

Published in final edited form as:

*Mol Neurobiol.* 2013 April ; 47(2): 552–560. doi:10.1007/s12035-012-8325-2.

## Molecular chaperones, alpha-synuclein and neurodegeneration

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### Abstract

Parkinson's disease (PD) is a devastating neurological condition that affects about 1% of people older than 65 years of age. In PD, dopaminergic neurons in the midbrain slowly accumulate cytoplasmic inclusions (Lewy bodies, LBs) of the protein alpha-synuclein ( $\alpha$ -syn) and then gradually lose function and die off. Cell death is thought to be causally linked to the aggregation/fibrillization of  $\alpha$ -syn. This review focuses on new findings about the structure of  $\alpha$ -syn, about how  $\alpha$ -syn cooperates with Hsp70 and Hsp40 chaperones to promote neurotransmitter release, and about cell-to-cell transfer of pathogenic forms of  $\alpha$ -syn and how Hsp70 might protect against this disease process.

### Keywords

alpha-synuclein; cysteine string protein; Hsp40; Hsp70; molecular chaperone; neurodegeneration; Parkinson's disease

### Introduction

Alpha-synuclein ( $\alpha$ -syn) is a neuronal protein that has been linked to several neurodegenerative diseases, referred to as synucleinopathies, such as Parkinson's disease (PD), dementia with Lewy bodies, multiple system atrophy and neurodegeneration with brain iron accumulation type 1 [1–3]. The common feature of these diseases is that intracellular inclusions of  $\alpha$ -syn form in neurons in an age-dependent process. In sporadic PD, alterations in  $\alpha$ -syn homeostasis with age lead to  $\alpha$ -syn inclusions (Lewy bodies [LB] and Lewy neurites [LN]) and the selective degeneration of dopaminergic neurons in the *substantia nigra*, which leads to postural instability, slowness of movement, and tremors. In familial PD, that is, for individuals who possess mutations (A30P, A53T or E46K) [4–6] or multiple copies of the  *$\alpha$ -syn* gene [7], LB pathology and the age of disease onset are earlier, i.e. 30 year versus 65 year for sporadic. Other than replenishing dopamine, no other therapeutic strategies can thwart this disease.

### The Structure and Function of $\alpha$ -Syn

$\alpha$ -Syn has long been considered as an intrinsically unfolded monomeric protein, but in light of new findings, this classification may no longer be valid. First, it is important to note the basic sequence elements of this protein. (i) The N-terminal domain (residues 1–65), which contains hydrophobic and cationic residues, can adopt an  $\alpha$ -helical conformation and interact with the surface of micelles and membranes that are enriched in anionic phospholipids [8]. (ii) The hydrophobic NAC domain (residues 66–95) controls the self-association of this protein. Because this domain is often found in Alzheimer's disease (AD)

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plaques, it was named the “non-A $\beta$  component of Alzheimer’s disease amyloid plaque” [9]. Fibers composed of the NAC domain can infect and kill healthy cells [10]. (iii) The C-terminal domain (residues 96–140) is unstructured and contains acidic amino acids and serine and tyrosine residues, which are subject to phosphorylation. The C-terminal domain may have chaperone activity [11].  $\alpha$ -Syn shares sequence similarity with 14-3-3 proteins [12] and a lipid binding motif found in a family of lipid binding proteins [13].

Recombinant human  $\alpha$ -syn purified from *E. coli* using denaturing conditions is an intrinsically unfolded monomeric protein [14] that has a propensity to adopt an  $\alpha$ -helical structure when bound to the surface of micelles or liposomes [15] (Fig. 1), and under certain conditions it slowly forms soluble aggregates and insoluble  $\beta$ -amyloid fibers [16–18].

Human  $\alpha$ -syn purified from red blood cells, or even from *E. coli*, using non-denaturing conditions is not a monomer [19,20]. Instead, it is a tetramer—a four-helix—bundle that resists aggregation. Tetrameric  $\alpha$ -syn binds to phospholipid membranes with higher affinity than monomers, and, the tetramers fail to form fibers over a 10 d incubation period, whereas the monomers do. This raises the possibility that conditions exist in cells that break up the tetramers, thereby releasing monomers that may slowly aggregate and fibrillize (Fig. 2). These early studies of monomeric  $\alpha$ -syn gave important insight into the self-association behavior of the monomer. The new studies are causing a paradigm shift that should result in a deeper understanding of the cellular events that lead  $\alpha$ -syn to aggregate and fibrillize.

