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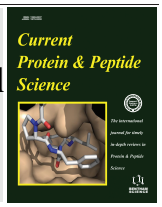
REVIEW ARTICLE

A Focus on the Beneficial Effects of Alpha Synuclein and a Re-Appraisal of Synucleinopathies



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Abstract: Alpha synuclein (α-syn) belongs to a class of proteins which are commonly considered to play a detrimental role in neuronal survival. This assumption is based on the occurrence of a severe neuronal degeneration in patients carrying a multiplication of the α-syn gene (*SNCA*) and in a variety of experimental models, where overexpression of α-syn leads to cell death and neurological impairment. In these conditions, a higher amount of normally structured α-syn produces a damage, which is even worse compared with that produced by α-syn owning an abnormal structure (as occurring following point gene mutations). In line with this, knocking out the expression of α-syn is reported to protect from specific neurotoxins such as 1-methyl, 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the present review we briefly discuss these well-known detrimental effects but we focus on findings showing that, in specific conditions α-syn is beneficial for cell survival. This occurs during methamphetamine intoxication which is counteracted by endogenous α-syn. Similarly, the dysfunction of the chaperone cysteine-string protein-alpha leads to cell pathology which is counteracted by over-expressing α-syn. In line with this, an increased expression of α-syn protects against oxidative damage produced by dopamine. Remarkably, when the lack of α-syn is combined with a depletion of β- and γ- synucleins, alterations in brain structure and function occur. This review tries to balance the evidence showing a beneficial effect with the bulk of data reporting a detrimental effect of endogenous α-syn. The specific role of α-syn as a chaperone protein is discussed to explain such a dual effect.

Keywords: Alpha synuclein, synucleinopathies, alpha synuclein aggregates, loss-of-function, co-chaperonine, neurodegeneration, neuroprotection.

1. INTRODUCTION

Alpha synuclein (α-syn) is a small (14.5 kDa) presynaptic protein consisting of 140 amino acids and it was originally isolated from synaptic vesicles and nuclear envelopes of the electric organ of Torpedo californica [1-3]. It belongs to the synuclein family, which includes two other proteins that are highly conserved in vertebrates, namely β- and γ-synucleins [1, 4-7]. In particular, α-syn is ubiquitously and heterogeneously expressed in both central and peripheral nervous system, encompassing a wide number of brain regions (isocortex, hippocampus, olfactory bulb, striatum, thalamus and cerebellum) [6, 8]. The protein is mostly found within synaptic terminals, where it often co-localizes with β- and γ-synucleins. A little amount is present in the cell body, dendrites or extra-synaptic sites along the axons [1, 8-11].

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Our understanding of the biology of α-syn has significantly increased under the impulse generated by the discovery, in 1997, of a single point mutation in α-syn (*SNCA*) gene, which may per se determine genetic Parkinsonism [12]. This gene mutation, which induces an altered primary structure of the native protein, was identified in an Italian kindred (the Contursi family) and in three non-related Greek kindreds, and it was associated with autosomal dominant inherited Parkinson's disease (PD) [12].

From that groundbreaking discovery, several *SNCA* alterations, which may contribute to the pathogenesis of PD have been identified. These include several point (missense) mutations (*i.e.* A30P, E46K, H50Q, G51D, A53E, A53T) which are classified as PARK1 type, while genomic duplications and triplications of *SNCA* are classified as PARK4 genetic parkinsonism [13-22]. These large gene rearrangements produce a higher amount of normally structured α-syn, which produces a dose-dependent damage, which in any case, is worse compared with that caused by point mutations (with an exception of the point mutation G51D which causes a devastating disorder overlapping with Multiple System

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Atrophy). Thus, in most cases, when normally structured α-syn is present in large amounts, it causes a brain damage, which is even worse than that induced by altered protein structure that is unique among all genetic forms of PD. In fact, *SNCA* multiplication produces a lethal form of PD, which significantly impairs life expectancy depending on the number of copies in excess. Thus, normally structured α-syn may be really deleterious for cell survival. In line with this, according to the autosomal dominant inheritance of α-syn point mutations, a gain of function is supposed to underlie the brain damage caused by the mutated protein in PARK1 patients. This is confirmed by experimental data from the last couple of decades regarding such a deleterious effect, as in the case of MPTP - [6, 23, 24], 6OHDA- [25], and rotenone-induced [26-28] parkinsonism. Therefore, it may be inferred that α-syn plays just a detrimental role for cell survival.

Despite α-syn represents the culprit of a variety of degenerative disorders named synucleinopathies, it still remains to be elucidated why, albeit its detrimental effects, evolution has preserved α-syn in the cell to serve any kind of functions, which may be beneficial instead. Noteworthy, in certain experimental conditions α-syn plays an opposite role which is critical to counteract cell damage. This is the case of methamphetamine-induced toxicity, where a depletion of α-syn dramatically exacerbates the nigrostriatal damage [6]. Similarly, the overexpression of α-syn may counteract the brain damage induced by a depletion of the chaperone cysteine-string protein-α (CSP- α) [29], or may prevent oxidative damage induced by dopamine (DA) self-oxidation products and parkin depletion [30]. Remarkably, its predominant expression at pre-synaptic terminals accounts for a physiological function in regulating synaptic vesicles dynamics, such as synapse maintenance, neurotransmitter release, and neuronal physiology and plasticity [31-33]. In line with this, α-syn associates with cell membranes and it preferentially binds to anionic lipids and high-curvature lipid vesicles, mostly small uni-lamellar vesicles (SUVs) and large uni-lamellar vesicles (LUVs) [34-38].

