Chaperone proteins as ameliorators of α-synuclein-induced synaptic pathologies: insights into Parkinson's disease

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α-Synuclein accumulation causes synaptic vesicle trafficking defects and may underlie neurodegenerative disorders: Neurodegenerative disorders, such as Parkinson's disease (PD) and other synucleinopathies, impact the lives of millions of patients and their caregivers. Synucleinopathies include PD, dementia with Lewy Bodies (DLB), multiple system atrophy, and several Alzheimer's Disease variants. They are clinically characterized by intracellular inclusions called Lewy Bodies, which are rich in atypical aggregates of the protein α-synuclein. While dopaminergic neurons in the substantia nigra are particularly susceptible to α-synuclein-induced aggregation and neurodegeneration, glutamatergic neurons in other brain regions (e.g. cortex) are also frequently affected in PD and other synucleinopathies (Schulz-Schaeffer 2010). Several point mutations in the α-synuclein gene (*SNCA*), as well as duplication/triplication of *SNCA*, are linked to familial Parkinson's disease. In animal models, these genetic alterations lead to overexpression and aberrant accumulation of α-synuclein within neurons, and eventually to neurodegeneration. Interestingly, in both animal models and human patients, α-synuclein aggregation often occurs at neuronal synapses and within axons prior to the appearance of larger aggregates (i.e. Lewy bodies) and other signs of neurodegeneration (Schulz-Schaeffer 2010; Volpicelli-Daley et al., 2011). The level of synaptic aggregation of α-synuclein is highly correlated with greater cognitive deficits in PD and DLB patients (Schulz-Schaeffer 2010). Thus, it is essential to understand how excess α-synuclein impacts synapses, as this may represent an early stage in the neurodegenerative disease progression and thus a viable target for therapeutic intervention, particularly with respect to cognitive impairment.

Under physiological conditions, α-synuclein is localized on presynaptic vesicles, and it modulates both neurotransmitter release via exocytosis and early stages of endocytosis (Sulzer and Edwards, 2019). When acutely or chronically overexpressed, mimicking conditions that occur in PD, α-synuclein causes activity-dependent synaptic vesicle trafficking defects, with predominant effects on synaptic vesicle endocytosis and reclustering (Nemani et al. 2010; Banks et al., 2020). These α-synuclein induced synaptic vesicle trafficking defects have been observed at both glutamatergic and dopaminergic synapses (Nemani et al., 2010; Banks et al., 2020). Thus the mechanisms by which excess α-synuclein impairs synaptic vesicle recycling represents an active area of investigation.

Over the last few years, our lab has developed the giant reticulospinal (RS) synapse of the sea lamprey (*Petromyzon marinus*), as a model system to study how excess α-synuclein impacts synaptic vesicle recycling. RS synapses are amenable to acute perturbation without inducing the known compensatory changes in presynaptic protein levels that are observed after chronic α-synuclein overexpression (Nemani et al., 2010). Using the lamprey RS synapse model, we have shown that synapses acutely treated with excess human α-synuclein exhibit profound ultrastructural changes, which include a severe reduction in the number of synaptic vesicles clustered at the active zone, expansion of the plasma membrane, increased number of large intracellular vesicular structures > 100 nm in diameter (putative endosomes called "cisternae"), and an increase in clathrin-coated pits and vesicles (Banks et al., 2020). This phenotype is consistent with α-synuclein impairing clathrin-mediated synaptic vesicle recycling, but until recently the mechanisms remained unknown.

Clathrin-mediated endocytosis involves the initial recruitment of clathrin adaptor and accessory proteins to the periactive zone to facilitate formation of a clathrin-coated pit (**Figure 1A**). The clathrin-coated pit progresses through several stages of membrane curvature and coat formation before the large GTPase dynamin separates it from the plasma membrane, resulting in a free clathrin-coated vesicle. The constitutively expressed Heat Shock Cognate (Hsc) Hsc70 chaperone and its co-chaperone auxilin/DNAJC6 then disassemble the clathrin coat, after which the uncoated synaptic vesicles are refilled with neurotransmitter and recycled back to the vesicle cluster (**Figure 1A**). Earlier this year, we reported in *eNeuro* that excess α-synuclein induced a dramatic and selective increase in the number of free clathrin-coated vesicles at synapses, with no change in clathrin-coated pits, indicating that α-synuclein specifically disrupted the clathrin uncoating step (**Figure 1B**) (Banks et al., 2020). We therefore hypothesized that α-synuclein may be interacting aberrantly with components of the clathrin uncoating machinery, thereby inhibiting clathrin uncoating and leading to impaired vesicle recycling (**Figure 1B**).

