine, and other chemicals necessary for the enzymatic conversion of tyrosine to L-DOPA. We found that addition of recombinant α-Syn inhibited L-DOPA production (unpublished data). It is known that only phosphorylated TH is active, and the phosphorylation and dephosphorylation of TH are primary in the signaling cascade regulating DA biosynthesis *(76).* Recently, a reciprocal interplay between α-Syn and 14-3-3 proteins for the regulation of TH activity has been suggested *(27).* The chaperone protein 14-3-3 binds to phosphorylated TH and is necessary for maximal phosphorylation of the enzyme *(77,78).* The 14-3-3 proteins also protect TH from dephosphorylation and increase the half-life of activated TH in neurons *(79).* Conversely, α-Syn has been shown to colocalize with and directly bind to TH, causing an overall net decrease in enzymatic activity and DA synthesis *(75).* α-Syn binds to the dephosphorylated form of TH and tends to maintain TH in an inactive form. The A30P and A53T mutant α-Syn have the same inhibitory effects on TH as the wild-type protein *(75),* leading to the proposal that altered regulation of TH by the α-Syn might not play a role in the pathogenesis of PD *(53).* However, in the pathological process, precipitation and aggregation of α-Syn might reduce the availability of cytoplasmic α-Syn, leading to an increase of TH activity mediated by 14-3-3 proteins. The imbalance between the effects of α-Syn and 14-3-3 proteins is highly deleterious because overproduction of cytosolic DA generates highly reactive species such as quinones and superoxide free radicals *(53,70).*

Baptista et al. recently reported that in the DA cells transfected by wild-type α-Syn, the expression of genes for DA synthesis were coordinately downregulated *(49).* These genes included GTP cyclohydrolase, sepiapterin reductase *(SR),* TH, and aromatic acid decarboxylase. Overexpression of mutant α-Syn did not change these gene expressions. The expression of the orphan nuclear receptor Nurr1 was also noticed in the α-Syn-transfected DA cells *(49).* Nurr1 has been shown to initiate transcription of TH *(80).* TH and other genes for DA synthesis have been found to have potential Nurr1-binding sites in the 5′ upstream sequence *(49).* Therefore, there is strong possibility that the transcription factor Nurr1 is responsible for the coordinate downregulation

