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α - synuclein and Parkinson's disease: the first roadblock

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Abstract

α-synuclein gene mutations are major underlying genetic defects known in familial juvenile onset Parkinson's disease (PD), and α-synuclein is a major constituent of Lewy Bodies, the pathological hallmark of PD. The normal cellular function of α-synuclein has been elusive, and its exact etiological mechanism in causing dopaminergic neuronal death in PD is also not clearly understood. Very recent reports now indicate that mutant or simply over-expressed α-synuclein could cause damage by interfering with particular steps of neuronal membrane traffic. α-synuclein selectively blocks endoplamic reticulum-to -Golgi transport, thus causing ER stress. A screen in a yeast revealed that α-synuclein toxicity could be suppressed by over-expression of the small GTPase Ypt1/Rab1, and that over-expression of the latter rescues neuron loss in invertebrate and mammalian models of α-synuclein-induced neurodegeneration. α-synuclein may also serve a chaperone function for the proper folding of synaptic SNAREs that are important for neurotransmitter release. We discuss these recent results and the emerging pathophysiological interaction of α-synuclein with components of neuronal membrane traffic.

Keywords: α-synuclein • endoplasmic reticulum • Golgi • membrane traffic • Parkinson's disease

Introduction

Parkinson's disease (PD) is an age-associated neurodegenerative movement disorder. The neuropathological basis underlying the clinical phenotypes of PD such as bradykinesia, tremor and rigidity, is the loss of dopaminergic neurons in the substantia nigra [1–2]. Most PD cases are late onset and sporadic/idiopathic. However, studies of genetic mutations occurring in cases of familial Parkinsonism have brought to light several genes that are linked to the disease [3–4]. These genes appear to be involved in a number of molecular pathways that may give rise to neuronal degeneration as seen in PD.

Oxidative stress is evident in the PD brain [5], and could indeed be a direct mode of cellular damage caused by MPTP, a neurotoxin that could precipitate PD symptoms, through the inhibition of mitochondrial complex 1. Autosomal recessive mutations in the gene *DJ-1* [6] have been identified as a possible cause of loss of protection against neuronal oxidative stress. DJ-1 is believed to protect cells against inhibitors of the mitochondrial complex

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1, which results in the generation of free radicals. Mutations in another gene, *PINK1*, were also identified as a cause of autosomal recessive Parkinsonism. Although the exact pathophysiological role of PINK1 is not yet clear, its localization to the mitochondria suggests that its function may be speculatively linked to oxidative stress resulting from mitochondrial activities [7–8]. DJ-1 and PINK1 physically associate and mutant DJ-1-PINK1 complex appear to potentiate the susceptibility of SH-SY5Y cells to MPP(+)-induced cell death [9]. Mutations in the *parkin* gene have also been linked to juvenile-onset PD. Parkin is an E3 ubiquitin-protein ligase, and patients with autosomal recessive mutation in parkin may accumulate misfolded proteins that ultimately lead to neuronal cell death [10–11]. Increasing evidence suggests that neurodegeneration in not just familial but also sporadic forms of PD may indeed be related to a defect in the capacity of the ubiquitin-proteasome system (UPS) to clear unwanted proteins [12]. Pathogenic mutant forms of DJ-1 have been shown to specifically but differentially associate with parkin [13]. Recent studies have shown that loss of dopaminergic neurons in *Drosophila* as a result of PINK1 deficiency is rescued by over-expression of parkin (reviewed in [14]). Thus, PINK1 and parkin may function in a common pathway in the pathogenesis of autosomal recessive PD. Most recently, 2 other genes associated with PD have been discussed - the lysosomal type 5 ATPase gene, ATP13A2 [15] and the leucine-rich repeat protein kinase 2 gene (LRRK2) [16], the latter of which is particularly interesting given its association with late-onset, sporadic PD.