Hsc70 as a target for α-synuclein-associated synaptic vesicle trafficking defects: To determine how excess α-synuclein impaired clathrin uncoating, we tested for direct interactions between α-synuclein and proteins that participate in the uncoating of clathrin-coated vesicles using biochemical methods. In pulldown assays, the highly conserved, alpha-helical N-terminus of α-synuclein was shown to directly interact with the C-terminus of Hsc70, the clathrin uncoating ATPase (Banks et al., 2020), consistent with prior findings (Redeker et al., 2012). This interaction was selective because under the same conditions, α-synuclein did not interact with other components of the clathrin coat including clathrin heavy chain, dynamin, or β-adaptin, a subunit of the AP2 adaptor complex (Banks et al., 2020). α-Synuclein also did not interact with other proteins that participate in clathrin uncoating, such as synaptojanin or auxilin/DNAJC6. These results suggested that excess α-synuclein may be interacting aberrantly with Hsc70 and inhibiting its function at synapses. *In vitro* uncoating assays comprising only the core uncoating machinery showed that excess α-synuclein does not directly impair the uncoating function of Hsc70 (Banks et al., 2020).

However, when introduced into the complex environment at synapses, excess α-synuclein inhibited the activity-dependent recruitment of Hsc70 to synaptic vesicle clusters. Thus, the presence of excess α-synuclein sequestered Hsc70 and drastically reduced its availability at synapses (**Figure 1B**). We reasoned that if α-synuclein sequesters Hsc70 and prevents its proper localization to synapses, then the synaptic defects might be rescued by exogeneously increasing the levels of Hsc70 at synapses. Supporting this idea, acute introduction of excess α-synuclein along with substoichiometric amounts of recombinant Hsc70 completely rescued the synaptic phenotype, resulting in synapses with normal numbers of synaptic vesicles, cisternae, and clathrin-coated vesicles (Banks et al., 2020). Thus, replenishing Hsc70 to synapses may have restored clathrin uncoating, thus improving the efficiency of synaptic vesicle recycling and ultimately ameliorating the vesicle trafficking defects caused by excess α-synuclein (**Figure 1C**). Another possibility is that excess α-synuclein impaired synaptic vesicle recycling by some other undetermined mechanism that was reversed by Hsc70. Further research is required to determine the precise mechanism(s) by which Hsc70 rescues these synaptic defects. Regardless of the mechanism, our study suggests that Hsc70 and clathrin mediated endocytosis could represent important targets for the development of novel therapeutics to treat synaptic and cognitive deficits in PD and DLB (Banks et al., 2020).

Chaperones as broader ameliorators of synuclein pathology: Chaperone proteins are an intriguing therapeutic target for PD due to their role in regulating protein folding, function, and aggregation. Members of the Heat Shock Protein (Hsp) Family and associated co-chaperones are present in increased levels in PD patient brains, and Hsp40, Hsp90, Hsp70 and Hsc70 are all components of Lewy Bodies. Furthermore, *in vitro* research shows that Hsc70 and Hsp70 can prevent α-synuclein fibril formation or disaggregate fibrils, which may help clear α-synuclein aggregates (Chaari et al., 2016; Gao et al., 2015). While it remains unclear whether Hsc/p70 accumulation in Lewy bodies is associated with toxicity and disease pathogenesis or alternatively the cells' protective response, this nonetheless could result in sequestration of Hsc70 away from other cellular compartments where its functions are required. In support of this idea, expression of human Hsp70 in a *Drosophila melanogaster* model of PD protected dopaminergic neurons from degeneration caused by α-synuclein overexpression without altering the α-synuclein inclusions (Auluck et al., 2002). In addition, introduction of aggregated α-synuclein incubated with Hsc70 into SHSY5Y cells was less cytotoxic than introduction of aggregated α-synuclein alone, with the largest rescue occurring in the presence of Hsc70 variants possessing an intact C-terminus (Chaari et al., 2016). Recently, Hsp110 overexpression was shown to increase levels of multiple chaperone proteins, including Hsc70, resulting in a reduction in α-synuclein aggregation in neuronal cell bodies and synapses, as well as a reduction in the pathological spread of aggregated α-synuclein throughout the brain, including the substantia nigra (Taguchi et al., 2019). Taken together, these findings implicate Hsc70 and increased chaperone function throughout neurons, including at synapses, as a viable strategy for improving outcomes in PD.