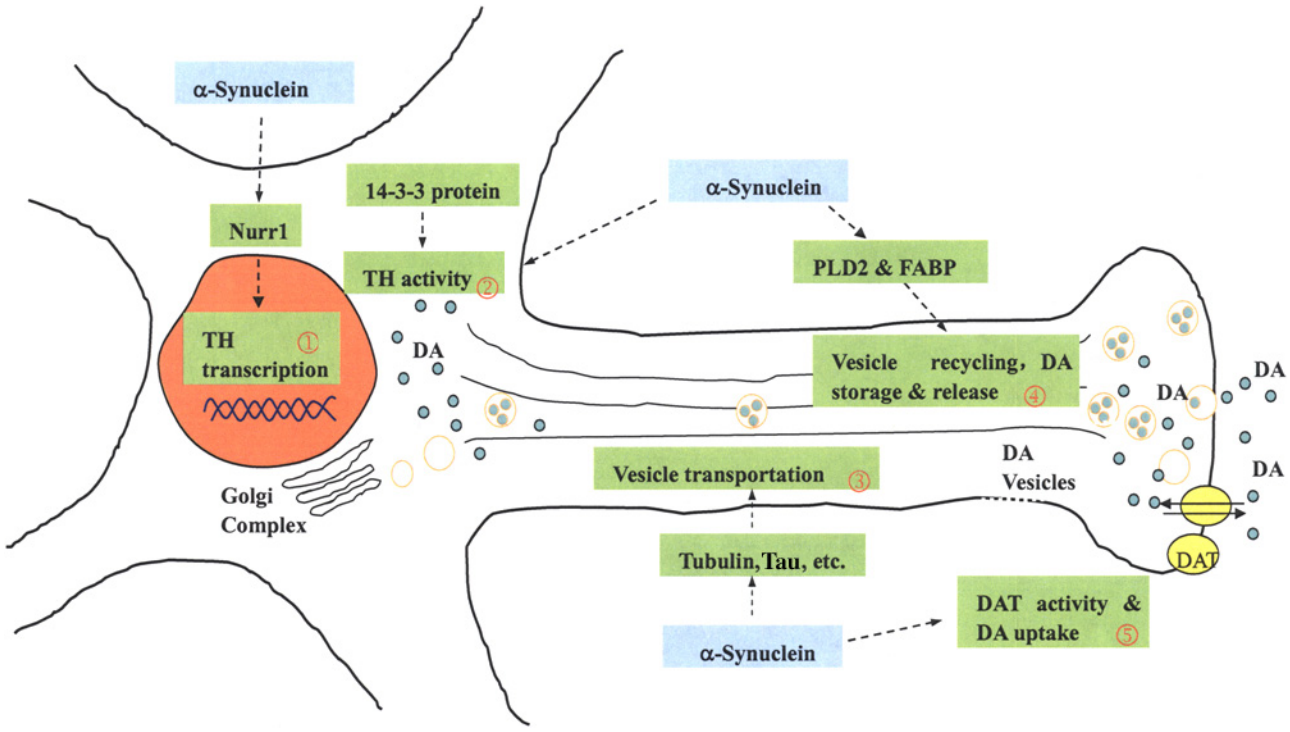

Fig. 1. Schematic representation of the action of α-Syn on dopamine metabolism. α-Syn affects almost every process associated with dopamine metabolism, including (1) inhibition of TH transcription probably by down-regulation of Nurr1 gene, (2) inhibition of TH activity through binding to the dephosphorylated form of TH and maintaining TH in an inactive form, (3) promotion of vesicle transportation by interacting several proteins such as tubulin and τ, (4) regulation of vesicle recycling, DA storage, and release by maintenance of a subset of presynaptic vesicles in the "reserve" or "resting" pools, and (5) reduction of DAT activity by forming a stable complex with DAT.

of the genes for DA synthesis. In α-Syn-transfected MES23.5 DA cells, it was also noticed that both TH protein and mRNA levels were dramatically reduced and the reduction of TH expression was not accompanied by cell injury, indicating that inhibition of TH expression is a normal function of α-Syn *(81)*.

Loss of Functions of α-Syn Disrupt the Homeostasis of DA Metabolism and Lead to a Vicious Cycle

Because α-Syn is involved in almost all of the processes related to DA metabolism, including DA synthesis, storage, release, and uptake, it is possible that loss of its functions might disrupt the homeostasis of DA metabolism. As described earlier, the presence of α-Syn in DA neurons in normal conditions tends to tone down the amount of cytoplasmic DA at nerve terminals, thereby limiting its conversion to highly reactive oxidative molecules. Conversely, loss of normal functions of α-Syn will favor the production of the highly reactive oxidative species in the cytoplasm of neurons through relief of the limitation mechanism exerted by α-Syn. There are several factors that might make α-Syn lose its functions. One of the factors is the mutation of the molecule. The PD-linked point mutations (A30P and A53T) have been shown to abolish the ability of α-Syn to