In the present review, we will focus on the multi-faceted roles of α-syn analyzing in detail its detrimental and beneficial effects and trying to balance these opposite outcomes in cell homeostasis. This analysis is aimed to provide a deeper insight on the significance of this protein for neuronal physiology and specifically in neurodegeneration. Furthermore, a critical analysis of the dual roles of α-syn will be implemented with its activity as co-chaperonine, which potentially operates within the cell or may be shared within a multicellular context. This may disclose the evolutionary drive leading to protein spreading, which should probably be reconsidered as a natural phenomenon which does not necessarily spread disease but may also help cell survival and sustain plasticity.

2. ALPHA-SYNUCLEIN RELATED NEUROPATHOLOGY

In human patients, the mutation of α-syn generates the degeneration of mesencephalic neurons belonging to the substantia nigra pars compacta (SNpc), mostly in the ventral tier of this nucleus, which mainly projects to the basal ganglia [39, 40]. Spared neurons, despite being viable, feature neuronal inclusions which stain specifically for α-syn [41, 42]. Remarkably, if one considers the neuropathology of the SNpc in most PD patients lacking any kind of mutations in *SNCA*, the occurrence of α-syn positive neuronal inclusions represents the hallmark for the pathological diagnosis of PD [43]. In most cases of PD, α-syn positive inclusions (roughly corresponding to what was once defined Lewy bodies) are not limited to DA neurons of the SNpc but they extend to a variable degree to other brainstem nuclei [44-49]. In fact, the presence of α-syn positive inclusions within spared neurons of the noradrenergic (NA) nucleus of Locus Coeruleus (LC) is now considered to be a key point in the diagnosis of PD [47, 50, 51]. This is in line with the consistently massive involvement of LC in PD, which may surpass the damage of SNpc neurons [39, 47, 52-55]. Apart from these two catecholamine-containing nuclei, α-syn aggregates may be present to a variable extent in a constellation of brainstem reticular nuclei, among which, the most frequently affected are the dorsal motor nucleus of the vagus, the nucleus of the solitary tract, the dorsal raphe nucleus, and the pedunculopontine nucleus. Recently, a clinical-pathological correlation, in which the severity of PD symptoms associates with the number of brainstem nuclei affected by α-syn pathology, has been hypothesized [48, 51]. Such a consideration led to redefine PD as a Monoamine Brainstem Disorder (MBD) in which an innumerous pattern of affected nuclei may justify the high variability of clinical symptoms occurring in multiple PD isotypes [47, 51]. In this way, the widespread α-syn pathology within the brainstem resembles the constellation of affected brainstem nuclei in von Economo's encephalitis lethargica [56, 57]. In fact, just like it was described for these patients, it is now well recognized that even in PD a number of symptoms affecting non-motor domains may be present. Among these non-motor symptoms (NMS), sleep disturbances occur very frequently [47, 58-60]. Sometimes REM sleep behavioral disorders (RBD) may even anticipate the onset of extra-pyramidal motor symptoms in a way that is reminiscent of what happened after the 1918's flu pandemia [61, 62]. These overlapping disorders are fascinating and they suggest that similar targets are shared in all PD-related disorders independently from the specific etiology. The presence of pathological α-syn accumulation within cells is not confined to the brainstem but it may extend far away to involve a variety of brain areas [42] and even the spinal cord [55, 63-65]. When spreading massively to cortical regions, α-syn produces a degenerative dementia associated with a parkinsonian movement disorder, known as Dementia with Lewy Bodies (DLB) [55, 66-69]. In this case, α-syn positive inclusions are present in a variety of neurons exceeding the monoamine systems [55, 69-74]. In other pathological conditions, the presence of α-syn aggregates occurs in glial cells as well, thus involving multiple brain areas. In this case, the substantia nigra, the striatum, the cerebellum, and preganglionic sympathetic neurons are markedly affected along with surrounding astrocytes. When this process involves oligodendrocytes and produces a de-myelinization in the affected areas, a pathological condition known as Multiple System Atrophy (MSA) is described. Among synucleinopathies also Pure Autonomic Failure (PAF, according to the Bannister classification) should be considered [75]. This consists in a pure orthosympathetic autonomic dysfunction in the absence of any movement disorders. This pathological