The first identified autosomal dominant mutations causing juvenile onset PD, however, are those associated with *α-synuclein* [1–4, 17], whose gene product is a major component of intraneuronal protein aggregates known as Lewy bodies [17], a pathological hallmark of most Parkinsonian brains. A53T and A30P are two missense mutations of the gene linked to familial PD, with E46K being another. Duplication and triplication of the *α-synuclein* gene is also linked to early onset of PD. α-synuclein is found in the presynaptic nerve terminal. The membrane-bound form exists in equilibrium with the soluble cytoplasmic form. Like the wild type, the A53T mutant accumulates at the plasma membrane, but A30P has poor membrane binding ability and is largely cytosolic. As a monomer, α-synuclein is unstructured when soluble, but has the propensity to form aggregates of insoluble fibrils, stabilized by β-sheet structures [2, 4]. A central hydrophobic region of α-synuclein facilitates self association and aggregation. The C-terminal tail of α-synuclein, on the other hand, inhibits aggregation, and a truncated form of the molecule is more aggregation-prone. Both the A53T [18] and the E46K [19], but not the A30P mutation, significantly promotes protofibril formation. α-synucleinopathic lesions contain a large amount of phosphorylated α-synuclein, and phosphorylation of α-synuclein at Ser129 promoted fibril formation *in vitro* [20]. When α-synuclein is over-expressed, most of the proteins end up as cytoplasmic inclusions rather than making their way to the cell membrane. Two distinct types of aggregates are observed, firstly large juxtanuclear inclusion bodies (containing α-synuclein fibrils) and the other smaller, non-fibrillar spherical α-synuclein aggregates that are scattered throughout the cytoplasm [21].

In spite of extensive studies, the exact cellular function of α-synuclein is still unclear, and the exact etiological mechanism by which over-expression of α-synuclein or its mutants could cause selective dopaminergic neuronal death in PD remains to be determined. Besides its abundant presence in PD's Lewy bodies, α-synuclein has also been implicated in other cellular mechanisms that result in loss of dopaminergic neurons, including UPS-mediated protein degradation and cellular transport. Pertaining to UPS defects, both an α-synuclein interacting protein, synphilin-1 [22], and a glycosylated form of α-synuclein, αSp22, are parkin substrates. αSp22 has been shown to accumulate in a non-ubiquitinated form in parkin-deficient PD brains [23].

Impaired axonal transport has been frequently proposed for neurodegenerative disorders associated with toxic aggregates, for example in Huntington's disease [24]. Involvement of vesicular membrane trafficking has also been implicated for various aspects of Alzheimer's disease [25], amyotrophic lateral sclerosis [26] and prion diseases [27]. A very recent report, as discussed below, has indeed pinpointed an association between the impairment caused by α-synuclein over-expression and a particular step of vesicular membrane traffic. The studies were initiated using a yeast cellular model for α-synuclein over-expression, results from which were then extended into animal neurons

in vitro and *in vivo*. The work is a good example of how a naïve cellular model could help in elucidating the mechanisms underlying the pathology of complex human diseases.

Expression of wild type and mutant α-synuclein in yeast cellular models

The complexity of disease etiology of genes in mammalian systems often necessitated the use of simpler model systems for their studies. The latter usually have the added advantage of established methods of genetic manipulation. The budding yeast *Saccharomyces cerevisiae* is one such model, and in spite of being unicellular, has all the essential key features of the mammalian system in terms of basic metabolism, intracellular signaling and membrane trafficking. In terms of impairment of cell function with regards to toxic aggregates, yeast has similar unfolded protein response and ubiquitin-proteosome based protein turnover that are perhaps better understood than their counterparts in mammalian cells. Indeed, Lindquist and colleagues have modeled prion disease [28–29] and Huntington's disease [30] in yeast. They are also the first to report the expression of wild type and mutant α-synuclein in yeast and characterize its cellular behavior and toxicity in this heterologous system [31–32]. The expressed wild type α-synucleins appeared to be targeted to the plasma membrane, and could form cytoplasmic inclusions at high concentrations, as it did when expressed in mammalian cells. The expressions of α-synuclein inhibited phospholipase D and induced the formation of lipid droplets. Vesicular trafficking, particularly endocytosis, appeared to be affected as well, as shown by the altered distribution of internalized FM4-64 dye (a tracer for looking at endocytosed material). A screen for synthetic lethality (*i.e.* for genes that if mutated are lethal only when expressed together with over-expressed α-synuclein) in fact revealed a few genes encoding components of the vesicular trafficking machinery that enhanced the toxic effect of α-synuclein [31–32]. Very similar observations with regard to the localization and toxicity of α-synuclein in yeast have also recently been observed by other workers [33], but there appear to be some disagreement in terms of formation of large aggregates.