A non-essential protein that is highly expressed in neurons,  $\alpha$ -syn localizes to the nuclear membrane and presynaptic nerve terminals [21], where it likely functions in synaptic vesicle recycling and neurotransmitter release [11]. Insights into  $\alpha$ -syn biology and pathobiology have come about from overexpression and knockout studies. Studies have shown that at elevated levels  $\alpha$ -syn inhibits vesicle trafficking [22], the proteasome [23], and even pokes holes in membranes [18,24]. Knockout studies in the mouse and rat have probed the effect of loss of function. In the mouse, although knocking out  $\alpha$ -syn fails to produce neurodegeneration [25–28], compared to control animals, reductions occur in the level of striatal dopamine [29] and the pool of undocked synaptic vesicles [27]. In the rat, virus-mediated RNAi-mediated silencing of  *$\alpha$ -syn* results in a rapid degeneration of dopaminergic neurons compared to control animals, suggesting that normal levels of  $\alpha$ -syn are required for the viability of these neurons [30]. The authors of this latter study cautioned that a therapeutic approach based on RNAi-mediated silencing of  $\alpha$ -syn in humans could backfire and exacerbate neurodegeneration. Taken together,  $\alpha$ -syn overexpression and knockout studies support a U-shaped toxicity curve: at the optimal level,  $\alpha$ -syn is non-toxic and carries out its normal function, whereas very low or very high levels trigger synaptic dysfunction and neurodegeneration. One way to look at such a U-shaped toxicity curve is that a loss-of-function occurs at low or zero levels of  $\alpha$ -syn, whereas a toxic gain-of-function occurs at high levels. Of course, an elevated level might promote self-association, which in turn could *decrease* the level of the active  $\alpha$ -syn species (whether monomer or tetramer) at presynaptic terminals, resulting in, oddly, a loss-of-function phenotype.

Expressed in many types of cells other than neurons,  $\alpha$ -syn is abundant even in red blood cells (RBCs) [31], although no one knows why. Possible functions of  $\alpha$ -syn in RBCs, which have no nucleus, no DNA, no RNA, no protein synthesis, and no mitochondria, are: (i) A *tether*— $\alpha$ -Syn binds membranes via its N-terminal  $\alpha$ -helical domain [32], whereas its flexible C-terminal tail, which does not bind the membrane, is free to bind other proteins like a chaperone (Fig. 1). It’s easy to imagine one end of an  $\alpha$ -syn molecule tightly bound to the plasma membrane while the other end grabs a cytosolic protein. A tether function could enable a soluble protein to modulate the activity of a membrane-bound protein. A possible tether function has been reported for  $\alpha$ -syn (see “Deletion of the Hsp40 Cysteine String

Protein- $\alpha$  (CSP $\alpha$ ) induces neurodegeneration;  $\alpha$ -Syn reverses this effect”). (ii) *A regulator of membrane fluidity* – The avid binding of  $\alpha$ -syn to the inner leaflet of the plasma membrane could alter the membrane fluidity in such a way as to increase the mechanical strength and even the lifetime of RBCs. An interesting parallel exists between  $\alpha$ -syn and Hsp12, which is a small heat shock protein (109 amino acids) expressed by the yeast *S. cerevisiae* and many other fungi [33,34]. It was recently reported that Hsp12, which is induced up to 100-fold upon heat shock, is an intrinsically unfolded chaperone that folds into an alpha-helical conformation upon binding to biological membranes [35]. A variety of experimental techniques showed that Hsp12 stabilizes the yeast plasma membrane and protects cells from high temperature stress. Hsp12 even has weak homology (< 20%) to  $\alpha$ -syn, although there is no evidence that Hsp12 forms pathological oligomeric structures like  $\alpha$ -syn. Perhaps in some types of cells, such as RBCs,  $\alpha$ -syn is a chaperone that stabilizes the plasma membrane against disruption by various stresses. (iii) *An anti-oxidant* – RBCs have evolved to hold large numbers of hemoglobin molecules. It is noteworthy that the *a-syn* gene and three heme biosynthetic genes *ALAS2*, *FECH*, and *BLVRB* show highly correlated gene expression profiles [36], suggesting that  $\alpha$ -syn plays some role in heme metabolism. Maybe  $\alpha$ -syn inhibits oxidative stress during heme and hemoglobin synthesis in RBC progenitor cells and this function is also required in RBCs. Perhaps not surprising, antioxidant functions of  $\alpha$ -syn have been reported [30,37–43].