overview, which is generally accepted when classifying synulceinopathies, is rather simplistic. In fact, the definition of synucleinopathies consists in multiple disorders of the CNS, where some neuronal systems are affected by α-syn aggregates. In keeping with this, other pathological conditions should be taken into account by using an extended definition of synucleinopathies. This is the case of Huntigton disease (HD), where despite being huntingtin the aberrant protein, large vacuoles accumulating within neurons (mostly in the neostriatum) feature α-syn aggregates along with huntingtin aggregates. Again, a number of disorders characterized by protein accumulation feature α-syn aggregates as well. This is the case of Alzheimer's disease (AD) [76-78], Frontotemporal Dementia (FTD) [79, 80], Progressive Supranuclear Palsy (PSP) [81], in which α-syn aggregates occur along with Tau accumulation. This should not surprise, since in the presence of α-syn mutations, inherited parkinsonism is also featured by Tau accumulation [82-85]. Apart from protein aggregates, the neuronal pathology related to α-syn is characterized by neuronal loss which happens in all the brain areas that we just described. In addition, when multiple copies of normally structured α-syn are present, a massive neuronal degeneration with a severe cell loss is described in the hippocampal region and other allocortical areas [86]. When analyzing classic α-syn positive inclusions, as they were originally described in PD patients, these are proteinaceous aggregates with no external limiting membrane where treatment with protease does not cleave α-syn [87, 88]. In this way, just like the pathological prion protein, aggregated α-syn is resistant to proteinase K and sarkosyl [89]. In addition, just like prion disorders, a multiple band of the protein is detected at SDS page immunoblotting. This is due to the formation of advanced glycation end-products (AGEs), where specific sugars covalently bind to α-syn. In these aggregates, α-syn often co-exists with ubiquitin, which justifies the previous description by McKeith of Lewy Bodies as ubiquitin-positive inclusions (this happened before the discovery of α-syn in PD) [90]. Indeed, Fritz Lewy provided the definition of Lewy Bodies (LB) in 1912, long time before the use of immunostaining. Thus, the original definition of LB corresponds to pale, eosinophilic cytoplasmic inclusions [42]. It sounds odd that we often treat these pathological hallmarks as a single pathological entity, since many α-syn positive inclusions do not stain for ubiquitin and some of them are not pale, eosinophilic structures. Thus, the staining with α-syn antibodies has modified the amount and the entity of what was once defined as LB, which may create some confusion despite being a guide for the perplex scientists. In fact, the present definition of LBs states that they are α-syn positive aggregates and that α-syn is the best marker to detect LBs thanks to its high sensitivity. However, if one considers the original definition of LB or the classification by McKeith, there is no reason to assume that all α-syn positive aggregates correspond to LBs. We feel that it is rather more correct to state that α-syn stains LBs and other proteinaceous aggregates which were previously unclassified. It is likely that even the molecular mechanisms leading specifically to LB formation, may or may not overlap with α-syn aggregates. In fact, the presence of ubiquitin witnesses for a tagging of a substrate (α-syn itself) to be delivered either to proteasome [87, 91] autophagy or autophagoproteasome [92-95] degradation. Instead, if one considers α-syn-positive/ubiquitin-negative proteinaceous material, there is no reason to assume that this will be eventually delivered for degradation. This is even more distant from the original Lewy's definition. In fact, the presence of pale eosinophilic cytoplasmic regions generally refers to a uniform variation of the cytosol architecture leaving unknown the specific nature of their content. Thus, we feel that it may be inappropriate to state that α-syn stains very selectively LBs; we rather consider more adherent to facts the definition that α-syn stains very many protein aggregates within PD brains.

5. CHALLENGING THE CLASSIC CONCEPT OF SYNUCLEINOPATHIES

In keeping with real observations and consistent definitions, the term synucleinopathy is rather misused. In fact, the original definition by Martì and coworkers [96] states that a synucleinopathy is a disorder in which α-syn aggregates are found within affected neurons. On the other hand, clinical nosography lists only a few disorders as synucleinopathies, which correspond to: PD, MSA, DLB and PAF. However, a fair pathological view-point includes other disorders in which α-syn aggregates occur consistently within affected neurons. This is the case of lipid storage disorders such as Gaucher's and Krabbe's diseases and Sanfilippo Syndrome, as well as HD, AD and Amyotrophic Lateral Sclerosis (ALS) [97-102]. Thus, the classic concept of synucleinopathies, which initially included only PD, MSA, DLB and PAF [96, 103, 104], should be rather considered as a continuously evolving spectrum of disorders which is indeed intermingled with the evolving concept of PD as a disease spectrum. New insights into the pathophysiological mechanisms behind neurodegenerative diseases, in the latest years have shown that disorders such as Krabbe's and Gaucher's diseases may themselves constitute a risk factor for developing PD [100, 102]. In detail, Krabbe's and Gaucher's diseases feature primary lysosomal dysfunction and marked glycolipids accumulation caused by a decreased glucocerebrosidase activity. The neuropathological evaluation of brains from Gaucher's and Krabbe's patients carrying *GBA1* and *GALC* gene mutations respectively, has revealed classical α-syn-positive, ubiquitinated Lewy inclusions [102, 105, 106]. To date, the carrier status of a heterozygous *GBA1* mutation is considered the most common genetic risk factor for α-syn aggregation-associated disorder in the brain [100]. The decreased glucocerebrosidase activity affects lipid raft function by interfering with the sorting and trafficking of proteins and lipids associated with the rafts. Thus, it has been postulated that glucosylceramides are responsible for directly stimulating the accumulation and promoting the oligomerization of α-syn [107], which is localized to these lipid rafts in neuronal cells [108]. Consequently, the buildup of α-syn would then fuel further impairment of the lysosomal and proteasomal degradative machineries [109, 110], eventually leading to a more generalized dysfunction and increased neuronal vulnerability to stressors (Fig. **1**). α-Syn has also been shown to bind specifically to gangliosides such as GM1 [111]. Mutations at the level of *GM1* cause a deficiency of beta-galactosidase, with resulting abnormal storage of acidic lipid materials in cells of the central and peripheral nervous systems, but particularly in the nerve cells. This leads to a perturbation of membrane dynamics due to the imbalance of

psychosine degradation, which accumulates within lipid rafts of the membrane, disrupting their architecture [112]. This mechanism is likely to induce localization of α-syn within pre-synaptic terminals and promote its aggregation [108]. α-Syn aggregates are found in Sanfilippo Syndrome as well, a storage disorder caused by α-N-acetylglucosaminidase (*NAGLU*) gene mutations. Similarly to what occurs in Gaucher's and Krabbe's diseases, a general impairment in the heparan sulfate clearance pathway is thought to cause α-syn pathological accumulation in this case [98].

