PERSPECTIVE

Impacts of increased α-synuclein on clathrin-mediated endocytosis at synapses: implications for neurodegenerative diseases

α-Synuclein causes synaptic pathologies in several neurodegenerative diseases: Parkinson’s disease (PD) is a neurodegenerative disease that impacts the lives of millions of people worldwide. A pathological hallmark of PD, as well as dementia with Lewy bodies (DLB) and several Alzheimer’s disease variants, is the appearance of intracellular inclusions called Lewy bodies, which contain high levels of aggregated α-synuclein. α-Synuclein is a presynaptic protein that normally associates with synaptic vesicle membranes and regulates synaptic vesicle trafficking under physiological conditions (Calo et al., 2016). However, in familial PD, multiplication and several point mutations in the α-synuclein gene (SNCA) ultimately lead to toxic aggregation of the α-synuclein protein and subsequent degeneration of dopaminergic neurons in the substantia nigra, although other brain areas are also affected (Schulz-Schaeffer, 2010). Recent studies indicate that toxic oligomerization and aggregation of α-synuclein also occurs in presynaptic boutons concurrently with or before Lewy body formation in both experimental models and human patients. When overexpressed, α-synuclein forms synaptic aggregates, which are associated with compromised neurotransmission in PD animal models (Nemani et al., 2010). These aggregates are toxic rather than Lewy bodies, and these presynaptic inclusions progressively correlated with greater cognitive decline in PD and DLB patients (Schulz-Schaeffer, 2010; Calo et al., 2016). Therefore, synaptic aggregation of α-synuclein likely plays a central role in triggering α-synucleinopathies, making the synapse a crucial place in which to study disease pathogenesis.

The classical model for α-synuclein toxicity suggests that misfolding of cytosolic monomeric α-synuclein leads to assembly of small toxic oligomers, followed by fission. (Figure 1A), indicating that increased levels of monomeric α-synuclein at synapses impair vesicle trafficking, as indicated by a loss of synaptic vesicles, and increased the number of endocytic profiles, including clathrin-coated inclusions (Hsc70) and its co-chaperone auxilin (Uncoating). Upon further investigation of the synaptic phenotype produced by increased levels of monomeric α-synuclein, we observed a selective increase in the number of functional CCVs, indicating a defect in the process of clathrin uncoating (Figure 1B) (Medeiros et al., 2017). This uncoating defect is caused at least in part by sequestration of Hsc70 because when exogenous Hsc70 was added back to the synapse, the defects in CCV uncoating and vesicle endocytosis were ameliorated (unpublished).

Monomeric and dimeric α-synuclein produce distinct effects on clathrin-mediated synaptic vesicle endocytosis: We next wanted to determine whether increasing α-synuclein dimers produced a similar – or distinct - phenotype at synapses compared with monomeric α-synuclein. For these experiments, we used recombinant α-synuclein dimers that were generated by covalent linkage of two human α-synuclein molecules, which allowed us to begin distinguishing the synaptic effects caused by an imbalance of α-synuclein oligomers (Pivato et al., 2012). Biochemically, recombinant α-synuclein dimers exhibited in vitro properties, including folding, fission, and lipid binding, that were similar to monomeric α-synuclein, albeit with slightly different efficacies (Pivato et al., 2012; Medeiros et al., 2017). Like monomeric α-synuclein, the α-synuclein dimers inhibited synaptic vesicle endocytosis and increased the number of clathrin-coated intermediates (Medeiros et al., 2017). However, unlike monomorphic α-synuclein, the dimers increased the number of CCPs that were still attached to the plasma membrane (Figure 1C). These structures presented as single CCPs (Busch et al., 2014; Xu et al., 2016) and/or vesicle reclustering (Nemani et al., 2010). The lampry giant synapses also provide the best evidence that increasing the number of oligomeric α-synuclein types may have different impacts at the synapse, suggesting that they may have distinct molecular targets that function within the clathrin pathway.

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rate (Cao et al., 2017). Thus, a growing body of evidence from both synaptic boutons and exhibited impaired synaptic vesicle endocytosis at synapses (Edvardson et al., 2012). In addition, increased expression of α-synuclein increases the number of CCPs that are still connected to the plasma membrane, indicating a defect in vesicle fission. Thus, α-synuclein monomers and dimers produce distinct effects on clathrin-mediated SV recycling.

**Mutations in genes associated with clathrin-mediated endocytosis are linked to PD and parkinsonism:** Our studies at lamprey synapses implicate clathrin-mediated endocytosis as a possible target of α-synuclein synaptic pathologies. Similarly, human genetics studies on PD are also pointing toward defects in the clathrin pathway as a possible underlying cause. For example, some patients with juvenile Parkinsonism carry a deletion in DNAJC6, the gene that encodes for auxilin, the Hsc70 co-chaperone that functions during CCV uncoating at synapses (Edvardson et al., 2012). In addition, increased expression of GAK, the ubiquitously expressed Hsc70 co-chaperone, is associated with risk of familial PD (Nagle et al., 2016). A missense mutation in synaptotagin 1 (R258Q), another protein that regulates clathrin uncoating at synapses, has also been linked to early onset parkinsonism (Krebs et al., 2013). Indeed transgenic mice carrying the synaptotagin 1 R258Q mutation accumulated CCVs within their synaptic boutons and exhibited impaired synaptic vesicle endocytosis, dystrophic axons, severe motor deficits, and increased death rate (Cao et al., 2017). Thus, a growing body of evidence from both animal models and human genetics indicates that defects in clathrin-mediated endocytosis are at least susceptibility factors in PD and parkinsonism, if not causal factors.

Going forward, it will be important to determine how higher molecular weight species and small toxic oligomers of α-synuclein impact synaptic vesicle trafficking and clathrin-mediated endocytosis at synapses. Understanding the molecular targets for each distinct α-synuclein species will be critical to these efforts. It will also be important to determine how the other human mutations associated with PD and parkinsonism affect synaptic structure and function, as has been done for the synaptotagin 1 R258Q mutation (Cao et al., 2017). Collectively, the current data suggest the possibility that synaptic deficiencies associated with increased levels of α-synuclein may be corrected by targeting aspects of the clathrin pathway. Determining how to ameliorate synaptic defects is a key step toward improving the symptoms in patients with α-synucleinopathies and many other related neurodegenerative diseases.

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