## Molecular chaperones

Cells expend considerable amounts of ATP to synthesize the multitude of proteins that constitute its proteome. To prevent the loss of the proteins due to inefficient folding, cells have evolved an amazing set of proteins, first dubbed ‘heat shock proteins’ (HSP) and now referred to as ‘molecular chaperones’, that help fold newly synthesized proteins, transport proteins into organelles, inhibit and reverse misfolding and aggregation, and degrade proteins that cannot be massaged back into shape [44–46]. Because of the complex organelle structure of the typical eukaryotic cells, not only does the cytosol contain molecular chaperones, but most organelles and compartments, such as nerve terminals, contain specialized chaperones and co-chaperones that participate in maintaining the protein homeostasis of each organelle and compartment. This review focuses on the role membrane-associated molecular chaperones play in maintaining  $\alpha$ -syn homeostasis.

Many neurodegenerative diseases are ‘proteinopathies’ because toxic protein aggregates and fibers accumulate in age-dependent process. Such diseases include Alzheimer’s, Huntington’s, Parkinson’s, amyotrophic lateral sclerosis, numerous ataxias, and prion diseases [1,47–50]. The accumulation of insoluble protein aggregates with age implies a failure of the cellular protein quality control system, which consists of molecular chaperones, the ubiquitin-proteasome system and the autophagy-lysosome system [51,52]. Neurons are particularly susceptible to aggregate formation, I suggest, for the following reasons. First, the high metabolic rate of neurons is conducive to protein aggregation. Neurons synthesize and consume more ATP per cell than most other types of cells [53]. Oxidative phosphorylation, which is responsible for most of the ATP synthesis in neurons, generates reactive oxygen species (ROS), which indiscriminately oxidizes lipids, DNA and proteins. Oxidized proteins often aggregate [54], and such aggregates may resist degradation by the proteasome and lysosome. Second, neurons are non-dividing cells that cannot dilute away protein aggregates by stealthily passing them to daughter cells. Aggregates that resist elimination have nowhere else to go; thus, they coalesce into inclusions (Lewy bodies), which may or may not be toxic per se, but certainly they can sequester essential proteins [55]. Third, neurotransmitter release at nerve terminals occurs on a millisecond time scale, with hundreds or thousands of events occurring per minute. For instance, dopaminergic neurons rapidly release dopamine into the synapse by a complex process involving

exocytosis of dopamine-containing vesicles in coordination with  $\text{Ca}^{+2}$  influx through the L-type calcium channels. This rapid neurotransmitter release (and reuptake), which involves numerous proteins, protein conformation changes, and reversible associations with membranes, has the potential to run amok if essential factors misfold or aggregate [56,57]. As shown below,  $\alpha$ -syn together with Hsc70/Hsp70 and two co-chaperones (cysteine string protein- $\alpha$ , CSP $\alpha$ , and small glutamine-rich TPR protein, SGT) are a chaperone machine that functions to maintain efficient neurotransmitter release at nerve terminals. (TPR stands for tetratricopeptide repeat, which is a protein-protein interaction domain.) Chaperone machine failure results in inefficient neurotransmitter release followed by gradual neurodegeneration. In general, by dissolving away harmful aggregates or inhibiting their formation, molecular chaperones are instrumental to maintaining protein homeostasis in all types of cells and especially neurons [58,59].

### **Hsp70 Protects from $\alpha$ -Syn-Induced Neurodegeneration**

The fruit fly (*Drosophila*) has been an invaluable organism to study PD [60]. In addition to analyzing the effect of the directed expression of human  $\alpha$ -syn on dopaminergic neurons, retinal degeneration and movement defects can also be analyzed in flies. This was the first model organism in which the importance of molecular chaperones, specifically Hsp70, vis-à-vis  $\alpha$ -syn-induced neurodegeneration was illuminated. Bonini and colleagues showed that human wild-type  $\alpha$ -syn triggers an age-dependent degeneration of dopaminergic neurons compared to control flies, which was reversed by the co-expression of human Hsp70 [61,62]. Interestingly, transgenic flies exhibited  $\alpha$ -syn inclusions, and these inclusions were still evident when human Hsp70 was co-expressed. Note that disrupting the function of the fly Hsp70 (called Hsc4) also accelerated neurodegeneration with or without the human  $\alpha$ -syn transgene [63]. This landmark study revealed that Hsp70 overexpression protects cells from  $\alpha$ -syn toxicity, that  $\alpha$ -syn inclusions are inert, and that the toxic species is probably soluble. Subsequent studies began to decipher the molecular details by which Hsp70/Hsc70 protects cells from  $\alpha$ -syn-induced neurodegeneration [64–72].