In Huntington disease (HD) the striatum is filled with α-syn aggregates which associate with huntingtin aggregates [97]. Phosphorylated α-syn aggregates co-localizing with superoxide dismutase 1 (SOD1) have been detected in an autopsy case of familial ALS [99]. This exemplifies another case where α-syn aggregation is suggested to be triggered by the interaction of mutant SOD1 protein with α-syn across axo-somatic synapses [99].

α-Syn aggregates have been reported to co-exist with Tau aggregates in classic Tauopathies such as AD and Lewy body variant of AD (LBVAD) [76-78, 113, 114]. Several genetic studies associate Microtubule-Associate Protein Tau (MAPT) H1 haplotype or *MAPT* variants (*i.e.* p.A152T) to an increased risk for developing PD and MSA [115-118]. However, other studies suggest that even wild-type Tau promotes *in vivo* α-syn aggregation and toxicity [119, 120]. This is consistent with evidence demonstrating that over-expressed Tau decreases proteasome activity by inhibiting histone deacetylase 6 (HDAC6), which in turn leads to an increased amount of α-syn aggregates [121].

This indicates a number of disorders in which α-syn aggregates may be found. Most of these disorders are not classified as synucleinopathies although we cannot see why. In fact, even in classic synucleinopathies the aggregation of α-syn may be not a primary event but rather the consequence of an altered cell metabolism (Fig. **1**).

Then, it comes natural to challenge the classic concept of "synucleinopathies" when referring to these kind of diseases. While figuring out that the classic definition does not always properly fit the real pathological issue, it becomes mandatory to scrutinize the biological significance of such an intriguing protein. This is actually a less discussed matter, which addresses the alternative though equally valid hypothesis that the aggregation of a-syn results in neurotoxicity through a

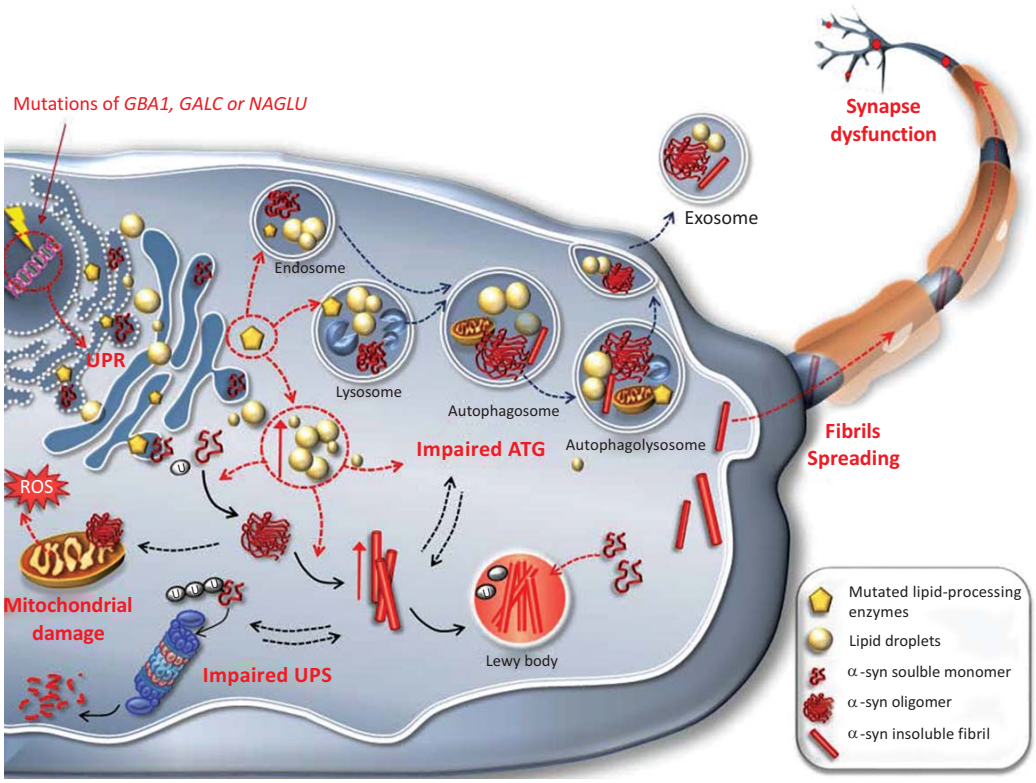

Fig. (1). Alpha synuclein aggregation in lipid storage diseases.
This cartoon depicts the cascade of the primary molecular events occurring in lipid storage diseases. This provides an example of how an impaired cell metabolism may directly promote α-syn aggregation through a mechanism of "loss of function". Specific mutations in *GBA1*, *GALC* or *NAGLU* genes in Gaucher's, Krabbes and Sanfilippo disease, respectively, cause a deficiency in the specific lipid-processing enzymes. This results in an abnormal production of acidic lipid material, which may accumulate in the endoplasmic reticulum and Golgi network thus contributing to an early impairment in the Unfolded Protein Response. An excess of lipids within lysosomes and cytoplasm leads to an impairment of the autophagy (ATG) machinery, and promotes oligomerization and fibrillization of α-syn in these compartments. The buildup of α-syn up to the formation of insoluble Lewy bodies (LBs), further impairs the ATG and proteasome (UPS) degradative machineries and contributes to mitochondrial disruption and release of reactive species of oxygen (ROS). Normally soluble α-syn may be trapped into LBs, resulting in a loss of α-syn function. Thus, a feed-forward vicious circle, while increasing the overall neuronal vulnerability, promotes the spreading of pathological α-syn, which can either move towards pre-synaptic terminals or be released outside the cell by exosomes.

loss-of-function mechanism, following sequestration of functional soluble a-syn into aggregates. In the next paragraph, the biological and beneficial face of a-syn will be portrayed.

