

Neurodegeneration and Protein Misfolding - What's Gone Wrong with the Cell?

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A range of events including genetic mutations, transcriptional / translational mistakes and protein:chaperone imbalance commonly result in misfolded proteins. The manner in which misfolding results in disease can be loosely divided into two groups: recessive loss of function and dominant gain of function. The former is more frequent and can involve mutations which result in non-native (misfolded) structures that either lack activity, fail to oligomerise, fail to be delivered to the appropriate subcellular compartment or are degraded by quality control mechanisms. Such is the case in cystic fibrosis. Normally, the cystic fibrosis transmembrane regulator (CFTR) polytopic protein folds in the endoplasmic reticulum (ER) prior to export to the plasma membrane. However, with the common CFTR- Δ F508 mutation, the resulting protein misfolds in the ER and is degraded by ER-associated degradation (1). In a heterozygote, the misfolded protein does not affect the wild-type CFTR protein and thus shows no symptoms as 50% active protein is typically sufficient. The CFTR- Δ F508 homozygote, however, produces no active CFTR protein and presents with cystic fibrosis.

Gain-of-function mutations change the gene product such that it attains a non-native structure and abnormal function. These mutations are characteristically dominant in nature and result in a number of diseases including forms of the late onset neurodegenerative diseases Parkinson's disease (PD), Huntington's disease (discussed in the article by Hatters in this Showcase on Research) and amyotrophic lateral sclerosis (ALS) / motor neuron disease (MND).

Identifying what critical cellular functions are impaired or disabled by a misfolded protein is crucial for unraveling the molecular basis of pathogenesis. Equally important is discerning if it represents a primary event in the disease pathology or a downstream secondary event.

Misfolded α -Synuclein and Parkinson's Disease

Misfolded α -synuclein (α Syn) has been identified as a central causal component of Parkinson's disease, a progressive neurological disorder involving the loss of dopamine-producing neurons in approximately 1% of people aged over 65. α Syn is especially prone to aggregation and is found as the primary component in Lewy bodies, the cytoplasmic inclusions that are a defining post-mortem feature of PD (2). Two other neurodegenerative diseases, dementia with Lewy bodies and multisystem atrophy, also display α Syn inclusions and are collectively referred to as α -synucleinopathies.

Early onset PD can result from dominant mutant α Syn alleles (A53T, A30P) (3) or gene duplication (4-6), which indicates that simple elevated expression of this

aggregation-prone protein can cause disease. α Syn is natively unfolded as a free monomer, can associate with lipids / membranes as well as self-associate / aggregate into insoluble fibrillar structures such as those found in Lewy bodies (7). It remains controversial as to whether these fibrillar structures or transient soluble oligomers (also referred to as protofibrils) are the pathogenic species of α Syn in PD. Consequentially, it is contentious as to whether α Syn inclusions such as Lewy bodies are toxic to neurons or, conversely, are neuroprotective by removing soluble oligomers from solution.

Elucidating the mechanisms underlying the cytotoxic effects of α Syn is essential for the development of treatments to ameliorate or prevent PD. Complicating this intent is that PD involves a complex combination of genetic susceptibilities and environmental factors (e.g. pesticide, Mn^{2+} exposure) for which a link between them has remained elusive. Loss-of-function mutations in other PD susceptibility (PARK) genes results in familial PD, but it remains unknown whether these deficiencies directly impact α Syn or instead represent distinct and α Syn-independent pathways towards neuronal death.

The Native Function of α Syn

α Syn is an abundant 140 amino acid protein that is widely distributed throughout the brain and observed in neuronal cell bodies and presynaptic neuronal terminals, where it can associate with synaptic vesicles. However, its expression is not restricted to neurons and it can be found in many other cell types.

Knowledge of α Syn's native function would likely suggest potential mechanisms for α Syn's cytotoxicity, but its function remains undefined. α Syn knockout mice display few defects, although dopamine release is enhanced upon initial stimulation, suggesting a negative regulatory role in neurotransmission (8). Normally, neurostimulation triggers the exocytosis of a pool of readily releasable synaptic vesicles docked and primed at the presynaptic terminal active site (9). A reserve pool of undocked / unprimed synaptic vesicles exists to allow rapid repeat neurotransmissions. The transition of reserve pool vesicles into readily releasable synaptic vesicles involves the assembly of essential SNARE complexes (9) and vesicle docking at active zones (Fig. 1A). If α Syn negatively modulates this process, then an absence of α Syn would produce a larger active pool and result in greater initial neurotransmitter release, but consequentially reduce the number of vesicles in the reserve pool, both of which were observed in α Syn null mice (Fig. 1B). It is possible that the native function of α Syn involves regulating the docking / priming of synaptic vesicles – a function that may overlap with its pathophysiological action (see below).