### **Deletion of the Hsp40 Cysteine String Protein- $\alpha$ (CSP $\alpha$ ) induces neurodegeneration; $\alpha$ -Syn reverses this effect**

Overexpression of Hsp70/Hsc70 chaperones is almost universally protective against toxic aggregates and filamentous proteins [61,66,68,73,74]; whereas, knocking out a chaperone rarely causes aggregate formation and cell death. Knocking out the chaperone CSP $\alpha$  is unique in that it causes neurodegeneration in several organisms. CSP $\alpha$  is a member of the Hsp40 family, which, via palmitoylation of its cysteine residues [75], localizes to the outer leaflet of presynaptic vesicles where it functions as a co-chaperone for Hsc70. There are several excellent reviews on J proteins [76–80] in general and neural J proteins [81–83] specifically.

Deletion of the *csp* gene in flies causes defects in synaptic transmission, which leads to paralysis and premature death [84]. Only 4% of flies with *csp* deleted reached adulthood at 25°C, whereas none reached adulthood at 29°C. Flies that survived to adulthood and were then exposed to 29°C became paralyzed and rapidly died. Electron microscopy analysis of the lamina region of photoreceptor terminals revealed that synaptic vesicles were visible in wild-type but not the *csp* mutant. The authors of the study concluded that CSP protein has a role in neurotransmitter release. The temperature dependence of the lethality was suggested to indicate that CSP is essential at high temperatures, like an inducible chaperone. This work was followed by three elegant studies from the same lab that illuminated the functions of CSP and  $\alpha$ -syn [11,85,86].

First, deletion of CSP $\alpha$  also causes rapid and progressive neurodegeneration in mice [85]. Analysis of tissue extracts from CSPs knockout (KO) mice revealed that deletion of CSP $\alpha$  significantly decreased the levels of the plasma membrane SNARE protein (SNARE, soluble NSF [N-ethylmaleimide-sensitive factor] attachment protein receptor) called SNAP-25, Hsp70, Hsc70 and even  $\alpha$ -syn. SNAREs catalyze vesicle-membrane fusion events in cells (for reviews on SNARE proteins, see [56,57,87]). The decrease in the levels of these four proteins upon CSPs deletion suggests a functional link between these proteins. The striking finding was that transgenic overexpression of human wild-type  $\alpha$ -syn abolished the lethal neurodegeneration in CSP $\alpha$  KO mice.  $\alpha$ -Syn overexpression promoted SNARE complex assembly, increased the levels of Hsp70 and Hsc70, but failed to increase the level of SNAP-25 [85]. The ability of three different human  $\alpha$ -syn species to block neurodegeneration in CSP $\alpha$  KO mice was evaluated and gave insight into the mechanism of protection. Given that wild-type  $\alpha$ -syn and A53T avidly bind membranes, whereas A30P does not [88], it was interesting that  $\alpha$ -syn and A53T blocked neurodegeneration in the CSP $\alpha$  KO mice whereas A30P did not. An important conclusion was that the membrane-binding ability of  $\alpha$ -syn, which is mediated by the N-terminal domain, is necessary to rescue neurodegeneration in CSP $\alpha$  KO mice

Second, using the mouse model to probe the mechanism by which  $\alpha$ -syn in functionally compensates for the loss of CSP $\alpha$ , it was discovered that the flexible C-terminal domain of  $\alpha$ -syn is also required to rescue neurodegeneration caused by deletion of CSP $\alpha$  [11]. It turns out that the C-terminus of  $\alpha$ -syn binds to the SNARE-protein synaptobrevin-2/vesicle-associated membrane protein 2 (VAMP2), which is lodged in the synaptic vesicle membrane. One model is that  $\alpha$ -syn, which is bound to the surface of a synaptic vesicle, acts as a non-classical chaperone that maintains synaptobrevin-2 in an active conformation that enables a synaptic vesicle to fuse with the presynaptic membrane. Alternatively,  $\alpha$ -syn could tether a presynaptic vesicle to the presynaptic membrane. In this model,  $\alpha$ -syn is bound to the presynaptic membrane while its C-terminal domain is bound to vesicular synaptobrevin-2. Whether  $\alpha$ -syn is a chaperone or a tether will await further experimentation.

