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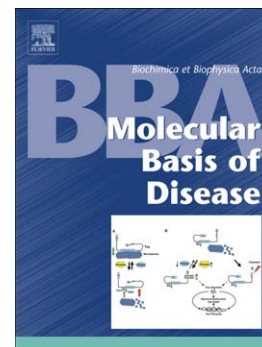
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**Molecular Mechanisms of  $\alpha$ -Synuclein Neurodegeneration**

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**ABSTRACT**

$\alpha$ -Synuclein is an abundant highly charged protein that is normally predominantly localized around synaptic vesicles in presynaptic terminals. Although the function of this protein is still ill-defined, genetic studies have demonstrated that point mutations or genetic alteration (duplications or triplications) that increase the number of copies of the  *$\alpha$ -synuclein (SCNA)* gene can cause Parkinson's disease or the related disorder dementia with Lewy bodies.  $\alpha$ -Synuclein can aberrantly polymerize into fibrils with typical amyloid properties, and these fibrils are the major component of many types of pathological inclusions, including Lewy bodies, which are associated with neurodegenerative diseases, such as Parkinson's disease. Genetic studies have clearly established that alteration in the  $\alpha$ -synuclein gene can lead to neuronal demise. Although there is substantial evidence supporting the toxic nature of  $\alpha$ -synuclein inclusions, other modes of toxicity such as oligomers have been proposed. In this review, some of the evidence for the different mechanisms of  $\alpha$ -synuclein toxicity is presented and discussed.

## Introduction

Parkinson's disease (PD) is a progressive, neurodegenerative disorder characterized by bradykinesia, resting tremor, cogwheel rigidity and postural instability [1,2] associated with the loss of dopaminergic (DA) neurons in the substantia nigra (SN) pars compacta [3,4]. Although the major pathological hallmarks of PD, Lewy bodies (LB) and Lewy neurites (LN), were originally observed in 1912 [5],  $\alpha$ -synuclein ( $\alpha$ -syn) was not identified as the major component of these proteinaceous inclusions until 1997 [6] following the discovery of PD kindred with point mutations in the  $\alpha$ -syn gene (*SNCA*) [7]. In addition to PD, the presence of  $\alpha$ -syn pathological inclusions is one of the defining features of several other neurodegenerative diseases, including dementia with Lewy bodies (DLB), LB variant of Alzheimer's disease and multiple system atrophy (MSA) [6,8-13]. Furthermore  $\alpha$ -syn inclusions are also found in a significant percentage of other neurodegenerative disorders, including neurodegeneration with brain iron accumulation type-1, Down's syndrome, progressive autonomic failure and familial and sporadic Alzheimer's disease [14-21]. Collectively these diseases have been defined as  $\alpha$ -synucleinopathies. This review will focus on the potential molecular mechanisms by which  $\alpha$ -syn may cause neurodegeneration.

## The $\alpha$ -Synuclein Protein

$\alpha$ -Syn is a small, highly charged 140-amino acid residue protein characterized by several major regions: 1) an amino-terminal region containing several imperfect KTKEGV repeats, 2) a hydrophobic center domain also referred to as the non-amyloid component (NAC) region, and 3) a highly negatively charged carboxy-terminus region (Figure 1).  $\alpha$ -Syn is a soluble, heat-stable

and natively “unfolded” protein [22,23]. It is predominantly expressed in central nervous system (CNS) neurons, where it is localized at presynaptic terminals in close proximity to synaptic vesicles [24-27] and can associate with lipid membranes by forming amphipathic  $\alpha$ -helices, as shown *in vitro* [22,28-31]. Although the function of  $\alpha$ -syn is still poorly understood, several studies suggest that it is involved in modulating synaptic transmission, the density of synaptic vesicles and neuronal plasticity [26,27,32-34], as well as provide a supportive role in the folding/refolding of SNARE proteins critical for neurotransmitter release, vesicle recycling and synaptic integrity [35]. However, knockout mouse models of  $\alpha$ -syn are not lethal, and brain morphology is intact, suggesting that  $\alpha$ -syn is not required for neuronal development and/ or that compensatory pathways are present [33,34]. *In vitro* studies have shown that the carboxy-terminal region of  $\alpha$ -syn is required for chaperone-like activity [36-38].  $\alpha$ -Syn can also associated with many proteins [39] and can regulate the activity of several enzymes, including tyrosine hydroxylase, the rate-limiting enzyme in dopamine production [40,41], mitogen-activated protein kinases (MAPKs) [42], and phospholipase D (PLD) [43,44].

### **Disease-Causing $\alpha$ -Synuclein Mutations**

The most direct and compelling evidence for a fundamental role of  $\alpha$ -syn in the pathogenesis of  $\alpha$ -synucleinopathies is the causal relationship between genetic mutations and disease. The mutation Ala53Thr resulting from a G to A nucleotide transition at position 209 of the *SNCA* ( $\alpha$ -syn) gene was first identified in a large Italian family (Contursi) and three small Greek families with autosomal dominant PD [7]. Thereafter, the Ala53Thr mutation was identified in at least 8 additional PD kindreds [45-48], and another autosomal dominant mutation (Ala30Pro) was identified in a German kindred [49]. The Glu46Lys mutation in  $\alpha$ -syn was

identified in kindred manifesting classical PD or PD with features of dementia associated with widespread Lewy pathology, referred to as DLB [50]. In addition, short chromosomal duplications or trisomies containing the *SCNA* gene, plus relatively short flanking regions on chromosome 4, were discovered in patients with PD or DLB [51-53], indicating that a 50 % increase in the expression of  $\alpha$ -syn is sufficient to cause disease.