Some workers have focused on other aspects and consequences of α-synuclein expression in yeast. α-synuclein appears to alter proteasome composition and impairs proteasome-mediated protein degradation as well as protein synthesis. It also reduced the ability of yeast cells to withstand stationary phase aging (a measure of viability after continuous culture at the stationary phase) [34–35]. Interestingly, however, Chen *et al.* [35] observed that α-synuclein seemed to have little effect on intracellular proteasome content or protein ubiquitination, and did not seem to increase the vulnerability of yeast cells to a variety of stressors. Flower *et al.* [36] observed that α-synuclein expression triggers changes that are reminiscent of mammalian cell apoptosis, including externalization of phosphatidylserine, reactive oxygen species (ROS) accumulation, and cytochrome c release from the mitochondria. Using ROS accumulation as a marker, the authors investigated various genes and drugs with respect to α-synuclein-induced apoptosis. Treatment with antioxidants, overexpression of Ssa3 (yeast Hsp70), or deletion of the yeast metacaspase gene *YCA1* abolishes the ability of α-synuclein to induce ROS accumulation. Deletion of *YCA1* also enhanced growth of α-synuclein-expressing cells. Griffioen *et al.* [37] observed that Zn^{2+} or Fe^{3+} ions enhanced α-synuclein-dependent growth inhibition. However, they also observed that deletion of *YCA1* did not ameliorate cytotoxicity but instead augmented reduction of growth. The reason for the contrasting observations made by the two groups above is not known. Another recent paper reported the expression of α-synuclein in the fission yeast *Schizosaccharomyces pombe* [38]. Contrasting to *S. cerevisiae*, α-synuclein did not target to the plasma membrane in fission yeast. Also, despite extensive aggregation, it was surprisingly nontoxic to fission yeast.

The variations in reported results serve as a reminder that studying of gene function in a simpler system is, while useful, not necessarily straightforward. However, there is a consensus in the data that over-expression of α-synuclein in yeast does recapitulate some key features observed in mammalian cells (for example inclusion body/aggregate formation), while other features (such as cellular stress and cytotoxicity) may be less obvious.

α-synuclein blocks ER-Golgi traffic in yeast cells

The remarkable usefulness of yeast as a simple genetic system to dissect the function of heterologous gene products is illustrated by a recent report from Lindquist, Bonini and colleagues [39], and has now α-synuclein narrowed down trafficking impairment caused by α-synuclein over-expression to at least one particular step of vesicular membrane traffic. Using the tight and highly inducible GAL4 promoter system, the authors were able to rapidly and synchronously induce α-synuclein expression and therefore examine very early events associated with that. ER stress is generally a result of misfolded proteins that accumulate in the ER. These proteins are translocated to the cytoplasm where they are removed *via* the ubiquitin-proteasome system. Cooper *et al.* showed that endoplasmic reticulum (ER) stress, manifested by the unfolded protein response [40], is one of the early cellular defects caused by the expression of human wild type α-synuclein and the A53T mutant. However, the reason for the induction of ER stress was not immediately apparent. One possibility is that α-synuclein impaired protein degradation [35] which would result in proteins accumulating in the ER not being adequately removed. However, the authors found that proteasome activity itself was not impaired in their model, suggesting that the cause for ER stress lies elsewhere. The clue lies in the fact that when the degradation of two proteins, namely the ER translocon component Sec61-2p and a soluble protein carboxypeptidase Y (CPY) were examined, *only the degradation of the latter* was affected by α-synuclein expression.

Why did α-synuclein expression selectively inhibit CPY degradation? One apparent difference between the itinerary of Sec61-2p and CPY is that the degradation of the latter requires its transport from the ER to the Golgi. The authors found that CPY ER-Golgi transport, as that of another secretory protein alkaline phosphatase, was inhibited within 3–4 h upon initiation of α-synuclein expression, with the A53T mutant causing a more rapid onset of trafficking block compared to wild type. Importantly, the earliest impairment of cell growth corresponded to this early impairment of ER-Golgi transport, which preceded the onset of ER stress.

In order to identify the genes or gene products that may be involved in this early transport arrest, the authors made use of the classical tools available to yeast geneticists. Using a strain with an intermediate level of α-synuclein expression, they conducted a genetic screen that would simultaneously look for genes that suppress and those that would enhance α-synuclein toxicity when over-expressed. They found that the largest and most effective bunch of suppressors were genes involved in vesicular transport, and intriguingly, were in fact acting at the same step in ER-Golgi transport (or are known suppressors for mutants defective in this transport step). These include Ypt1p (a member of the Rab family small GTPase known to mediate ER-Golgi transport [41], Ykt6p (an N-ethylmaleimide sensitive factor attachment protein receptor, or SNARE, molecule that is involved in ER-Golgi transport) [42] and Erv29p (a known cargo receptor that functions in ER exit) [43]. Also found with this group is Ubp3p (an ubiquitin protease) and Bre5p (a cofactor of Ubp3p) that are known to be required for the deubiquitination of Sec23 [44], a component of the coat protein II complex that mediates ER-Golgi transport. (For a comprehensive list of suppressors and enhancers identified, see table S1 of Cooper *et al.* [39]).