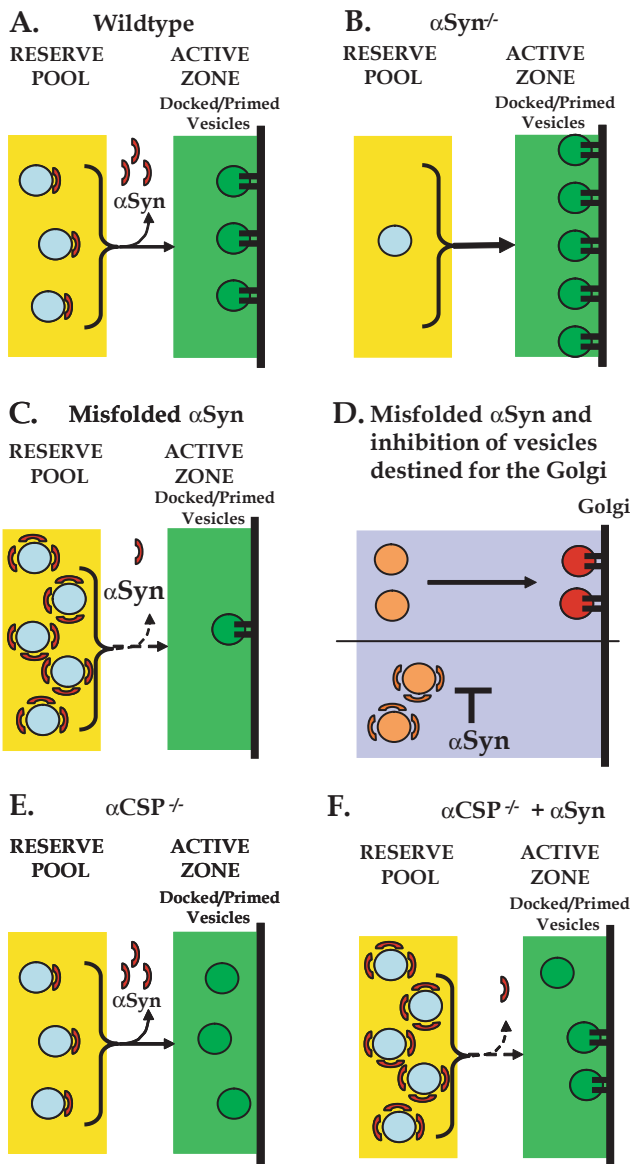


Fig. 1. Possible native and disease associated functions of α Syn.

A. Wildtype. α Syn (red arcs) associates with synaptic vesicles in the reserve pool (grey circles) and negatively modulates the transition to primed vesicles (green circles), containing functional SNARE complexes (represented by "="; see (9) for more details) docked with the pre-synaptic membrane in the active zone.

B. α Syn knockout (α Syn^{-/-}) cells lack this negative regulator and therefore a greater number of synaptic vesicles exit the reserve pool, producing an increase in primed vesicles in the active zone.

C. Misfolded α Syn, resulting from mutations, elevated expression or modification, associates with synaptic vesicles, but perhaps fails to dissociate at the appropriate rate / stage, resulting in reduced neurotransmission and eventual neurodegeneration.

D. Misfolded α Syn impeding vesicles bound for the Golgi. ER-derived vesicles destined for the Golgi (orange circles) are bound by α Syn, which prevents docking / fusion with the Golgi.

E. α CSP^{-/-} cells fail to efficiently form functional SNARE complexes (green circles lacking "=") and display reduced neurotransmission and eventual neurodegeneration.

F. α CSP^{-/-} + α Syn. Overexpression of α Syn may impair the forward transition of unprimed synaptic vesicles from the reserve pool, coincidentally providing more opportunity for the impaired assembly of the SNARE complex in CSP^{-/-} mice and thus partially suppress the CSP^{-/-} phenotype.

How is α Syn Killing the Cell?

How might misfolded / aggregated α Syn harm cells? Models include aggregates sequestering essential proteins, negative interactions with lipids / membranes or ineffective proteasomal attempts to degrade aggregates, resulting in proteasomal inhibition. The broad range of factors implicated in PD does not assist in narrowing the choices: oxidative stress, ER stress, vesicle trafficking defects, mitochondrial dysfunction, impaired proteasomal degradation or inhibited lysosomal degradation / autophagy. How are these varied and complex factors related to α Syn, which are primary or secondary events and what is their inter-relationship, if any?