### **Formation of $\alpha$ -Synuclein Amyloidogenic Inclusions.**

$\alpha$ -Syn filaments (10-15 nm-wide) are the major ultrastructural component of pathological inclusions characteristic of synucleinopathies [6,12,13]. These inclusions can occur in cell bodies such as LBs that are present in neurons of patients with PD and DLB or such as glial cytoplasmic inclusions (GCIs) that form in oligodendrocytes of individuals with MSA.  $\alpha$ -Syn inclusions can also present in the processes of affected cells, forming for example dystrophic neurites or large axonal swellings termed neuroaxonal spheroids [12,13].

*In vitro* studies have shown that recombinant soluble  $\alpha$ -syn can readily polymerize into amyloidogenic fibrils that are structurally similar to those in human brains [54-57]. Polymerization of  $\alpha$ -syn is a nucleation dependent process, i.e. fibril formation displays a lag phase followed by a rapid increase in fibril formation [57-60], and this lag phase is dramatically reduced by the addition of a “seed” or “nucleus” of pre-aggregated  $\alpha$ -syn [59]. The polymerization of  $\alpha$ -syn is associated with a dramatic conformational change from random coiled to predominantly  $\beta$ -pleated sheet [61-64], and it has been proposed that  $\alpha$ -syn progresses from an unordered monomer through partially folded intermediates, and finally elongates into “mature” filaments [58].

The central hydrophobic region in  $\alpha$ -syn is necessary for fibrillization [64,65] and this region is buried within the fibril core, as demonstrated by immuno-electron microscopy analysis and proteinase K resistance assays [64,66,67]. The negatively charged carboxy-terminus negatively regulates fibril formation such that deletions of this region promotes fibril formation [62,67]. The presence of the amino-terminal region also reduces fibrillogenesis, as deletions of some of the repeats can accelerate filament formation [68].

The Ala53Thr and Glu46Lys  $\alpha$ -syn proteins show increased rates of self-assembly and fibril formation [54-55,57,60,69-72]. This is consistent with studies showing that the Ala53Thr  $\alpha$ -syn preferentially adopts a  $\beta$ -sheet conformation [73]. These *in vitro* data suggest that the Ala53Thr and Glu46Lys  $\alpha$ -syn mutations could be pathogenic because of their increased the propensity to form pathological inclusions. In fact,  $\alpha$ -syn, which contains both the Gly46Lys and Ala53Thr mutations, fibrillizes more rapidly than the Ala53Thr mutation alone, and this protein is even more likely to conform to the amino-terminal structure of  $\alpha$ -syn in pathological inclusions, as detected by conformational-specific antibodies [74].

While some reports suggest that Ala30Pro  $\alpha$ -syn forms fibrils more slowly than WT  $\alpha$ -syn [69], this finding is not consistently observed by others [55,57]. In addition, the Ala30Pro mutation appears to affect  $\alpha$ -syn properties independent of protein aggregation. The Ala30Pro mutation may partially impaired the ability of  $\alpha$ -syn to bind to brain vesicles [35,75], likely due to a decreased likelihood to form  $\alpha$ -helices [35,73]. However, it does not significantly prevent  $\alpha$ -syn localization to presynaptic terminals [76]. This mutation can also directly impair the *in vitro* chaperone-like activity of  $\alpha$ -syn [36], and studies in mice indicate that Ala30Pro  $\alpha$ -syn is deficient in the ability of refolding SNARE proteins [35], which may be due to its reduced ability to interact with vesicles.



### Protofibrils/ Oligomers Toxicity Hypothesis

The polymerization of  $\alpha$ -syn from unstructured monomer to mature amyloid fibrils rich in  $\beta$ -sheets proceeds through the formation of several altered-sized oligomers and polymers that can be visualized and assayed by electron microscopy, atomic force microscopy and size-exclusions chromatography (Figure 2) [69,77]. Several of these intermediates (as well as products that may not culminate into fibrils) have been described as spheres (2-6 nm in size), chains of spheres (also termed protofibrils) and rings resembling circular protofibrils (also termed annular protofibrils) [69,77,78].

Several findings have suggested that protofibrils or some forms of  $\alpha$ -syn oligomers may be toxic. This hypothesis is analogous to the proposal that amyloid assembly intermediates of other amyloidogenic proteins such as the A $\beta$  peptide involved in Alzheimer's disease may be toxic [77,79]. The initial observation that Ala30Pro  $\alpha$ -syn may have a tendency to accumulate as oligomers instead of mature fibrils led to the suggestion that  $\alpha$ -syn may have a similar toxic mechanism [69,77]. In addition, the formation of such oligomers *in vitro* is shown to increase leakiness of synthetic lipid vesicles [80]. Two mechanisms have been proposed to explain this effect of  $\alpha$ -syn oligomers on membrane permeability: 1)  $\alpha$ -syn annular oligomers may integrate into membrane resulting in the formation of pores or channel-like structures that could cause uncontrolled membrane permeability [77-79,81-83], and 2) oligomers enhance the ability of ions to move through the membrane bilayer, without the formation of pores [84].