The specificity of the Ypt1p-requiring step is apparently essential, and other Rabs that mediate transport steps distal to the ER-Golgi step could not suppress α-synuclein toxicity. On the other hand, Gyp8p [45], which is a Rab GTPase activating protein (GAP) with a substrate specificity for Ypt1p (and deactivates it), was shown to be an enhancer of α-synuclein toxicity. A dominant negative form of Ypt1p likewise enhanced α-synuclein toxicity. A negative dominant form of the yeast ER-Golgi t-SNARE associated protein Sly1p (encoded by the *SLY1-20* mutant gene), is a suppressor of Ypt1p defect [46]. This yeast mutant background correspondingly suppressed both growth defect and CPY trafficking defect resulting from α-synuclein expression.

Exactly how α-synuclein expression affects Ypt1 function is however unclear at the moment. The genetic screen is probably most sensitive in detecting factors that are limiting for the ER-Golgi transport step in question. The authors showed that in cells with α-synuclein cytoplasmic inclusions, HA-tagged Ypt1p is localized to these. It is therefore possible that the α-synuclein cytoplasmic inclusions were able to sequester away cytoplasmic Ypt1 from its role in ER-Golgi transport. α-synuclein binds to

a myriad of proteins, and may conceivably bind Ypt1 fortuitously, although that remains to be investigated. Pull-down studies with members of the Rab protein family have shown that α-synuclein from the mutant human A30P trangenic mice interacts with Rab3a, Rab5, and Rab8 [47]. Rab3a has also been shown to bind α-synuclein in human samples of multiple system atrophy [48]. α-synuclein therefore does appear to have a propensity to interact with members of the Rab family.

The fact that Ypt1 is identified as a suppressor, and not Sar1p (which is responsible for COPII coat assembly and vesicle budding) [49] indicate that the process affected by α-synuclein may have more to do with vesicle docking and fusion rather than vesicle budding. An alternative scenario to the hypothesis of Ypt1p being simply sequestered away from its physiological role by α-synuclein, is therefore that the cytoplasmic inclusions formed by α-synuclein may associate with ER-Golgi transport vesicles, and thereby prevent their docking and fusion with the target Golgi membrane. α-synuclein has a known affinity for lipid rafts, and binds to lipid rafts isolated from cultured cells and purified synaptic vesicles [50]. α-synuclein's known association with synapse and synaptic vesicles may be in line with the above notion [51], although in this regard it is not obvious how Ypt1p over-expression and the *SLY1-20* mutation could act as α-synuclein toxicity suppressors. It would in any case be worth checking for any altered localization of SNARE proteins as well as membrane tethering factors [52–53] in the α-synuclein expressing yeast cells. For that matter, it could be interesting and important to check the effect of α-synuclein expression on ER-Golgi transport SNAREs and the tethering factors p115 and GM130 [54] in mammalian cells.

Cooper *et al.* had previously also showed that α-synuclein expression affects endocytosis based on analysis with FM4-64 staining. For unclear reasons, endocytosis is also perturbed in the yeast strain harboring a temperature sensitive *ypt1-3* mutation. α-synuclein expression may therefore have secondary effects on cellular membrane traffic as a result of the initial ER-Golgi transport block, again a possibility worth more elaborate analysis in mammalian cells.

The fact that the SNARE Ykt6p could rescue α-synuclein neurotoxicity is also remarkable. Ykt6p has multiple localizations in yeast cells and was first implicated as an essential gene in yeast ER-Golgi transport [42]. It was subsequently shown to be also an important component for yeast vacuole homotypic fusion [55]. In mammalian neurons, Ykt6 is found in a unique compartment that is distinct from the Golgi [56] and at much higher levels than other cells. This suggests it has a special role in neurons beyond the general biosynthetic Golgi-to-vacuole transport. It would be interesting to see if α-synuclein could interact with Ykt6, or the Ykt6 containing compartment, and whether α-synuclein competes with Ykt6 for its binding partners.

