Journal Club

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Prime Time for α-Synuclein

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Review of Larsen et al. (http://www.jneurosci.org/cgi/content/full/26/46/11915)

Parkinson's disease (PD) is the most common neurodegenerative movement disorder, afflicting ~1% of individuals over the age of 65. PD results primarily from a severe and selective devastation of dopaminergic neurons in the substantia nigra pars compacta, although neuropathology extends to other brain regions. Overwhelming evidence implicates the presynaptic protein α-synuclein (α-syn) in the pathogenesis of PD. α-Syn is a major constituent of Lewy Bodies, cellular inclusions that are the pathological hallmark of PD. Furthermore, several missense mutations in the α-syn gene and duplication or triplication of the wild-type locus are linked with early-onset PD in rare familial forms of the disease. Overexpression of α-syn, or PD-associated α-syn mutants, in mouse, rat, fly, worm, and even yeast suggest that excess accumulation of α-syn leads to cellular toxicity (Moore et al., 2005; Lee and Trojanowski, 2006).

Despite intense study, the precise function of α-syn remains unresolved. However, a growing body of evidence suggests a role in the maintenance of synaptic vesicle pools (Murphy et al., 2000; Cabin et al., 2002), activity-dependent dopamine release (Abeliovich et al., 2000), or as an auxiliary chaperone for the assembly of specific soluble *N*-ethylmaleimide-sensitive factor attached protein receptor (SNARE) complexes that drive vesicle fusion with the plasma membrane (Chandra et al., 2005). Despite these indications, single and double knock-outs of α-, β-, and γ-synuclein are viable and exhibit very little (if any) phenotype under basal conditions (Chandra et al., 2004). However, synucleins may perform subtle regulatory functions and might even become essential under specific situations of stress or injury. Indeed, an essential function for α-syn has been revealed in cysteine-string protein-α-deficient mice (Chandra et al., 2005).

An intriguing new study by Larsen et al. (2006) in *The Journal of Neuroscience* provides new insights into α-syn function. Previously, the authors demonstrated that high levels of human α-syn are cytotoxic to PC12 cells (Stefanis et al., 2001). To study α-syn function, they generated a new stable PC12 cell line that expressed low, nontoxic levels of wild-type human α-syn or the PD-associated A30P α-syn mutant. In these cells, α-syn expression caused no gross morphological defects and did not affect viability [Larsen et al. (2006), their Fig. 1*A* (http://www.jneurosci.org/cgi/content/full/26/46/11915/F1)] but rather resulted in a significant decrease in stimulation-dependent dopamine (DA) release [Larsen et al. (2006), their Fig. 1*B* (http://www.jneurosci.org/cgi/content/full/26/46/11915/F1)]. This result was strikingly complementary to the increase in evoked DA release observed in α-syn knock-out mice (Abeliovich et al., 2000) and reinforces the notion that α-syn can function as a negative regulator of DA release.

The hunt was on for how α-syn inhibited DA release. First, the authors showed that the α-syn-dependent inhibition of DA secretion was not attributable to decreased Ca^{2+} entry or decreased sensitivity to Ca^{2+} [Larsen et al. (2006), their Fig. 2 (http://www.jneurosci.org/cgi/content/full/26/46/11915/F2)]. Next, they speculated that α-syn overexpression might decrease the number of dense core vesicles (DCVs) docked at the plasma membrane and available to release their contents (DA). However, when the number and location of DCVs was assessed by electron microscopy, α-syn-expressing cells actually had more docked vesicles than control cells [Larsen et al. (2006), their Fig. 3 (http://www.jneurosci.org/cgi/content/full/26/46/11915/F3)]!

This phenotype was reminiscent of the increase in docked DCVs after exposure to botulinum toxin A, a metalloendopeptidase that cleaves certain SNAREs (Jahn and Scheller, 2006), suggesting that perhaps fusion events were reduced by α-syn expression. Alternatively, α-syn might decrease the amount of DA released per vesicle fusion event. To test these possibilities directly, the quantal size, or amount of DA released by each vesicle fusion event was measured. For these analyses, primary cultures of chromaffin cells isolated from adrenal glands of wild-type, α-syn-overexpressing transgenic mice, or α-syn knock-out mice were used [Larsen et al. (2006), their Fig. 4 (http://www.jneurosci.org/cgi/content/full/26/46/11915/F4)]. Analysis of individual fusion events re-