Direct *in vivo* data supporting the "toxic oligomeric  $\alpha$ -syn hypothesis" are still relatively limited and most of the evidence is circumstantial. There are studies in cultured cells that support this notion, but others demonstrated a lack of association between intracellular oligomer and

toxicity (see the section on “Cell culture studies of  $\alpha$ -synuclein toxicity” below). There is also a paucity of animal studies to directly support this hypothesis, but this could be related to the difficulties in monitoring these species *in vivo* due to their transitory nature.

### Cell Culture Studies of $\alpha$ -Synuclein Toxicity

Several studies in cultured cells indicate that the expression of mutants of  $\alpha$ -syn (Ala53Thr or Ala30Pro) can sensitize cells to toxic challenges; however, the results of these studies are not unequivocal [85-96]. In some studies, toxicity induced by the expression of mutant  $\alpha$ -syn is associated with the formation of  $\alpha$ -syn aggregates [88,89,91,97]. The over-expression of wild-type (WT)  $\alpha$ -syn in some settings has also been reported to render cells more vulnerable to cellular challenges [42,94,97-103]. In striking contrast, other studies have shown that expression of WT  $\alpha$ -syn can protect against cellular stresses [88,99,104-109]. Nevertheless, several different mechanisms, including proteasomal inhibition, affects on signal transduction pathways, mitochondrial alterations, increased levels of free radicals, and membrane clustering of dopamine transporter resulting in increased dopamine uptake, have been proposed as toxic mechanisms associated with the expression of WT or mutant  $\alpha$ -syn [42,90,92,96,98-100,110].

Several studies have shown that the extracellular addition of *in vitro* generated  $\alpha$ -syn oligomers to cultured cells can lead to toxicity [111,112]. Although these studies are artificial, there is some data to suggest that  $\alpha$ -syn that may normally be secreted by cells or released due to cell death can be directly or indirectly toxic to other adjacent cells (see [113] and references therein). Some studies suggest that the intracellular formation of  $\alpha$ -syn oligomers in H4 neuroglioma cells is associated with toxicity [114]. Conversely, others studies have shown that

the formation of abundant Ala53Thr  $\alpha$ -syn oligomers in SH-SY5Y cells induced by increasing intracellular catecholamine levels is not associated with toxicity [115].

The reasons for the discrepancies in the results between the toxic or protective effects of  $\alpha$ -syn are not clear, but cell types, the promoters and transfection methods used to overexpress  $\alpha$ -syn, the use of tagged protein versus native  $\alpha$ -syn, the nature of the toxic stimulus utilized, and the level of expression, may be important factors [110].

### **Studies of $\alpha$ -Syn Toxicity in Yeast (*Saccharomyces Cerevisiae*)**

Although no orthologue of  $\alpha$ -syn exist in yeast, expression of untagged WT  $\alpha$ -syn or WT  $\alpha$ -syn-EGFP in the yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) can inhibit cell growth and may result in cell death [116-119]. When expressed at low levels or upon initial expression at high levels,  $\alpha$ -syn is localized to the plasma membrane, but when expressed at high levels it subsequently forms cytoplasmic inclusions that is associated with toxicity [116-118]. Expression of Ala53Thr  $\alpha$ -syn results in similar distribution profiles and toxicity. In contrast, Ala30Pro  $\alpha$ -syn displays both plasma membrane and diffuse cytoplasmic localizations, does not form inclusions, and demonstrates much reduced cell growth inhibition [116,117]. The altered distribution of Ala30Pro  $\alpha$ -syn could be due to its reduced affinity for lipid membranes and/or targeting to the vacuole for degradation [119]. Further studies show that the  $\alpha$ -syn inclusions in yeast are not comprised of amyloid-like fibrils, but instead are  $\alpha$ -syn associated with clusters of vesicles [116,120]. The expression of  $\alpha$ -syn in *S. cerevisiae* impairs endoplasmic reticulum (ER) to Golgi vesicle trafficking, which leads to the accumulation of these membranous vesicles [116,118,120]. A genome-wide screen identified several suppressors of these defects in ER-Golgi trafficking, including the Rab guanosine triphosphatase Ypt1p, which also suppresses  $\alpha$ -

syn toxicity [118]. Although the importance of these findings as it relates to the pathobiology of  $\alpha$ -syn in humans is not completely clear, there is some experimental evidence suggesting that the deleterious effects of  $\alpha$ -syn in this pathway may be relevant to disease. Expression of Rab1 (the murine ortholog of *YPT1*) in *Drosophila* rescues toxicity induced by expressing WT and Ala53Thr  $\alpha$ -syn in DA neurons [118]. Similarly, expression of Rab1 can rescue the demise of DA neurons induced by over-expressing Ala53Thr  $\alpha$ -syn in cultured rat midbrain primary neurons [118].