4. PHYSIOLOGICAL AND BENEFICIAL ROLE OF α-SYN

A growing body of evidence reported in literature, converges in validating the implication of α-syn in a variety of physiological processes encompassing regulation of synaptic vesicles' trafficking, neurotransmitter release, gene expression and stress response (by counteracting oxidative stress and several toxic insults) (Fig. **2**). In this paragraph, while revising the above-mentioned aspects, we report some salient findings where accordingly to its physiological role, a-syn behaves as a neuroprotective agent.

4.1. Membrane Curvature and Synaptic Vesicles' Homeostasis

α-Syn's astonishing conformational plasticity and it's affinity for binding membranes, especially highly curved ones [122, 123], as well as its ability to actively alter their shape/curvature [124], confers to α-syn a crucial role in synaptic vesicle homeostasis and endo-/exocytosis. For instance, α-syn is involved in the regulation of several processes and properties of synaptic membranes such as the size of the vesicular pool, vesicular trafficking to- and docking with the presynaptic membrane and clathrin-associated formation of synaptic vesicles [125] (Fig. **2**). In detail, experimental evidence demonstrates that synucleins are key in maintaining the functional endocytic rate of synaptic vesicles. Biochemical and ultrastructural analyses revealed that synucleins are involved in early stage clathrin-mediated endocytosis, before

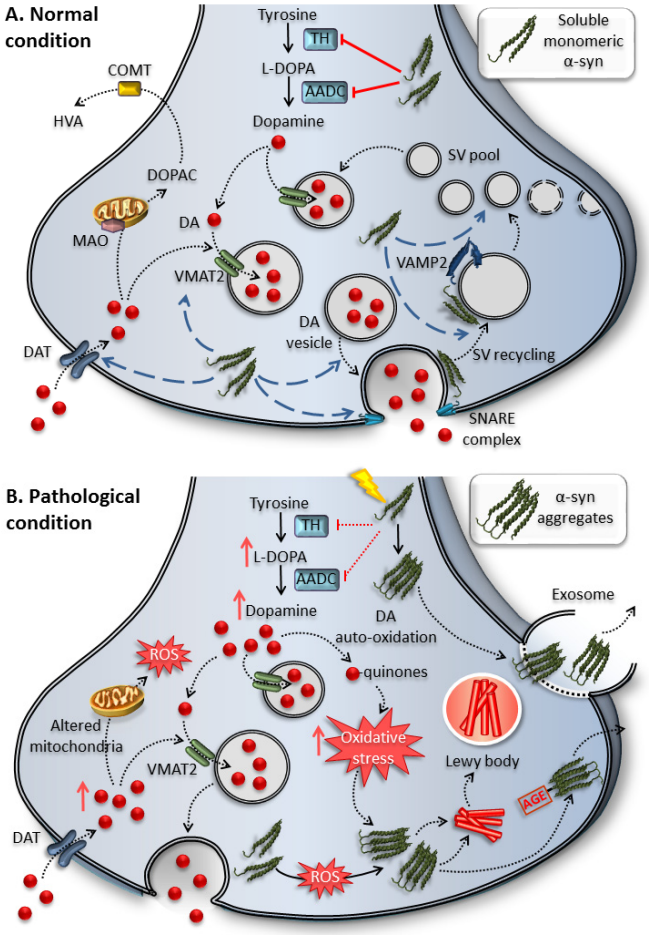

Fig. (2). Physiological role of alpha synuclein in DA metabolism.

(**A**) α-Syn has been reported to regulate a variety of physiological processes and properties of synaptic membranes such as the size of the vesicular pool, synaptic vesicles' trafficking, docking and recycling, as well as neurotransmitter release. In fact, α-syn promotes membrane bending by interacting with a variety of proteins such as synaptobrevin-2/VAMP2. Moreover, α-syn is reported to control neurotransmitter release by co-chaperoning SNARE-complex assembly. For instance, α-syn is involved in the regulation of DA biosynthesis and handling. Under normal conditions, α-syn inhibits the activity of TH and AADC and increases DAT and VMAT2.

(**B**) Upon either specific genetic mutations or environmental factors (low pH, oxidative stress, iron), α-syn is modified and sequestered into aggregates with a "loss of function". Thus, a loss of TH inhibition increases DA synthesis, reduces the uptake of DA and impairs synaptic vesicle trafficking and recycling. An increased DA synthesis in the absence of vesicle storage, augments unsequestered cytosolic DA which readily auto-oxidizes to form DA-quinone and reactive oxygen species (ROS), which contribute to the formation of α-syn aggregates. This vicious cycle of α-syn aggregates and oxidative stress may contribute to DA neurons degeneration. Similarly, these aggregates may be transmitted intercellularly as AGE-α-syn aggregates and/or exosomes.

clathrin and dynamins action, and they act by promoting membrane bending [126]. This is in line with the findings that α-syn can sense and generate membrane curvature during endocytosis, similarly but less efficiently to endophilin [124, 127, 128]. *In vitro* membrane tubulation assay showed that these properties are shared between all the members of the synuclein family. Interestingly, the authors demonstrated that α-syn concentration at synaptic level (2-5 μM), is in the range needed to promote tubulation. This is expected to generate a robust stimulus to sustain membrane curvature since membrane tubulation increases linearly for low doses of α-syn up to 7 μM [128]. This activity is shared by all synucleins including β and γ [128], which allows a wide compensatory range when α-syn is not working.