bind to the lipid vesicles *(35)*, and overexpression of A53T mutant α-Syn in differentiated MESC2.10 DA cells has been found to result in downregulation of the vesicular DA transporter (VMAT2), enhanced cytoplasmic DA fluorescence, and increased intracellular levels of superoxide *(53)*. In addition, it was demonstrated that the inhibitory effect of α-Syn on the activity of hDAT was also lost by A53T mutation of the protein, and DA accelerated both the production of reactive oxygen species and cell death in hDAT and wild-type or A30P, but not A53T, coexpressing cells, raising the possibility that loss of inhibition of hDAT by α-Syn could lead to more DA uptaken into the cells and increased levels of oxidative stress *(81)*. Another factor that can make α-Syn lose its normal functions is the abnormal aggregation of α-Syn into protofibrils, because α-Syn formation into protofibrils might decrease the bioavailability of the physiological form of the molecule. This can again increase the toxic potential of DA at nerve terminals, because the protein can no longer participate in the intricate physiological processes necessary for the maintenance of healthy DA neurons. Starting from the formation of α-Syn protofibrils, it is clear that free radicals, such as free iron or iron-centered radicals *(29)*, oxidized DA *(15)*, and catecholamines structurally related to DA *(15)*, accelerate and stabilize the formation of α-Syn protofibrils by inhibiting the conversion of toxic soluble protofibrils into insoluble fibrils *(15)*. Oxidative stress induced by loss of normal functions via formation of α-Syn protofibrils can, in turn, exacerbate α-Syn aggregation and protofibril formation. As a result, the DA neurons get into a vicious cycle, in which α-Syn protofibrils, abnormal DA metabolism, and oxidative stress interact with each other, leading to a final selective degeneration of the neurons. There are several factors in PD that can trigger the vicious cycle among aggregated α-Syn protofibrils, abnormal DA metabolism, and oxidative stress. Mutations in the α-Syn gene can accelerate the formation of α-Syn protofibrils *(17)* and trigger the vicious cycle. The levels of protofibrils and aggregated forms of α-Syn are also likely to be modulated through the ubiquitin–proteasome pathway *(83)*. Inhibition of the pathway can increase the levels of aggregated forms of α-Syn. There are two genes linking to familial PD in this pathway *(84)*. One is the *parkin* gene, which is a E3 ligase required to transfer ubiquitin onto proteins targeted for proteasome degradation. Another one is UCH-L1 (ubiquitin carboxyl-terminal hydrolase L1) gene. Mutations of both genes will compromise the degradation system and render α-Syn accumulated in the cytoplasm and promote the formation of α-Syn protofibrils, thereby triggering the vicious cycle. Although a causal link between a decrease in proteasomal degradation and high amounts of α-Syn aggregates has not yet been demonstrated, aggregation of α-Syn is clearly facilitated by increased concentration of α-Syn, as shown both in vitro *(21)* and in transgenic animals *(85,86)*. Environmental toxins are some other factors that might trigger the vicious cycle occurring in DA neurons in PD. Both MPTP and rotenone can induce parkinsonism in human and animals *(87,88)*. By inhibiting complex I in mitochondrial respiratory chain in DA neurons, both the toxins can induce increased expression and aggregation of α-Syn. PD is a neurodegenerative disease apparently linked to aging. It has been demonstrated that the aging brain is accompanied by decreased mitochondrial function and increased oxidative stress *(89)*. Evidence shows that oxidative stress will promote α-Syn aggregation by modification of the molecule *(90)*, again increasing the risk of leading the DA neurons into a vicious cycle.

Conclusion

Although α-Syn is implicated in the pathogenesis of PD, α-Syn itself is not toxic. Instead, by maintaining the homeostasis of dopamine metabolism, it helps DA neurons sustain a healthy state (Fig. 1). However, because of the molecular characteristic, α-Syn can easily form aggregated molecules such as the protofibrils

under the influence of both genetic and environmental factors. Aggregation into protofibrils will make α-Syn lose its normal functions in maintaining the homeostasis of dopamine metabolism. This will increase the levels of cytoplasmic DA and favor the production of highly reactive oxidative molecules in the DA neurons. On one hand, DA in the cytoplasm can stabilize the α-Syn protofibrils, the toxic form of α-Syn. On the other hand, the reactive oxygen species produced by DA metabolism in the cytoplasm will promote further aggregation of α-Syn into the protofibrils. As a result, α-Syn protofibrils, abnormal DA metabolism, and oxidative stress interact with each other, leading to a final selective degeneration of the neurons. In this vicious cycle, α-Syn seems to play a central role. Any factors such as gene mutations, environmental toxins, and aging that promote the formation of α-Syn protofibrils will drive the DA neurons into the vicious cycle. Preventing the formation of α-Syn protofibrils will be an alternative therapeutic approach for PD patients.

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