### **Studies of $\alpha$ -Syn Toxicity in *Drosophila Melanogaster***

There are no known orthologs of  $\alpha$ -syn in *Drosophila Melanogaster*; nevertheless the ability to use this organism to identify genetic modifiers and to conduct studies of neurodegeneration in a shorter timeframe than in mammals has compelled the development of  $\alpha$ -syn transgenic (tg) flies. Expression of human  $\alpha$ -syn in *Drosophila* is reported to result in a selective age-dependent neuronal loss of DA neurons, locomotor dysfunction and cytoplasmic inclusions, some of which are composed to 7-10 nm wide filaments with additional granular material similar to LBs [121]. Co-expression of the chaperone heat-shock protein (Hsp) 70 can suppress neuronal degeneration, while a dominant negative form of Hsp 70 increases DA neuronal loss [122]. In addition, a drug that inhibits Hsp 90, a negative regulator of heat shock response, also rescues DA neuronal loss. However, these initial observations in  $\alpha$ -syn tg flies have been challenged since locomotor dysfunction could not be replicated by others [123]. In addition, the loss of DA neurons is not observed when a whole-mount immunohistochemistry approach is used, compared to sequential paraffin sectioning used in the other studies [123],

suggesting that expression of  $\alpha$ -syn may not result in DA neuronal death but in other toxic effects that could alter neuronal morphology or the size of DA neurons.

### **Studies of $\alpha$ -Syn Toxicity in *Caenorhabditis elegans***

There is also no known ortholog of  $\alpha$ -syn in the worm *Caenorhabditis elegans* (*C. elegans*), but the potential utility of this organism to quickly identify genetic modifiers compelled studies to develop  $\alpha$ -syn tg models. However, the effects of expressing WT and mutants of  $\alpha$ -syn in DA neurons of *C. elegans* have been controversial. Lakso and colleagues report that expression of WT or A53T human  $\alpha$ -syn using pan-neuronal or DA neuronal promoters cause a reduction in the number of DA neuron cell bodies and processes [124]. Conversely, Kuwahara and colleagues do not observe a demise of DA neurons using a DA-specific promoter to express human WT, A30P or A53T  $\alpha$ -syn, although a decrease of neurites is noted [125]. It is suggested that the apparent reduction in DA neurons observed by Lakso and colleagues could be due to a reduction in the expression of the tg-expressed marker used to monitor DA neurons, resulting from using the same promoter to express  $\alpha$ -syn [125]. Nevertheless, Kowahara and colleagues report that the tg expression of A53T or A30P  $\alpha$ -syn results in a reduction in DA levels associated with impairment in locomotor rate in response to food, which in *C. elegans* is attributed to the function of DA neurons [125].

### **Mouse Tg Models of $\alpha$ -Syn Toxicity**

Several tg mouse models expressing either WT or mutant (Ala53Thr and Ala30Pro)  $\alpha$ -syn have been reported. Masliah and colleagues reported on the first  $\alpha$ -syn tg mouse lines that were generated [126]. In these mice neuronal expression of WT human  $\alpha$ -syn is driven using a

platelet-derived growth factor- $\beta$  (PDGF- $\beta$ ) promoter, which results in the formation of amorphous, non-filamentous  $\alpha$ -syn neuron aggregates in the neocortex, the hippocampus, and occasionally in the SN. A subset of  $\alpha$ -syn inclusions is also ubiquitin positive, which is characteristic of authentic human  $\alpha$ -syn inclusions. However, in contrast with typical  $\alpha$ -syn inclusions in PD, a significant portion of the inclusions in these mice are located in the nucleus. The formation  $\alpha$ -syn aggregates in PDGF- $\beta$ / $\alpha$ -syn tg mice is also associated with a modest reduction in striatal tyrosine hydroxylase-positive terminals. Interestingly, over-expression of  $\beta$ -syn, a protein with close homology to  $\alpha$ -syn, but unable to polymerize into amyloid fibrils [64], by transgenic cross breeding, reduces the numbers of  $\alpha$ -syn inclusions, ameliorates motor impairment and results in a partial rescue of striatal tyrosine hydroxylase-positive terminals suggesting that  $\beta$ -syn may prevent  $\alpha$ -syn aggregation [127].

Since Alzheimer's disease pathology and PD often coincide in patients, the effects of accumulating amyloid- $\beta$  (A $\beta$ ) peptide, the major component of senile plaques characteristic of Alzheimer's disease, on  $\alpha$ -syn pathobiology has been assessed using tg mice. The PDGF- $\beta$ / $\alpha$ -syn tg mice have been cross-bred with a tg mouse model of Alzheimer's disease line, where a disease-causing mutant form of the human amyloid precursor protein (APP) is expressed resulting in the production and accumulation A $\beta$  extracellular inclusions [128]. The expression of A $\beta$  peptide in these bigenic mice is shown to potentiate neuronal and presynaptic terminal loss, motor impairments and the formation of fibrillar intraneuronal  $\alpha$ -syn when compared to PDGF- $\beta$ / $\alpha$ -syn tg mice. These findings provide important information supporting the notion that A $\beta$  peptide, which is predominantly extracellular, can promote the formation of intraneuronal  $\alpha$ -syn aggregates.

The PDGF- $\beta$ / $\alpha$ -syn tg mice also have been used to generate bigenic tg mice that overexpress rat Hsp 70, and Hsp 70 expression is found to mitigate the formation of  $\alpha$ -syn aggregates, suggesting that Hsp 70 may have a role in refolding or degrading misfolded  $\alpha$ -syn [129].