Third, digging deeper into the role of CSP $\alpha$  in synaptic transmission, it was revealed that SNAP-25 is a client of the CSP $\alpha$ -Hsc70-SGT chaperone machine [86]. In addition to the decrease in SNAP-25 level in CSP $\alpha$  KO mice, knocking out CSP $\alpha$  triggers an increase (40%) in ubiquitinated forms of SNAP-25 and Hsc70, but not other neuronal proteins. This suggests that SNAP-25 is destabilized and subject to degradation by the proteasome when CSP $\alpha$  is deleted. In support of this hypothesis, the CSP $\alpha$ -Hsc70-SGT chaperone complex binds to monomeric SNAP-25 but not to assembled SNARE complexes containing SNAP-25. In the absence of CSP $\alpha$ , apparently a larger fraction of SNAP-25 molecules adopt an off-pathway, inactive, and potentially toxic conformation (SNAP-25\*) that is tagged with ubiquitin for degradation. The CSP $\alpha$ -Hsc70-SGT chaperone machine facilitates the folding of SNAP-25, which minimizes its degradation, and thus promotes SNARE complex assembly and membrane fusion. The function of  $\alpha$ -syn may be to maintain SNARE-complex assembly in a presynaptic terminal during aging.

## Cell-to-cell Transfer of Chaperones and $\alpha$ -Syn

In the 1980's, studies were published showing that cells release Hsc70/Hsp70 molecules in response to heat shock, and that the released chaperones are taken up by neighboring cells [89,90]. Hsp70 was even found to form ion channels in membranes [91,92]. Although the mechanisms of chaperone release have not been fully elucidated, three mechanisms have been proposed [93,94]: (i) Chaperones are released from dead cells. (ii) Chaperones are released from living cells via exosomes, which are small (50 nm – 100 nm) vesicles that



arise from multivesicular bodies within cells [95]. (iii) Chaperones escape living cells by forming pores in the cell membrane [92]. Whatever the mechanism, the function of extracellular Hsc70/Hsp70 could depend on the specific compartment, but ideas include priming or modulating the immune response [96,97], killing tumor cells [98], signaling stress, and even, more recently, inhibiting the conversion of  $\alpha$ -syn into toxic conformers in the extracellular milieu in the brain [99]. Mechanisms (i–iii) probably also govern the release of  $\alpha$ -syn from cells [24,100–103].

The idea of PD spread, i.e., the cell-to-cell transfer of  $\alpha$ -syn, came from a postmortem analysis of the brains of PD subjects and age-matched healthy controls. Braak and colleagues found that sporadic PD progresses in six distinct stages [104]. In the earliest pre-symptomatic stage, LBs immune-reactive for  $\alpha$ -syn and ubiquitin form in the anterior olfactory bulb and the vagus nerve. The LB pathology slowly spreads up the brain stem in to cortical areas, ultimately affecting sensory and motor areas of the brain. Why the olfactory bulb and enteric nerves are particularly susceptible to LB formation is not known. One possibility is that an inhalant or a food triggers LB formation. For reviews on PD spread see [105–108].

Some individuals with severe PD received fetal stem cell transplants in order to slow down the progressive loss of striatal dopamine. Postmortem analysis of such subjects revealed an amazing finding. It was discovered that 11–16 years after transplantation the grafted neurons had LB-like inclusions that stained positively for  $\alpha$ -syn and ubiquitin [109,110]. In contrast, in PD patients who had received similar grafts, but who had died much sooner after transplantation (18 months), the grafts showed no signs of PD. One explanation is that dying neurons release a toxic form of  $\alpha$ -syn, which is subsequently taken up by healthy neighboring cells. Inside the healthy cells, the toxic conformers act as a template to convert endogenous, non-toxic  $\alpha$ -syn into a toxic, self-propagating conformation. The results support the hypothesis that, like the human prion protein,  $\alpha$ -syn can adopt two very different conformations, i.e., one is non-infectious, the other is infectious. The non-infectious conformation is probably the stable,  $\alpha$ -helical tetramer, whereas the infectious conformation probably has solvent-exposed  $\beta$ -sheets.

If PD is transmissible, then pathogenic forms of  $\alpha$ -syn that are secreted by infected cells should be taken up by healthy cells and trigger their demise. Using a variety of clever techniques that monitor uptake of exogenous  $\alpha$ -syn by healthy cells, several labs have verified this hypothesis [10,99,111,112]. Fragments of preformed  $\alpha$ -syn fibrils are endocytosed by primary mouse neurons in culture, and these fibrils trigger soluble endogenous  $\alpha$ -syn to form LB and LN-like inclusions in various parts of the neurons [10]. These inclusions stain for  $\alpha$ -syn, phosphorylated  $\alpha$ -syn and ubiquitin. The NAC domain is the minimum fragment that propagates LB pathology. Strikingly, several days after the fibrils were added to the culture medium, decrements in the levels of SNARE proteins (VAMP2, SNAP-25, and CSP $\alpha$ ) occur, with resultant loss of neuronal function and ultimately cell death. These SNARE proteins are either degraded when endogenous  $\alpha$ -syn aggregates or they are sequestered in the LBs and LNs. These experimental systems will be extremely powerful to unraveling the mechanism of PD spread.