Moreover, α-syn is reported to participate in the control of neurotransmitter release via interactions with members of the SNARE family. More specifically, it was suggested to promote SNARE-complex assembly through a non-enzymatic mechanism, by binding to phospholipids via its N-terminal and to synaptobrevin-2 via its C-terminal [129]. Despite α-syn's role in SNARE-complex assembly still remains controversial, several studies focused on the role of α-syn in co-chaperoning SNARE when acting in combination with other proteins. For instance, α-syn strongly interacts with CSPα, a synaptic vesicle protein with a co-chaperone activity. This pre-synaptic co-chaperone is essential for neurotransmitter release and neuronal survival [130, 131]. Deletion of CSPα in mice results in a loss of the proper SNARE complex assembly and produces neurodegeneration, thus resulting in a lethal phenotype [132, 133]. Remarkably, the deletion of α- and β-synucleins exacerbates CSPα KO lethal phenotype, which is consistently reversed by a transgenic overexpression of α-syn [29]. In detail, α-syn overexpression prevents the neurodegeneration caused by CSPα deficiency, such as spinal cord gliosis, neuronal cell death, and decreased neuromuscular junction average size. Moreover, overexpression of α-syn rescues locomotor deficits and motor behavior and increases CSPα KO mice survival [29]. Again, overexpression of α-syn rescues altered synaptic structure and function yielded by the complete deletion of all endogenous synucleins (α-, β- and γ-) [134]. In line with this, singularly transfecting synuclein null cultures of hippocampal neurons with each of synucleins (either α-, β- or γ-) prevents impaired synaptic vesicles endocytosis, thus supporting the functional redundancy and the mutual compensatory effect of synucleins in synaptic homeostasis [126].

All this evidence strongly suggests a role as a co-chaperone protein similar to CSPα for all synucleins, which may work within each cell and across a cell population [135]. Notably, α-syn's cell-to-cell transmission, from the donor towards the acceptor cell, either between neurons or from neurons to glial cells [135, 136], occurs without any sign of cell membrane damage and leakage nor cell death [135].

4.2. Handling Dopamine Release

Accumulating attention has been paid to the potential role of α-syn as a regulator of dopamine (DA) biosynthesis and handling (Fig. **2**). Several *in vivo* studies have adopted synuclein's deletions to investigate the impaired homeostatic regulation of DA levels. An extended deletion encompassing α- and β-synucleins turned out to reduce striatal DA terminals in the nigrostriatal system [136, 137]. In line with this, the striatal monoamines quantification by HPLC and coulometry performed in the dorsal striatum from synuclein deficient mice revealed a decrease in DA levels compared with controls [137]. In addition, the triple synuclein deletion furtherly reduces striatal DA terminals while altering brain structure [138]. Even the single α-syn KO may produce degeneration, as it was demonstrated in mice lacking α-syn, which displayed an enhanced damage of DA nigrostriatal terminals induced by methamphetamine (METH). Compared to wild-type littermates, α-syn KO mice resulted more sensitive to METH neurotoxicity and they exhibited a threefold loss of DA amount [6].

In keeping with a role as a co-chaperone, a loss of a-syn function has been postulated to disinhibit TH and AADC enzymes [33, 139-141], which triggers increased DA synthesis, reduction of the amount of VMAT2 on synaptic vesicles [142] and impaired synaptic vesicle trafficking and recycling [35, 126, 129, 143-145]. These events result in a net increase of cytosolic DA which auto-oxidizes to produce toxic adducts such as DA-quinone and ROS [146]. This may be furtherly accompanied by the sequestration of normally soluble α-syn into growing aggregates, resulting in the definitive loss of α-syn function and of its regulated-proteins [147]. In fact, an overexpression of α-syn prevents DA self-oxidation and the accumulation of toxic DA metabolites induced by a loss of the co-chaperone parkin [30], which strengthens the evidence for a physiological role of α-syn in DA homestasis.

4.3. Counteracting Oxidative Stress and Toxic Insults

The scenario of a beneficial and protective role of a-syn in decreasing cell vulnerability in response to several neurotoxic insults, has been continuously amplifying as reported by several studies [148-151]. For instance, α-syn protects against oxidative stress [151, 152], apoptotic stimuli [148], and various environmental toxic agents such as the pesticides paraquat and rotenone [149, 150].

In keeping with neuroprotective properties, a very recent study shows that α-syn overexpression ameliorates phenotype and neuromuscular junction pathology in the Smn2B/- mouse model of Spinal Muscular Atrophy (SMA) [153]. The decreased disease severity, accompanied by increased weight gain and extended life span, suggest that α-syn may act as a motor neuron disease phenotype modifier exerting a neuroprotective effect in motor neuron pathology. In line with this, transgenic mice for mutated α-syn are reported to develop a motor neuron disorder [154].

Despite conflicting data still exist, all this evidence about α-syn neuroprotective effects, which extend beyond PD and synucleinopathies, calls for further investigation regarding the physiological role of this protein.

4.4. Genetic and Epigenetic Modulation

α-Syn has been found to also have nuclear localization [155-157], which suggests it may play further yet undiscovered roles related to the regulation of gene expression. Epi-

genetic-mediated alterations, such as abnormal methylation or miRNA dysregulation, have been reported to affect the expression and/or the aggregation of α-syn, and in turn, abnormal spreading of α-syn into the nucleus may influence the epigenetic and genetic landscape by interacting with chromatin, histones and DNA regions [158]. In nigral dopaminergic neurons of *SNCA* transgenic mice the levels of protein kinase C delta (PKC δ) and of p300 were found down-regulated suggesting that α-syn may be protective in this experimental model [159]. Moreover, α-syn expression has been reported to drastically decrease the apoptotic response of neuronal cells by down-regulating p53 expression and transcriptional activity, and this effect gets lost when soluble α-syn becomes sequestered in aggregates [160].