Other studies have used a Thy-1 promoter to drive the neuronal expression of WT, Ala30Pro or Ala53Thr human  $\alpha$ -syn in tg mice [76,130-132]. In some of the Thy-1 tg mouse lines, expression of WT or Ala53Thr  $\alpha$ -syn results in the appearance of perikaryal and neuritic accumulations of  $\alpha$ -syn and age dependent motor impairment associated with the degeneration of ventral root axons and muscle denervation [130]. A subset of  $\alpha$ -syn inclusions in these mice are argyrophilic and immunoreactive for ubiquitin, but they lack the filamentous characteristics of authentic human  $\alpha$ -syn inclusions. Kahle and colleagues generated Thy-1 tg mouse lines expressing human WT or Ala30Pro  $\alpha$ -syn. These Thy-1/WT- $\alpha$ -syn and Thy-1/Ala30Pro- $\alpha$ -syn tg mice are reported to initially developed detergent-insoluble somatodendritic accumulations of human  $\alpha$ -syn that is not associated with any obvious phenotype [76,131,133]. With aging the Thy-1/Ala30Pro- $\alpha$ -syn tg mice develop neuronal cytoplasmic fibrillar “amyloidogenic” inclusions that resemble the properties of authentic  $\alpha$ -syn inclusions as evidenced by thioflavin S-reactivity, proteinase K resistance and ultrastructure studies [132]. These inclusions are predominantly abundant in the midbrain, brainstem, amygdala and spinal cord [132,133]. From these studies reported by Kahle and colleagues, it is unclear if the Thy-1/WT- $\alpha$ -syn tg mice develop age-dependent pathological features similar to the Thy-1/Ala30Pro- $\alpha$ -syn transgenic mice or if these changes are specific and exacerbated by the Ala30Pro mutation [131,132]. The formation of amyloidogenic inclusions in Thy-1/Ala30Pro- $\alpha$ -syn tg mice is associated with deterioration in locomotor performance that progressed to spastic paralysis of the hind limbs

[128]. Furthermore, the specific formation of amyloidogenic inclusions in the amygdale of Thy-1/Ala30Pro- $\alpha$ -syn is correlated with a decline in cognitive performance [133].

Tg mice have also been generated that expressed either WT, Ala53Thr or Ala30Pro human  $\alpha$ -syn using the murine prion protein promoter (PrP) [134,135]. PrP  $\alpha$ -syn tg mice expressing Ala53Thr  $\alpha$ -syn, but not those expressing equivalent levels of WT or Ala30Pro  $\alpha$ -syn, develop amyloidogenic, 10-15 nm filamentous  $\alpha$ -syn inclusions in neurons (i.e. axonal spheroids, LB-like and LN-like lesions) that replicate many of the biochemical and histological features of authentic human synucleinopathies [134,135]. These  $\alpha$ -syn inclusions are predominantly observed in the spinal cord, brain stem, deep cerebellar nuclei, and the thalamus. Similar to Thy-1/Ala30Pro  $\alpha$ -syn transgenic mice, the hippocampus and the SN are spared. Also similar to Thy-1/Ala30Pro  $\alpha$ -syn transgenic mice, PrP/Ala53Thr  $\alpha$ -syn transgenic mice display an age-dependent severe motor phenotype that includes reduced ambulation, paralysis of the extremities usually beginning at a hind limb that progress to quadriparesis and arched back posture. These phenotypic features coincide with the accumulation of filamentous intracytoplasmic  $\alpha$ -syn neuronal inclusions. The degeneration of motor axons is likely the main cause of the motor phenotype in the PrP/Ala53Thr  $\alpha$ -syn tg mice, as dramatic Wallerian degeneration of ventral roots was observed [134]. Ultrastructure studies show that  $\alpha$ -syn filamentous axonal inclusions can trap mitochondria and impair axonal transport leading to axonal swelling containing vacuoles, vesicles and mitochondria [134]. The formation of  $\alpha$ -syn inclusions is also associated with motor neuron loss [136]. The increase propensity of Ala53Thr  $\alpha$ -syn to polymerize into fibrils compared to WT or Ala30Pro  $\alpha$ -syn [54,55,57] is the most likely explanation for the formation of neurotoxic inclusions in PrP/Ala53Thr  $\alpha$ -syn tg mice, while PrP/WT  $\alpha$ -syn tg or PrP/Ala30Pro  $\alpha$ -syn tg mice with similar expression do not display pathology. Since the age-



dependent phenotypic changes and pathologies in Thy/Ala30Pro  $\alpha$ -syn tg mice are similar to those in PrP/Ala53Thr  $\alpha$ -syn tg mice, but PrP/Ala30Pro  $\alpha$ -syn tg mice are not affected, it is possible that Thy/Ala30Pro  $\alpha$ -syn tg mice have higher expression levels than PrP/Ala30Pro  $\alpha$ -syn tg mice. However, a direct comparison has not been performed. This possibility is further supported by PrP/Ala30Pro  $\alpha$ -syn tg mice by Sudh f and colleagues that display similar pathological and phenotypic features as described above for PrP/Ala53Thr  $\alpha$ -syn tg mice [35,137].