One model for cell-to-cell transfer of  $\alpha$ -syn is that dysfunctional cells secrete an infectious form of  $\alpha$ -syn that then propagates to other cells. Another model is that normally functioning cells occasionally, or rarely, secrete the non-infectious, tetrameric  $\alpha$ -syn, which causes problems as follows. I propose that the concentration of  $\alpha$ -syn is so high inside cells that the *tetramer* is thermodynamically favored. If  $\alpha$ -syn tetramers are released by a small fraction of healthy cells, then because of a dilution effect the thermodynamically favored species in the extracellular milieu is the monomer, which is prone to aggregate/fibrillize. Of

course, most, but not all, monomers will be degraded by extracellular proteases. But, over time, small quantities of infectious  $\alpha$ -syn seeds could form, which could then infect, propagate in, and kill healthy neighboring cells (Fig. 3). In short, the non-infectious  $\alpha$ -syn tetramer should be favored inside neurons, whereas the potentially toxic monomer should be favored outside neurons. Keeping  $\alpha$ -syn inside and preventing seed formation outside neurons are potential therapeutic approaches.

The potential of chaperones, especially Hsp70, to protect neurons from toxic extracellular seeds of  $\alpha$ -syn is best illustrated by a recent study that showed that Hsp70 can inhibit the cytotoxicity of extracellular  $\alpha$ -syn. Using a novel assay to detect  $\alpha$ -syn secreted by H4 neuroglioma cells into the tissue culture medium (CM), it was shown that H4 cells secrete toxic oligomeric forms that can be taken up by neighboring cells [99]. When Hsp70 was overexpressed the level of  $\alpha$ -syn oligomers in the CM was decreased by 64%, although the level of intracellular  $\alpha$ -syn was unaffected. This means that intracellular Hsp70 inhibits the formation and release of toxic oligomeric forms of  $\alpha$ -syn. Another possibility is that, when overexpressed, Hsp70 is secreted into the CM and that extracellular Hsp70 also inhibits the formation of toxic  $\alpha$ -syn oligomers in the extracellular milieu. This possibility was tested using an enzyme-linked immunosorbent assay to detect Hsp70. It was discovered that H4 cells co-overexpressing Hsp70 and  $\alpha$ -syn showed significant levels of Hsp70 in the CM compared to control cells in which these proteins were not overexpressed. Additional controls ruled out the release of Hsp70 by dead cells. This raises the possibility that Hsp70 and  $\alpha$ -syn are secreted together. An issue is whether ATP and co-chaperones are secreted with Hsp70; for it would be unusual for Hsp70 to perform work on substrates without ATP and co-chaperone help. Overall, this study showed that Hsp70, whether intracellular or extracellular, can inhibit the formation of toxic conformations of  $\alpha$ -syn that can infect neighboring healthy cells (Fig. 4). Therapeutic approaches that increase Hsp70 levels in the brain are one way to inhibit the formation of toxic seed-like conformations of  $\alpha$ -syn.

## Summary

The major recent findings in the PD field were that  $\alpha$ -syn is a stable tetramer, the CSP $\alpha$ -Hsc70-SGT chaperone machine, in concert with  $\alpha$ -syn, promotes neurotransmitter release, and  $\alpha$ -syn can infect and kill healthy cells. With respect to chaperones, it will be interesting to see whether  $\alpha$ -syn tetramers form spontaneously or whether chaperone help is required. Given that Hsp70 protects cells against  $\alpha$ -syn in so many models, drugs that increase Hsp70 levels or drugs that mimic the action of Hsp70s have great potential to inhibit PD spread. The mechanisms by which cells secrete chaperones and  $\alpha$ -syn need to be elucidated.

## Acknowledgments

This work was supported by the NIH grant NS057656.