5. THE FATE IN THE STRUCTURE

In the context of this multi-faceted behavioral mechanism, detailing not only the physiologic role of a-syn, but even peculiar features in its structure, may be essential in providing potential explanations about its susceptibility to pathological misfolding and aggregation. For instance, studies aimed to unravel the structural properties of native brain α-syn, provide a clear demonstration on how fate in the structure may be closely predictive of spontaneous misfolding and aggregation properties. Studies so far reported in literature have postulated two main theories about the nature of endogenous α-syn. The vast majority define it as a natively unfolded monomer of about 14 kDa acquiring an α-helical secondary structure only upon binding to lipid vesicles [1, 2, 161-163]. In contrast, in 2011 Bartels and coworkers [164] reported that endogenous α-syn occurs mainly as a stably folded tetramer of about 58 kDa. Analytical ultracentrifugation, electron microscopy and *in vitro* cell crosslinking under non-denaturing conditions from neuronal and non-neuronal cell lines, brain tissue and living human cells, revealed that native α-syn possesses α-helical structure without lipid addition but it owns a greater lipid-binding capacity than recombinant α-syn. Differently from recombinant monomers, these native human tetramers underwent little or no amyloid-like aggregation *in vitro*. Thus, they proposed that α-syn misfolding and aggregation event is actually preceded by the destabilization of the helically folded tetramers [164]. In 2013 instead, the monomeric state of native brain α-syn was reconfirmed by Burré and coworkers [165]. They demonstrated that native α-syn, directly purified from mouse brain, is a largely unstructured monomer, which aggregates time-dependently. The authors detected a molecular mass of ~63 kDa, which is close to that predicted for a folded tetramer, but Filtration Gel migration and Size-Exclusion Chromatography coupled with Multi-Angle Laser-Light Scattering (SEC-MALS) argued against a folded multimeric conformation [165]. These results sustain the theory following which a labile rather than a stable conformational state, is what renders α-syn much more prone to aggregation. In this intrinsically disordered unfolded state, a plethora of dynamic changes, especially significant post-translational modifications, occur at the level of a-syn, thus playing a crucial role in regulating its function and conformation (Fig. **3**). The C-terminus and the N-terminus, which contain a large number of charged residues, have been identified as the two main regions being particularly susceptible to acetylation, phosphorylation, oxidation, nitration, ubiquitination and truncation [166-170]. Certain conditions have been reported to favor conformational modifications and drive a pathological cascade of conversion from α-syn self-assembly up to insoluble fibrils and oligomers formation [171, 172]. These range from environmental factors such as low pH [173], oxidative stress [174] and iron [175, 176], to genetic mutations [177, 178], and eventually to binding or interacting molecular partners [179, 180], including the same DA [181]. Nonetheless, in this context, oligomers or protofibrils may accelerate the conversion of physiological α-syn into aggregates by acting as seeds [182]. In line with this, several recent studies in mice have shown that stereotaxically injected aggregates (but not monomers) of α-syn, can spread to neuroanatomically connected regions and initiate *de novo* aggregation [183-185]. A recent study where a new Proximity-Ligation-Assay technique was set up for detecting oligomeric α-syn in human PD post-mortem brain tissues, demonstrated that disease-associated α-syn species show an intermediate proteinase K sensitivity, suggesting they are oligomers in a very early stage of aggregation [186]. They clearly appeared to be folded differently than physiological presynaptic α-syn but had not yet acquired a highly compact structure resistant to prolonged digestion by proteinase K [186]. These observations fit the hypothesis following which interacting α-syn molecules are an early and necessary step allowing the misfolded species to transfer their pathogenic folding to newly recruited units in a prion-like route [187].

These data suggest that events triggering misfolding of α-syn can be in part drawn back at the level of its intrinsic structural features, which in certain conditions may consequently contribute to an aberrant conformation (Fig. **3**).

CONCLUSION

The dynamic and plastic chameleon-like structure of α-syn coupled with its potential in performing so many critical physiological functions configure it as a double edged sward, which better exemplifies the intriguing paradigm of this multi-faced protein. When analyzed from this point of view it becomes easier to envision how the biological background and the complex interaction with so many other molecular factors, may affect the amount, conformation and localization of normal α-syn, leading to a loss of its biologic function up to a gain in neurotoxic properties. Remarkably, as we revised here, there are many studies providing evidence about a neuroprotective role of α-syn in several experimental settings. On the other hand, when mutated or simply up-regulated, α-syn may target further membranous structures, regulatory molecules or even whole molecular pathways, and this presumably accounts for its toxicity. This is why continuous studies and overall reappraisals of the biological background where these interactions take place are essential to shed light on α-syn toxicity. Most notably, this implies the need for compelling treatment options, which while battling aggregated pathological forms of a-syn, should also preserve its physiological function. In line with this, in the latest years, natural compounds such as curcumin and its analogs [188] are emerging as potential drugs able to inhibit a-synuclein fibrillization and to stabilize it in a non-toxic state.