Using the PrP/Ala30Pro  $\alpha$ -syn tg mice, a dramatic increase in the level of the lipid-binding protein Apolipoprotein E (ApoE) coincides with the motor impairment and motor neuron loss associated with  $\alpha$ -syn inclusions [137]. These findings are particularly interesting since specific ApoE genotypic isoforms are important risk factors for Alzheimer's disease. Further, ApoE can modulate the formation of  $\alpha$ -syn pathology, since breeding these PrP/Ala30Pro  $\alpha$ -syn tg mice on an ApoE null background delays motor disease, while increasing survival, although these processes are not completely abolished [137]. These findings indicate that ApoE can be an important modulator of  $\alpha$ -syn aggregation and related pathogenesis, although the mechanisms are not clearly defined.

Since septin-4 (Sept4), a member of the septins family of polymerizing GTP binding proteins that serve as scaffolds that can anchor or stabilize other molecules, is present in  $\alpha$ -syn pathological inclusions in human brains and interacts with  $\alpha$ -syn [138], the effect of Sept4 on  $\alpha$ -syn pathobiology has been investigated *in vivo* in the PrP/Ala53Thr  $\alpha$ -syn tg mice described above. The breeding of PrP/Ala53Thr  $\alpha$ -syn tg mice on a Sept4 null background results in an exacerbation of locomotor deterioration and neuronal loss associated with  $\alpha$ -syn inclusion

formation [139]. These data suggest that Sept4 may act as a suppressor of  $\alpha$ -syn aggregation and resulting neurodegeneration.

Counter intuitively, the transgenic mice described above do not develop substantial SN DA neurons pathologies. For reasons that remain enigmatic, it appears that, in contrast to humans, this population of neurons in mice is resilient to the formation of  $\alpha$ -syn inclusions and degeneration. This notion is consistent with the lack of pathology even when the a tyrosine hydroxylase promoter, which drives express specifically in catecholaminergic neurons, is used to generate WT, Ala53Thr or Ala30Pro  $\alpha$ -syn tg mice [140].

Since  $\alpha$ -syn inclusions in human brain contain C-terminally truncated  $\alpha$ -syn [112,141-143], which may be generated by incomplete 20 S proteasome degradation or calpain cleavage [112,144-145], and C-terminal truncated  $\alpha$ -syn fibrillizes faster *in vitro* (see above), Tofaris and colleagues created tg mice expressing C-terminal truncated  $\alpha$ -syn [146]. These tg mice express human  $\alpha$ -syn missing the last 20 amino acids (i.e.  $\alpha$ -syn 1-120) driven by a rat tyrosine hydroxylase promoter on an  $\alpha$ -syn null background. In this model,  $\alpha$ -syn 1-120 is expressed in DA neurons of the SN and olfactory bulb, resulting in aggregates with either granular and fibrillar morphologies. Shrunken neuronal perikarya and swollen axons of DA neurons are observed, but without significant neuronal loss. In another effort to study the effect of truncated  $\alpha$ -syn, transgenic mice were generated using rat tyrosine hydroxylase promoter to express Ala53Thr human  $\alpha$ -syn 1-130 (i.e. lacking the last 10 amino acids) [147]. Animals that express this truncated protein have a significant loss of nigral DA neurons, which is not observed in a similar tg line expressing full-length human  $\alpha$ -syn. However, the loss of DA neurons is shown to occur during embryogenesis without the formation of  $\alpha$ -syn inclusion. These findings support

that this form of truncated  $\alpha$ -syn can be toxic in nature, but it does not provide insights in the typical age-dependent neurodegeneration associated with human diseases.

As GCIs in oligodendrocytes are key pathological features of MSA, several tg mouse lines expressing WT human  $\alpha$ -syn in oligodendrocytes were generated. Kahle and colleagues used a proteolipid protein (PLP) promoter to drive express  $\alpha$ -syn specifically in oligodendrocytes, and these mice develop detergent-insoluble aggregates with histological profiles that resembled GCIs [148]. These mice demonstrate increased sensitivity to striatonigral degeneration and olivopontocerebellar atrophy induced by the mitochondrial inhibitor 3-nitropropionic acid [149]. Expression of human  $\alpha$ -syn using a murine myelin basic protein (MBP) promoter results in the specific expression of  $\alpha$ -syn in oligodendrocytes with progressive accumulation of filamentous inclusions associated with disruption of myelin lamina and demyelination [150]. In these mice, the accumulation of  $\alpha$ -syn in oligodendrocytes results in decreased dendritic density and loss of DA projections in the basal ganglia. In one of the MBP/ $\alpha$ -syn tg mouse lines expressing the highest levels of  $\alpha$ -syn, severe neurological features, including ataxia and seizure activity, resulting in premature death, is observed.

Transgenic mice expressing WT human  $\alpha$ -syn in oligodendrocytes also have been generated using a murine 2', 3'-cyclic nucleotide 3'-phosphodiesterase (CNP) promoter to drive expression [151]. The transgene is specifically expressed in oligodendrocytes resulting in age-dependent cytoplasmic brain and spinal cord accumulations and the formation of fibrillar inclusions. These inclusions are associated with demyelination, demise of oligodendrocytes, age-dependent motor impairment and brain atrophy. Injury to oligodendrocytes results in secondary neuronal degeneration including accumulation of perikaryal hyperphosphorylated

neurofilaments, degeneration of axonal terminal, neuronal loss, and formation of neuronal inclusions comprised of endogenous mouse  $\alpha$ -syn.