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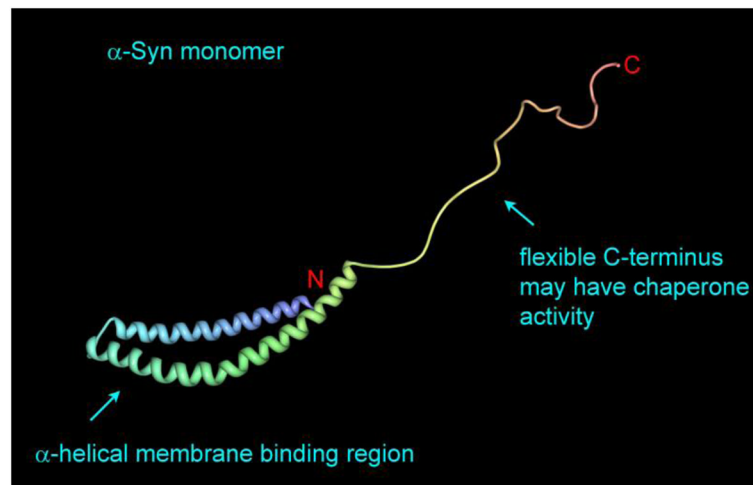
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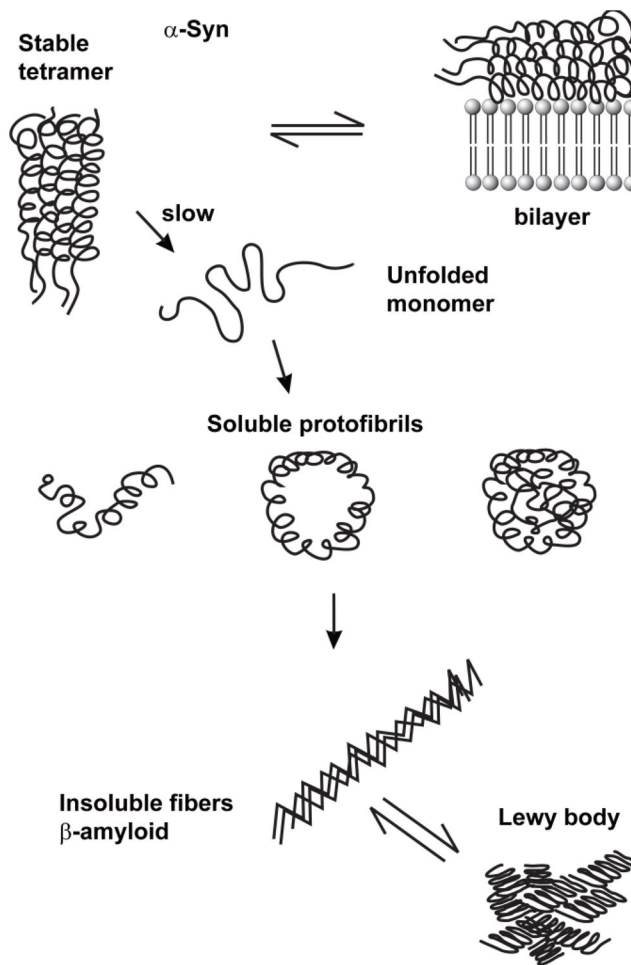
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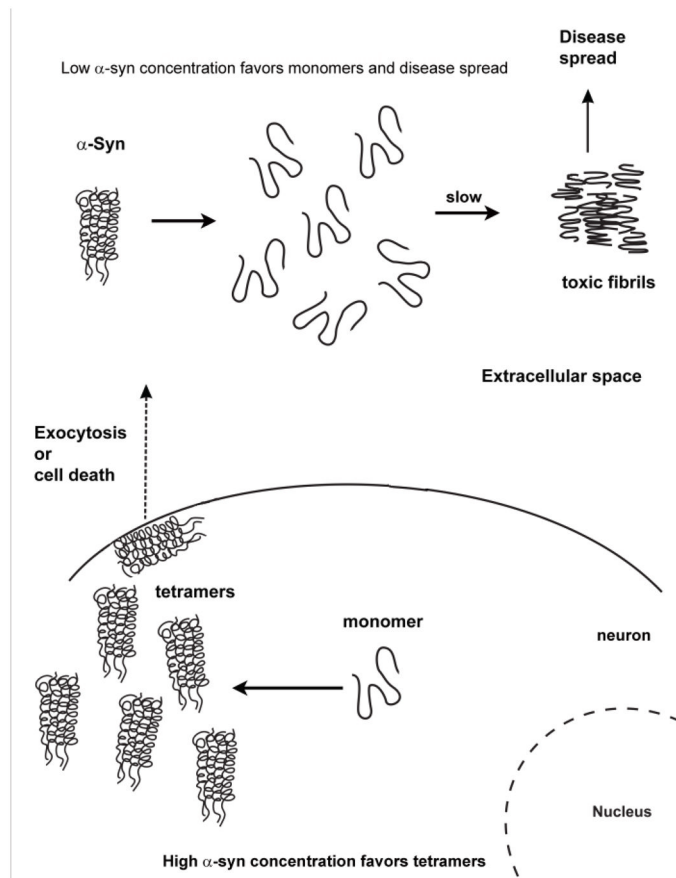