Ypt1/Rab1 rescues α-synuclein-induced dopaminergic neuron loss

The key translation of the discovery made in yeast above lies in its confirmation in PD models involving neurons. The authors tested the ability of Rab1 to rescue α-synuclein-associated dopaminergic neuron loss in three invertebrate and mammalian models of PD. A model of *Drosophila* transgenically expressing α-synuclein [57], with or without co-expression of Rab1, the mouse homologue of Ypt1p, was first examined. Flies were aged and dopaminergic neurons in the dorsomedial cluster were quantified by immunostaining for tyrosine hydroxylase (TH). Rab1 co-expression rescued TH-positive neuron loss in a robust manner, and suppression of α-synuclein toxicity by Rab1 is as good as that of Hsp70, which was the strongest suppressor previously identified using the fly α-synuclein transgenic model [57]. The authors also examined the effect of co-expressing α-synuclein alone or with Rab1 on dopaminergic neurons in a *C. elegans* transgenic model [58]. Again, a strong rescue effect of dopaminergic neuron loss was observed, which is comparable to torsinA, an ER-associated chaperone and the strongest suppressor identified with the worm transgenic system [59]. The authors went on to demonstrate that Rab1 over-expression could protect mammalian neurons against α-synuclein toxicity. Rat midbrain primary neurons in culture were infected with lentivirus expressing α-synuclein (wild type and A53T) with or without Rab1 co-expression, and viability of dopaminergic neurons were quantified by immunostaining for TH and the dendritic marker MAP2. Rab1 co-expression robustly attenuated the neuronal loss caused by α-

synuclein expression alone. It appears then that the finding of Rab1 as a suppressor of α-synuclein toxicity in yeast cells is of physiological (or pathophysiological) relevance to dopaminergic neurons of higher organisms.

The ability of Rab1 to rescue α-synuclein neurotoxcity has not yet been tested in an *in vivo* mammalian model, such as that involving lentiviral mediated expression in the rat substantia nigra [60]. Such experiments may go some distance in telling us if Rab1 or Rab 1 regulator could be potentially useful therapeutic targets.

Dopaminergic neurons are particularly susceptible to α-synuclein expression

A hallmark of neurodegenerative diseases in general is that only a particular subset of neurons is affected in each case. As α-synuclein is rather ubiquitously expressed in the brain, the selective susceptibility of dopaminergic neurons to its toxicity calls for an explanation, which would be important for PD prevention or treatment. α-synuclein interacts with a plethora of molecules, including monoamines. Monoamine metabolism is associated with oxidative stress, and may promote aggregation and neuronal damage. Although dopamine itself may not be toxic at physiologically relevant doses, it has been proposed that other dopamine metabolites may play a role in α-synuclein aggregation [61]. Dopamine-α-synuclein adducts could in fact stabilize α-synuclein protofibrils [62]. It was also recently shown that over-expression of different types of α-synuclein mutants could directly disrupt vesicular pH of chromaffin vesicles and lead to increased cytosolic species of catecholamines [63]. Dopamine synthesized in the cytoplasm is taken up into synaptic vesicles *via* the VMAT2 transporter [64], and are presumably much more stable in these vesicles (where monoamine oxidase is not present). The inhibition of ER-Golgi transport could speculatively result in an inhibition of delivery of VMAT2 (and for that matter other synaptic vesicle components) to the synapses and accumulation of cytosolic dopamine that could be neurotoxic when metabolized. As dopamine helps stabilize protofibril formation by forming a dopamine-α-synuclein adduct, this may well cause a vicious circle where dopamine and α-synuclein enhance each others' toxicity. It should be of interest to examine these possibilities in cultured mammalian neurons.

α-synuclein as a chaperone synaptic SNARE complex assembly

α-synuclein over-expression in dopaminergic neurons is obviously detrimental [59, 65], but its expression in the brain is suggestive of a physiological role at normal levels. α-synuclein knockout mice are viable, fertile and have no gross CNS morphological or neurological abnormalities [66-67]. Sudhof and colleagues [68] showed that although mice with transgenic expression of wild type and mutant *human* α-synuclein developed neurodegeneration with age with partial penetrance, transgenic expression of the *murine* gene does not result in neurodegeneration. Even over-expression of human α-synuclein had been shown to protect against paraquat induced neurodegeneration [69]. α-synuclein delivered by protein transduction using the TAT sequence were also shown to be protective against oxidative stress and other cellular injuries [70].