Recently, the first tetracycline-regulated conditional tg mice (tet-off) expressing human wt  $\alpha$ -syn was described [152]. To drive inducible expression in specific neuronal populations these mice have been cross-bred to tg mice expressing tetracycline-controlled transactivator under the control of the hamster PrP or calcium/calmodulin-dependent protein kinase II $\alpha$  (CaMKII $\alpha$ ) promoter. In one of these inducible PrP/ $\alpha$ -syn tg mouse lines,  $\alpha$ -syn is expressed in the olfactory bulb, cortex, basal ganglia and cerebellum, while in another inducible PrP/ $\alpha$ -syn tg line expression is predominantly confined to olfactory bulb. In one of the inducible CaMKII $\alpha$ / $\alpha$ -syn tg mouse lines,  $\alpha$ -syn is expressed in the olfactory bulb, cortex, basal ganglia, hippocampus, thalamus and substantia nigra, including DA neurons. These CaMKII $\alpha$ / $\alpha$ -syn tg mice exhibit reductions in SN DA neurons and hippocampal neurogenesis, without the presence of  $\alpha$ -syn aggregates. They demonstrate a progressive motor decline as assayed by rotarod that can not be reversed by turning off tg  $\alpha$ -syn expression.

### **Studies $\alpha$ -Syn Over-expression in Adult DA Neurons using Viral Delivery**

Several studies have used viral vectors to express  $\alpha$ -syn in adult rats or monkeys nigral DA neurons. One advantage of this approach is that it mitigates the possible effects of early developmental expression of  $\alpha$ -syn with the possibility of compensatory mechanisms. Expression of human WT, Ala30Pro or Ala53Thr  $\alpha$ -syn in rat or monkey nigral DA neurons using adeno-associated viral vectors that stably express the transgene (> 6 months) results in substantial and specific demise of these DA neurons (30-80% loss), concurrent with the formation of cellular  $\alpha$ -syn inclusions and dystrophic neurites [153-155]. Similar results are observed when using a

lentiviral-based vector system to express  $\alpha$ -syn proteins in rat nigral DA neurons; however, over-expression of rat  $\alpha$ -syn is much less toxic [156].

### General remarks

Although genetic and pathological studies have clearly demonstrated the importance of  $\alpha$ -syn in the etiology of PD, several different mechanisms of toxicity have been proposed. These can be grouped into 3 categories based on the monomeric/polymeric nature of the proposed toxic species.

First, simple increases in intracellular abundance of monomeric  $\alpha$ -syn have been proposed as a mode of neuronal toxicity. Some studies in cultured cells many support this notion; however, these findings are not unanimous and the loss of DA neurons in the SN or other types of neurons has not been observed in tg mice that simply over-express high levels of  $\alpha$ -syn. In PD or DLB patients with duplication or triplication of the  $\alpha$ -syn gene, where  $\alpha$ -syn expression is increased by 50% or 100%, respectively,  $\alpha$ -syn pathological inclusions always coincide with disease (see [157] and references therein). In addition, some studies have suggested that  $\alpha$ -syn expression may be increased in specific brain areas or types of neurons in individuals with sporadic PD, but these findings have been challenged in other reports (see [158] and references therein).

Secondly, based on *in vitro* data discussed above, some forms of  $\alpha$ -syn oligomers and protofibrils have been proposed as potent toxic species. However, this hypothesis still lacks solid direct *in vivo* studies documenting toxicity linked to the present of  $\alpha$ -syn oligomers, although some models using cultured cell supports this notion. Conversely, biochemical studies

have shown that the presence of some forms of  $\alpha$ -syn oligomers in the midbrain of PrP/Ala53Thr  $\alpha$ -syn tg mice without any evidence of toxicity to DA neurons [115].

Lastly, the notion that the aberrant polymerization of  $\alpha$ -syn into filaments, which eventually form large intracytoplasmic inclusions, can cause the dysfunction and the demise of neurons or oligodendrocytes has been supported by various experimental models, as described above. Furthermore, the involvement of  $\alpha$ -syn aggregates in the dysfunction and demise of neurons is suggested by the correlations between severity of dementia and LB density in patients with DLB [159-162]. In addition, it is likely that a profusion of smaller  $\alpha$ -syn aggregates in the form of neuritic and pre-synaptic  $\alpha$ -syn inclusions have a predominant role in impairing normal neuronal function [74,128,163-165].  $\alpha$ -Syn aggregates may impair proteasome function [166], and they may act as "sinks," incidentally recruiting other necessary, cellular proteins from their normal cellular functions.  $\alpha$ -Syn inclusions can impair cellular functions by obstructing normal cellular trafficking (including disruption of ER and Golgi apparatus), by disrupting cell morphology, by impairing axonal transport, and by trapping cellular components (eg. mitochondria).