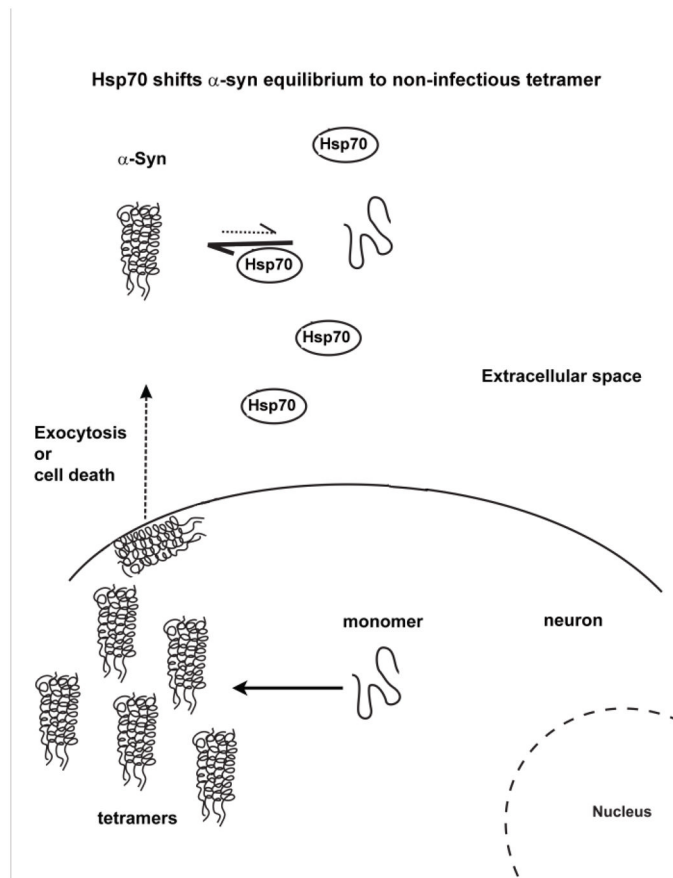
**Fig. 1.** Monomeric  $\alpha$ -syn bound to a micelle. The N-terminal residues (1–95) form two  $\alpha$ -helices that adopt a hairpin-like structure, while the C-terminal residues are unstructured. Protein data bank file: 1XQ8 [32].



**Fig. 2.** Model for  $\alpha$ -syn aggregate/fibril formation. The  $\alpha$ -syn tetramer is thermodynamically favored inside cells because of the high expression level of this protein. The tetramers could dissolve into unfolded monomers because of post-translational modifications, dilution, or unknown factors. Unfolded monomers can aggregate into soluble high molecular mass protofibrils and insoluble  $\beta$ -amyloid fibers. Emerging evidence indicates that a pathogenic conformation of  $\alpha$ -syn is transmitted from cell-to-cell, and this transmission causes disease spread through the brain. For simplicity, in Figs. 2–4 the  $\alpha$ -syn tetramer is depicted as a four-helix bundle where each monomer is an  $\alpha$ -helix with a flexible tail. In the physiological  $\alpha$ -syn tetramer, each monomer has been suggested to adopt the hair-pin-like structure shown in Fig. 1 [20].



**Fig. 3.** Model of  $\alpha$ -syn toxicity and disease spread. The model stipulates that a high concentration of  $\alpha$ -syn inside cells favors the non-infectious tetramer whereas a low concentration outside the cells favors the potentially toxic monomer. Probably most of the monomeric  $\alpha$ -syn in the extracellular space is degraded by proteases; nevertheless, in time, toxic  $\alpha$ -syn conformations can form and infect neighboring cells.



**Fig. 4.** Hsp70 protects against toxic extracellular forms of  $\alpha$ -syn. Hsp70/Hsc70 chaperones are known to be secreted, perhaps via exosomes, from a variety of cells in response to stress. Hsp70/Hsc70 chaperones protect against  $\alpha$ -syn-induced cell death in a variety of organisms, and Hsp70 was recently shown to reduce the infectivity of extracellular  $\alpha$ -syn. The figure illustrates that in the extracellular milieu Hsp70 shifts the  $\alpha$ -syn equilibrium to the non-infectious tetramer.