One approach is to determine what cellular functions are impaired in cell-based model systems in which α Syn is inducibly expressed to cytotoxic levels. However, where and how does one first look for impaired cellular function and once found, how is it ascertained whether the dysfunction represents a primary initiating event in the disease pathology or a downstream secondary consequence? Furthermore, in choosing to investigate a particular cell function (e.g. mitochondrial dysfunction), one is displaying a bias for a disease hypothesis involving the mitochondria. Instead of examining an individual

cellular process for dysfunction, one can perform large-scale, unbiased approaches in *Saccharomyces cerevisiae* (yeast), a simple eukaryotic cell model that possesses much of the same basic cellular machinery as neurons. A yeast model of PD was developed in which induction of α Syn resulted in cytotoxic aggregates (Fig. 2) and broad, unbiased, genome-wide, discovery-based approaches used to investigate the basis of cell death. In addition to avoiding a predisposition towards a particular disease hypothesis, this approach has the potential to detect convergence of disease-modifying pathways that would otherwise be difficult to detect, something very relevant in PD where many genetic and environmental factors influence the disease.

The first approach involved α Syn expression in an ordered collection of yeast knockout mutants representing ~80% of all yeast genes. These loss-of-function mutants were screened for candidates that displayed enhanced α Syn-induced toxicity and the mutants identified were enriched in vesicle-mediated transport, i.e. mutants impaired for vesicular trafficking were more sensitive to α Syn expression (10). Importantly, these genes showed specificity to α Syn as they did not significantly overlap with genes whose absence sensitised yeast to the toxic

effect of Huntington's protein. A subsequent approach involved expressing α Syn in yeast cells containing a custom overexpression library of every yeast gene and screening for genes whose overexpression suppressed α Syn induced cell death. The predominant category of suppressors encoded proteins participating in vesicular transport between the ER and Golgi, including Rab1, a key regulatory component for this transport step (11). This converged with parallel findings that delivery of cargo from the ER to the Golgi is the first observable defect in yeast expressing toxic levels of α Syn (11). The early onset trafficking block and the suppression of cell death by trafficking genes strongly supports that this trafficking defect is likely a primary event in α Syn toxicity. Such a transport block would in turn cause ER stress, a condition associated with PD, which was observed at later time points. ER stress can induce oxidative stress, another condition associated with PD.

These yeast discoveries acted as a foundation from which to extend into neuronal-based PD models where α Syn-induced dopaminergic neuron loss in rat midbrain primary neurons was significantly rescued by virus-driven Rab1a overexpression (11). The yeast model has also provided a discovery platform with which to rapidly and inexpensively screen for small molecules that ameliorate the toxicity.

Misfolded α Syn and Vesicular Trafficking Defects

The α Syn-induced defect in trafficking between the ER and Golgi in yeast is likely at the stage of vesicle tethering / docking / fusion at the Golgi (11) and when viewed in conjunction with α Syn cell culture models (12), one can envisage significant overlap between α Syn's native and pathophysiological roles (13). α Syn may act in a regulated manner to modulate the transition of synaptic vesicles from reserve pool to primed vesicles at the active site. If so, misfolded α Syn may fail to remove the temporary restraint, resulting in reduced neurotransmission and eventual neurodegeneration (Fig. 1C). In an equivalent manner, misfolded α Syn may inappropriately interact with vesicles destined for the Golgi, impairing their delivery and causing the ER-Golgi transport block observed (11) (Fig. 1D).

Paradoxically, α Syn overexpression in mice protects against the degeneration of nerve terminals that occurs in the absence of CSP α , a proposed Hsc70 co-chaperone thought to assist in folding and refolding synaptic SNARE proteins (14). The absence of CSP α inhibits SNARE protein complex assembly at the pre-synaptic junction (Fig. 1E) and α Syn transgenic expression restored SNARE complex assembly while the absence of endogenous α Syn aggravated the CSP α -defect (14). It has been proposed that α Syn, acting as a chaperone, restores SNARE complex assembly (13,14). Instead, I speculate that the overexpression of α Syn may impair the forward transition of unprimed synaptic vesicles from the reserve pool, coincidentally providing more opportunity for the impaired assembly of the SNARE complex in CSP α ^{-/-} mice to form and thus partially suppress the CSP α ^{-/-} phenotype (Fig. 1F). The absence of α Syn, which exacerbates the CSP α ^{-/-} phenotype, would remove the α Syn brake, potentially accelerating the forward transition of unprimed vesicles, further reducing the opportunity for SNARE complex assembly in CSP α ^{-/-} mice, and account for the enhanced neurodegeneration.