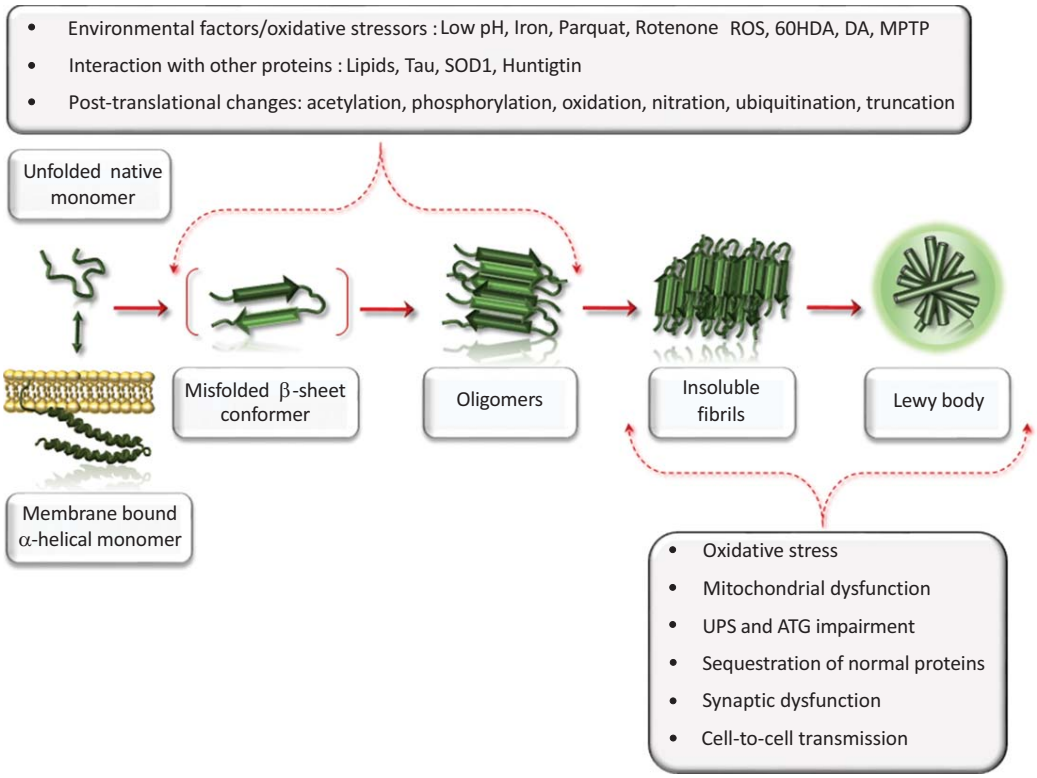

Fig. (3). Disruption of alpha synuclein conformational equilibrium: causes and consequences.
This cartoon provides a schematic summary about the mechanisms affecting α-syn conformation, and those resulting from a loss of α-syn function. α-Syn is a natively unfolded monomer that acquires α-helical secondary structure upon binding to lipid membranes. In a physiological state, a dynamic equilibrium exists between these two conformations. Several environmental factors, oxidative stressors, post-translational changes and interactions with other proteins may directly favor α-syn misfolding and drive a pathological cascade of conversion from self-assembly up to insoluble fibrils formation. In this aberrant conformational state α-syn may propel a generalized impairment of cell homeostasis consisting in synaptic dysfunction, oxidative stress, mitochondrial disruption, impairment of cell-clearing mechanism, sequestration of normal proteins into insoluble LBs, and cell-to-cell spreading of misfolded and aggregated α-syn conformers.

LIST OF ABBREVIATIONS

AADC	=	Aromatic Amino Acid Decarboxylase
AD	=	Alzheimer's Disease
AGEs	=	Advanced Glycation End-products
ALS	=	Amyotrophic Lateral Sclerosis
ATG	=	Autophagy
COMT	=	Catechol-O methyl Transferase
CSP- α	=	Cysteine-string Protein-α
DA	=	Dopamine
DAT	=	Dopamine Transporter
DLB	=	Dementia with Lewy Bodies
DOPAC	=	3,4-Dihydroxyphenylacetic Acid
FTD	=	Frontotemporal Dementia
GALC	=	Galactocerebrosidase
GBA	=	Glucocerebrosidase Beta
HD	=	Huntigton Disease
HDAC6	=	Histone Deacetylase 6
HVA	=	Homovanilic Acid
LBs	=	Lewy Bodies
LBVAD	=	Lewy body Variant of AD
MAO	=	Monoamine Oxidase
MAPT	=	Microtubule-Associate Protein Tau
MBD	=	Monoamine Brainstem Disorders
METH	=	Methamphetamine
MPTP	=	1-Methyl, 4-phenyl 1,2,3,6-tetrahydropyridine
MSA	=	Multiple System Atrophy
NAGLU	=	α-N-acetylglucosaminidase
PAF	=	Pure Autonomic Failure
PD	=	Parkinson's Disease
PKC δ	=	Protein Kinase C Delta
PSP	=	Progressive Supranuclear Palsy
RBD	=	REM Sleep Behavioral Disorders
ROS	=	Reactive Oxygen Species
SMA	=	Spinal Muscular Atrophy
SNpc	=	Substantia Nigra Pars Compacta

SOD1 = Superoxide Dismutase 1

SV = Synaptic Vesicles

TH = Tyrosine Hydroxylase

UPS = Ubiquitin Proteasome System

VAMP2 = Vesicle-associated Membrane Protein 2

VMAT2 = Vesicular Monoamine Transporter 2

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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LR wrote the paper and made artwork; CLB provided a critical review of the literature; FL contributed to write the paper and made artwork; FB contributed to review literature; SG provided a review of the literature; FF wrote the paper and provided a critical review of the whole manuscript.

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