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vealed that the number of molecules released per fusion event was unaffected by α-syn expression (quantal size) [Larsen et al. (2006), their Table 1 (http://www.jneurosci.org/cgi/content/full/26/46/11915/T1)]. In contrast, the total number of fusion events in cells from α-syn-expressing mice was significantly decreased [Larsen et al. (2006), their Fig. 5*B* (http://www.jneurosci.org/cgi/content/full/26/46/11915/F5)]. Notably, the chromaffin cells from the A30P α-syn transgenic mice showed a similar decrease in vesicle fusion events, suggesting that perhaps not every α-syn function requires the protein to associate with membranes, because this mutant is partially defective in phospholipid binding (Jo et al., 2002). Together, these data are consistent with a role for α-syn as a regulator of the releasable pool of vesicles at the synapse (Fig. 1).

Thus, in a series of elegant experiments using multiple approaches, Larsen et al. systematically dissect the role of α-syn in regulating neurotransmitter release. They rule out vesicle formation or morphological docking as well as Ca^{2+}-triggered fusion and suggest that α-syn inhibits "priming," a reaction that transfers morphologically docked vesicles to a fusion-competent state. This entails the initiation of SNAREpin formation, where a vesicle-SNARE on the vesicle begins to engage its cognate target-SNARE complex on the target membrane. SNAREpin assembly then reaches a critical state, which is poised to catalyze bilayer mixing but is frozen by regulatory factors that preclude membrane fusion until Ca^{2+} entry (Jahn and Scheller, 2006). Curiously, in another context, α-syn acted to promote SNARE complex formation (Chandra et al., 2005). Clearly, additional work will be required to decipher the precise molecular details of how α-syn might modulate vesicle priming, but the new study by Larsen et al. opens the way toward finally establishing the function of α-syn.

An emerging concept from the work of Larsen et al. as well as other laboratories is that the toxic and normal functions of α-syn might be more closely related than previously suspected. A role for α-syn at the priming step in vesicle fusion at the synapse may very well be related to the types of vesicular transport defects observed in yeast cells overexpressing α-syn (Cooper et al., 2006). It is tempting to speculate that α-syn, when expressed at physiological levels, functions as a negative regulator of vesicle fusion and neurotransmitter release at the synapse. However, its accumulation beyond a certain threshold might lead to an inappropriate deployment of this function at the synapse or perhaps promiscuous inhibition of additional trafficking steps. Such alterations may play an important, but underappreciated role in the early stages of PD and be particularly deleterious to dopaminergic neurons.

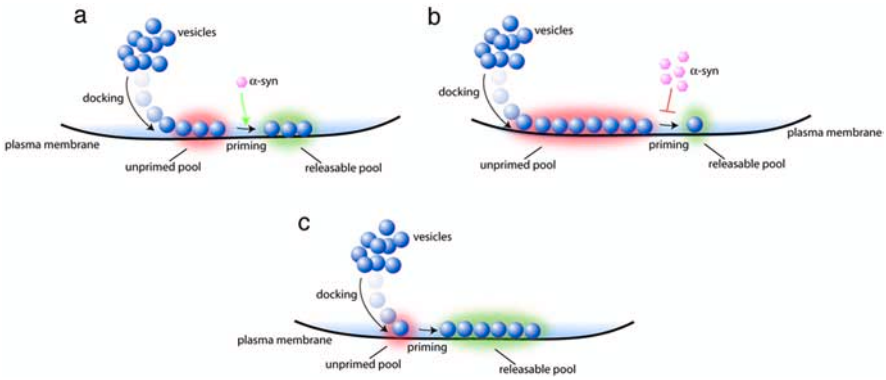

Figure 1. A model for α-syn as a regulator of vesicle priming. ***a***, In the presence of wild-type levels of α-syn, vesicles dock with the plasma membrane but are not yet fusion competent (unprimed pool) until primed (releasable pool). α-Syn functions as a regulator of this priming step (green arrow). ***b***, In the presence of high levels of α-syn, vesicles continue to dock with the plasma membrane but now linger in a fusion-incompetent state because of an inhibition of priming. Consequently, the unprimed pool of vesicles increases, and the releasable pool of vesicles decreases, leading to an increase in the number of docked vesicles and a decrease in exocytosis. ***c***, In the absence of α-syn (e.g., mouse knock-out), vesicles dock with the plasma membrane, but priming is dysregulated, leading to excessive priming and a build-up in the releasable pool of primed vesicles, potentially explaining the increased evoked neurotransmitter release observed in neurons from α-syn-deficient mice (Abeliovich et al., 2000).

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