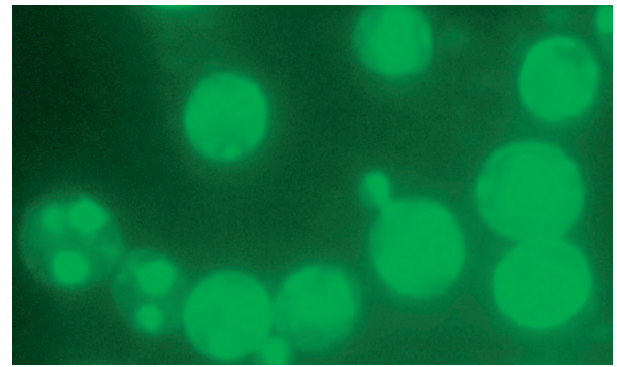


Fig. 2. Yeast cells expressing α Syn-GFP forming cytotoxic intracellular aggregates.

How do Cells Reduce the α Syn Risk?

How do neurons protect themselves from misfolded / aggregated α Syn and do these systems fail with age and thus are responsible for the late onset of these diseases? Molecular chaperones can reduce aggregation and *in vivo* Hsp70 overexpression reduced the amount of misfolded, aggregated α Syn species in α Syn transgenic mice and suppressed neurodegeneration in α Syn-based *Drosophila* models of PD (15,16). Factors that diminish the ubiquitin-proteasome system or autophagy-lysosome pathway, the two critical mechanisms to remove abnormal proteins, would elevate α Syn concentration closer to an aggregation threshold. Defects in both of these systems have been implicated in PD models (17,18).

Why the Apparent Selectivity of Neuronal Loss?

α Syn is expressed in many tissues, yet only particular neurons are lost and this has driven the field to account for the apparent selectivity. Oxidative stress is likely a contributing factor as it is closely associated with PD, while dopamine-producing neurons are under oxidative stress due to the metabolism of dopamine. Oxidative stress modification of α Syn enhances its propensity to aggregate and this may account for dopaminergic neurons being lost in PD. Similarly, mitochondrial complex I inhibitors (MPTP and rotenone) cause oxidative stress and induce PD-like symptoms in animal models. It is possible that the loss of other PARK genes (e.g. DJ-1) may result in PD by sensitising neurons to oxidative stress. Dopamine is also capable of directly reacting with α Syn to form adducts that retard the conversion of oligomers / protofibrils to fibrils. Cytosolic dopamine might therefore promote accumulation of the proposed toxic α Syn oligomers that could contribute to the vulnerability of these neurons in PD (19).

While the dopamine metabolism-induced oxidative stress provides an attractive model for the selective vulnerability of the dopaminergic neurons, it does not account for more recent findings that non-dopaminergic neurons are also lost in PD, consistent with a broad range of non-motor symptoms observed in PD (20). The appeal of the α Syn-induced vesicular trafficking defect is that it is not restricted to specific cell types as trafficking is essential to practically all cells, especially neurons. The fact that α Syn inclusions have not yet been found in cells outside the brain might be related to the relatively short lifetime of these cells compared to that of neurons.



Conclusions and Future Research

While α Syn-induced cellular dysfunction may involve multiple mechanisms, the above model involving vesicular trafficking defects, effective suppression by a Rab protein in multiple PD models and an overlap of α Syn's possible native and disease functions is appealing. Utilising the α Syn yeast PD model, in parallel with α Syn cell culture models, we aim to demonstrate that misfolded α Syn interferes with vesicle docking / fusion at the Golgi and elsewhere and to identify the molecular mechanism responsible. The broad and unbiased nature of the α Syn yeast PD model also permits us to screen for undiscovered susceptibility factors (both genetic and environmental) that very likely influence the propensity of α Syn to misfold and may assist in accounting for the vast majority of PD that is idiopathic. In addition, we seek the function of another PD susceptibility (PARK) gene while also investigating a direct functional connection between it and α Syn. Together, these studies provide a step forward towards the long-term possibility that genetic loci and environmental risk factors linked to PD interact with each other. Our success with these approaches has prompted us to investigate the basis for misfolded superoxide dismutase-1 induced cell death in ALS / MND.

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