In both cases above, α-synuclein's protective effect may result from its ability to activate Hsp70, and the structural similarity between α-synuclein and small heat shock proteins has been pointed out [71]. There is therefore an apparent dissociation between α-synuclein deposition and neurodegeneration, and this suggests that its involvement in human neurodegenerative processes may arise not only from a gain of toxic function, but perhaps also from a loss of protective function. Could this be a chaperone function, and what molecular process might α-synuclein be chaperoning?

Sudhof and colleagues have also been studying another synaptic vesicle protein, cysteine string protein alpha (CSPα), which contains a DNA-J domain characteristic of Hsp40 chaperones. CSPα-deficient mice develop an age-dependent, progressive and fatal sensorimotor disorder, with degeneration of neuromuscular junctions and Calyx synapses with death at approximately 2 months of age [72]. CSPα is not essential for the normal operation of Ca^{2+} channels or exocytosis but acts as a presynaptic chaperone that maintains continued synaptic function. Interestingly, the authors showed that transgenic α-synuclein prevents this lethal neurodegeneration of CSPα-deficient

mice and rescues their motor impairment phenotype [68]. Expectedly, the CSPα-deficient phenotype is exacerbated in a background of α/β-synuclein double knockout. α-synuclein does not, however, function as a co-chaperone of CSPα. The two proteins do not interact with each other and α-synuclein does not stimulate Hsc70 activity like CSPα. α-synuclein may therefore not functionally replace CSPα-deficiency, but may rescue the detrimental consequence of the deficiency in a cell autonomous manner. This rescue activity apparently requires the membrane bound form of α-synuclein, and is lost in the A30P mutant which is deficient in phospholipids binding.

What then are the CSPα substrates that α-synuclein might also be acting on? The authors performed quantitative immunoblotting analysis to examine the levels of several brain proteins that might be altered in CSPα-deficient mice. They noticed a rather significant and unique reduction in the synaptic SNARE SNAP-25, which is not even seen in mouse lacking VAMP2 [73], the putative SNARE partner of SNAP-25. Transgenic α-synuclein expression did not reverse this decline of SNAP-25 levels in CSPα-deficient mice, even at 13 months of age, in spite of its apparent ability in keeping these mice alive far beyond the lethal time period of 2–3 months. The authors could not detect direct interaction between the synaptic SNAREs with either CSPα or α-synuclein. However, they found a significant decrease in synaptic SNARE complex assembly (assessed by two different methods) in CSPα-deficient mice, which was reversed by transgenic wild type α-synuclein expression, but not by the A30P mutant. α-synuclein, therefore, may aid synaptic SNARE complex assembly, functioning as an auxiliary molecular chaperone that complements CSPα. The findings of Sudhof and colleagues highlighted a potential dual role of α-synuclein in neurodegeneration, particularly in sporadic late-onset PD. Whichever cellular stress and insults that could cause α-synuclein aggregation could also diminish its physiological chaperone function.

Concluding remarks

The reports discussed above illustrate the emerging pathophysiological interaction of α-synuclein with components of neuronal membrane traffic, and more such interactions may be revealed in time to come. While transgenic analyses in mouse were typically revealing, the usefulness of yeast as a simple genetic system that allows screening of genetic suppressors and enhancers have effectively identified a cellular process that is a direct target of α-synuclein toxicity. The faster growth rate of yeast and easier manipulation in culture would facilitate screening of drugs that might counter α-synuclein toxicity, and such attempts have already identified some flavonoids as potentially useful in this respect [37]. Yeast may well yield basic molecular and cell biological aspects of the α-synuclein story that other approaches have missed.

As it is, studying the effect of α-synuclein over-expression in yeast has revealed an additional molecular mechanism that might result in neuronal death and the onset of PD. Besides oxidative stress and UPS defects, disruption of vesicular transport could therefore contribute to PD onset. It is likely that all three (and perhaps other) pathways are interrelated, and collectively contribute to sporadic PD. A defect in one may well exacerbate or trigger the development of defects in other pathways, culminating in neuronal death or chronic degeneration. For example, defects in the UPS system caused by parkin could further aggravate the ER stress caused by α-synuclein. Although no one particular genetic determinant can be identified for sporadic PD, it is likely that the plethora of environmental and genetic factors work together *via* common mechanisms to generate cellular defects that result in dopaminergic neuronal death. More such mechanisms and their inter-relations would undoubtedly be revealed in the near future.

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