Despite the strong experimental evidence, efforts to develop chaperone based therapeutics have been largely unsuccessful thus far, possibly because they were focused on increasing general chaperone activity, rather than on specific chaperone supported processes. Hsc70 has multiple roles in the cell and its function in uncoating of clathrin-coated vesicles, unlike its general chaperone functions in protein folding, is unlikely to be substituted by other closely related chaperones (Sousa and Lafer, 2015). It is possible that neurodegeneration may not be solely

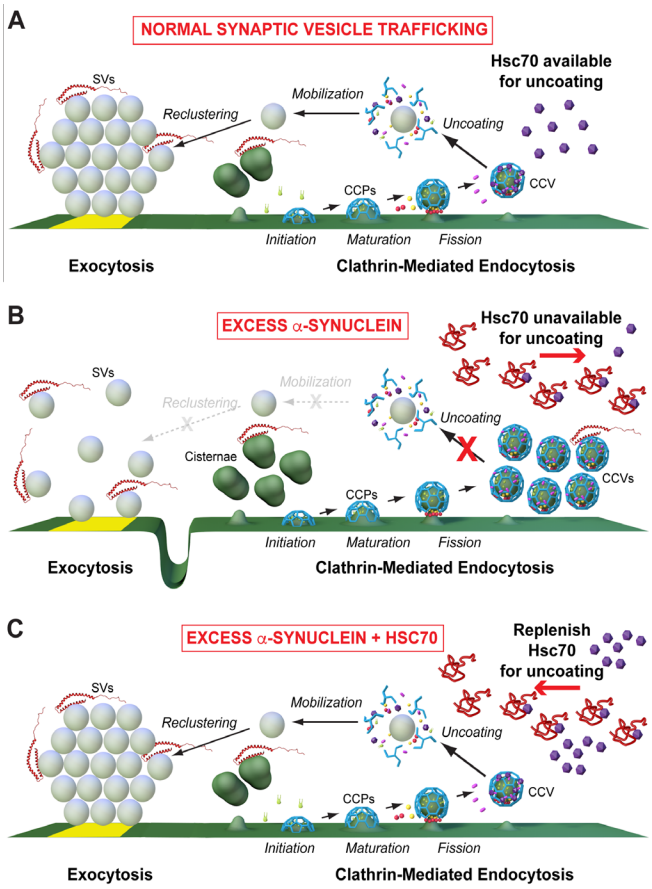

Figure 1 | **Heat Shock Cognate 70 (Hsc70) ameliorates α-synuclein-induced synaptic deficits.**
(A) During normal synaptic vesicle (SV) trafficking, SVs are efficiently recycled via clathrin-mediated endocytosis. (B) After introduction of excess α-synuclein, vesicle recycling is severely impaired. Our recent study indicated deficits in the process of uncoating clathrin-coated vesicles (CCVs), likely due to sequestration of Hsc70. (C) In support of this model, replenishing Hsc70 rescued the synaptic deficits, thereby restoring synaptic vesicle trafficking. Graphics generated by Jack Cook and Tim Silva (Woods Hole Oceanographic Institution). Adapted from Banks et al. (2020). CCPs: Clathrin-coated pits.

associated with α-synuclein aggregate formation, but may also be due to α-synuclein associated synaptic vesicle trafficking defects leading to more widespread neuronal dysfunction (Sulzer and Edwards, 2019; Banks et al., 2020). Therefore, Hsc70 is uniquely positioned to address both a need to increase general chaperone activity and mitigate disruptions to synaptic vesicle trafficking. Going forward, it will be important to further understand the possible mechanisms for Hsc70 rescue of α-synuclein-induced synaptic defects.

Interestingly, mutations in several other clathrin uncoating proteins at synapses, including auxilin/DNAJC6 and synaptojanin, have been linked to familial PD with juvenile or early onset characteristics. Mice harboring the homozygous R258Q mutation in synaptojanin, which has been identified in multiple families, develop neurological symptoms, exhibit movement deficits, and have excessively high numbers of clathrin-coated vesicles at dopaminergic and glutamatergic synapses, consistent with vesicle trafficking defects (Cao et al., 2017). This growing body of evidence from both animal models and human genetics implicates defects in clathrin-mediated endocytosis at synapses, in particular clathrin uncoating, as strong contributors in PD etiology. Given that clathrin-mediated endocytosis and its molecular players such as Hsc70 participate in other membrane trafficking events throughout the neuron, our findings may have broader implications for more widespread cellular impacts of α-synuclein, which currently also include ER stress and mitochondrial dysfunction. Going forward, it will be important to evaluate Hsc70 and other chaperone proteins for their potential to mitigate synaptic defects caused by α-synuclein, as this could be important for sparing synaptic function and slowing neurodegeneration, thereby improving cognitive function in the synucleinopathies and other related neurodegenerative diseases.

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