It is important to emphasize that the different alternative mechanisms of  $\alpha$ -syn toxicity based on the different forms of  $\alpha$ -syn polymers are not necessarily mutually exclusive. The presence of any form of  $\alpha$ -syn polymer, from small oligomers to amyloid fibrils, are abnormal and may be problematic for the normal activities of cells, thereby resulting in neurodegeneration.

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## FIGURE LEGENDS

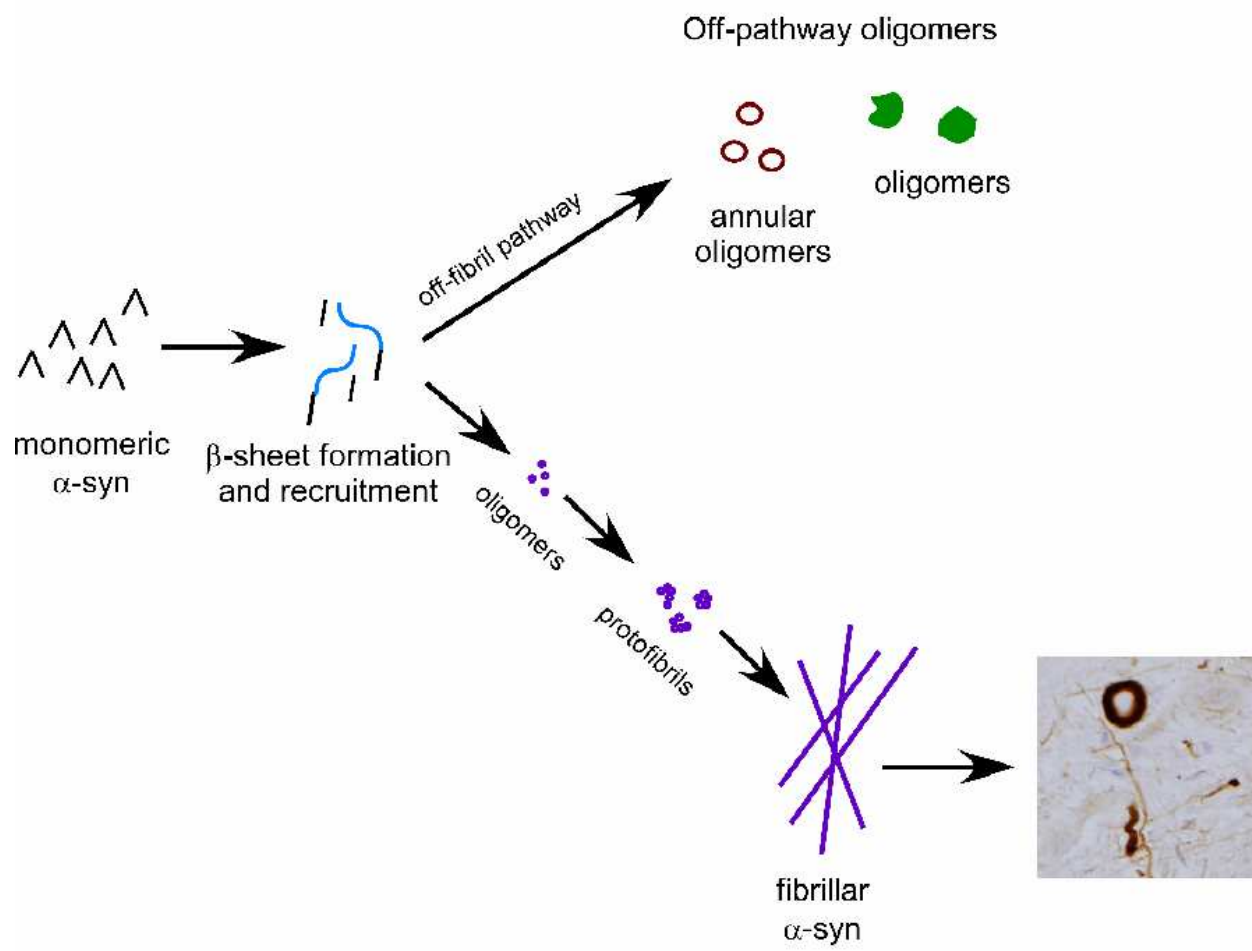
**Figure 1. Amino acid sequence and regions of  $\alpha$ -synuclein.**  $\alpha$ -Syn is composed of: 1) an amino-terminal domain (black) containing several imperfect KTKEGV motifs (blue underline); 2) a hydrophobic center (purple) termed non-amyloid component (NAC); and 3) a negatively charged carboxy-terminus (green). Three familial mutations in  $\alpha$ -syn (red) have been identified in patients with PD.

**Figure 2. Formation of  $\alpha$ -synuclein polymeric intermediates and fibrils.**  $\alpha$ -Syn in native, monomeric form is mostly unstructured. Under certain conditions  $\alpha$ -syn can undergo structural changes, resulting in  $\beta$ -pleated sheet formation. This form of  $\alpha$ -syn can take two pathways, one which is off of the fibrillar pathway, and the other which will eventually form mature fibrils. The off-fibril pathway can result in the formation of annular or other forms of oligomers that will never develop into mature fibrils. The fibrillar pathway undergoes intermediate stages, which include protofibrils, before maturing into long strands and becoming LBs or LNs.





Figure 2



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