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Review

Probing yeast for insights into neurodegenerative disease: ORFeome-wide screens for genetic modifiers of α-synuclein cytotoxicity

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Several of the most devastating neurodegenerative disorders, including Parkinson's disease and dementia with Lewy bodies, belong to the synucleinopathy class of common neural disorders. A synucleinopathy is characterized by brain tissue plaques formed by the aggregation of misfolded protein—mainly misfolded α-synuclein. α-Synuclein has been extensively studied as the primary protein aggregate in brains afflicted by Parkinson's disease, but the toxic mechanism in which it is involved remains largely enigmatic. Fortunately, a simple but innovative yeast model of synucleinopathy has made possible high-throughput screens for genetic modifiers of α-synuclein toxicity. Deftly interpreted through the use of computational algorithms, these screens could reveal the genetic regulatory networks that underlie synuclein toxicity *in vivo*, and may enable therapeutic strategies to target the genetic root of neurodegeneration.

Key words: Parkinson's disease, α-synuclein, high-throughput screen, synucleinopathy.

INTRODUCTION

Protein misfolding and aggregation, problems typically associated with neurodegenerative diseases such as Creutzfeldt-Jakob disease and bovine spongiform encephalopathy, may also be responsible for the synucleinopathy class of neurodegenerative disorders in humans. One of the most clinically pervasive synucleinopathies is Parkinson's disease (PD). About 1 - 2% of the general population over the age of 65 is thought to be affected by PD (Goedert, 2001). Since its first description by James Parkinson in 1817, PD and related disorders identified as Parkinsonisms have been diagnosed as movement disorders typified by muscle rigidity, resting tremor, and bradykinesia (Goedert, 2001). More recently, PD has been discovered to result from the selective degradation of dopaminergic neurons in the substantia nigra pars compacta, part of the midbrain basal ganglia that is associated with motor control. Importantly, pathological samples from the substantia nigra are studded with dense protein aggregates called Lewy bodies, which upon further analysis were found to be chiefly composed of natively-unfolded α-synuclein protein (Ross and Poirier, 2004)—the hallmark of synucleinopathy. It is believed that α-synuclein misfolding and aggregation are responsible for familial and idiopathic PD early pathological features, which include mitochondrial defects and vesicle mistrafficking.

In a rare case of familial PD, families originating from the village of Contursi Terme in Southwestern Italy contained heritable point mutations in SNCA, the α-synuclein gene locus, with over 90% penetrance. This rare case strengthened the association between α-synuclein and PD, but the most common idiopathic forms of PD are neither familial nor caused by point mutation; rather, unknown genetic and environmental factors are thought to play a role in PD etiology (Goedert, 2001). The neuropathology community is continually eluded by how and why α-synuclein accumulates in diseased brain tissue, and whether it does so inheritably or sporadically. To begin to understand the genetic mechanisms by which α-synuclein confers toxicity, investigators seek to identify genes responsible for producing proteins that enhance or suppress—modify—toxicity *in vivo*.

SCREENING FOR MODIFIERS OF α-SYNUCLEIN CYTOTOXICITY

Conducting a screen for α-synuclein toxicity modifiers is nearly impossible in mammals; mammalian neurons are cumbersome specimens to genetically manipulate, culture, and screen in large quantities. To the surprise of many, the solution to this screening problem came in the form of a tiny organism that lacks neurons. Outeiro and Lindquist (2003) first described a screenable model for α-synuclein toxicity in *Saccharomyces cerevisiae*, a single-celled budding yeast. Despite the enormous evolutionary gap separating yeast from humans, expressing α-synuclein, a distinctly mammalian protein, in *S. cerevisiae* recapitulates many mammalian toxic phenotypes. Outeiro and Lindquist (2003) revealed that toxicity is dependent upon the number of copies, or "dose," of α-synuclein expressed.

Following this discovery, Lindquist and colleagues developed a technique to use the yeast model in screens for genetic modifiers of α-synuclein toxicity. Rapid screening was made possible by expressing α-synuclein in combination with the overexpression of a gene or genes from a yeast open reading frame (ORF) library, then assessing resultant toxicity by a cell survival assay. Genes that increased cell survival when over expressed along with α-synuclein expression were deemed toxicity suppressors, while genes that decreased cell survival when over expressed along with α-synuclein expression were deemed toxicity enhancers (Cooper, 2006). Modifier gene "hits" uncovered by these screens were then verified by over expression in higher organisms such as nematodes, fruit flies, and rats to verify findings from the yeast model in organisms with neurons.

In early screens, Cooper et al. (2006) demonstrated that overexpression of the yeast gene *Ypt1* can rescue yeast from α-synuclein toxicity. When *Rab1*, the mammalian homolog of *Ypt1*, was then over expressed *in vivo* in rat brains, dopaminergic neurons were rescued from degeneration normally induced by α-synuclein expression. Since forward ER-Golgi trafficking genes such as *Rab1* suppressed toxicity and negative traffic regulating genes enhanced toxicity in the yeast model, Cooper et al. (2006) noted that vesicle misregulation may be an early step in the molecular etiology of PD and other synucleinopathies. The early screening experiments have definitively provided evidence that α-synuclein toxicity in yeast depends upon the expression levels of vesicle trafficking genes. It was hypothesized that these results may have implications for the neuron: If dopamine is improperly trafficked to synaptic vesicles, it could remain in the cytosol and oxidize to reactive oxygen species that impair mitochondrial function and precipitate cell death. Dopamine in the cytosol is prone to autooxidation or metabolism by monoamine oxidase, the products of which impair intracellular protein function; in contrast, vesicular dopamine is protected from oxidation (Watabe and Nakaki, 2007). The results of Mosharov et al. (2006) suggest that overexpression of α-synuclein, particularly known pathological mutants of α-synuclein, causes dopamine to leak from its vesicles into the cytosol. Thus, at least when considering the role of α-synuclein, ER-Golgi trafficking in yeast may be analogous to synaptic vesicle trafficking in mammals.

Three years after the screens identified *Ypt1* as a suppressor, Gitler et al. (2009) discovered a strong genetic interaction between α-synuclein and *YPK9*, a yeast ortholog of the PD-linked gene *PARK9*. *YPK9* and *PARK9* rescued yeast cells and dopaminergic neurons, respectively, from α-synuclein toxicity. Furthermore, Gitler et al, (2009) demonstrated that *PARK9* can also protect cells from manganese toxicity. Epidemiological studies have documented an increase in Parkinsonism among miners and welders occupationally exposed to manganese (Jankovic, 2005). Exposure to the manganese-containing pesticide maneb has also been implicated as a causal event in the etiology of PD (Thiruchelvam et al., 2000). The *PARK9* discovery has therefore uncovered what could be the first direct connection between a genetic basis for neurotoxicity and environmental exposure to manganese.

THE SEARCH FOR MECHANISM

Despite the uncertainties surrounding the genetics of synucleinopathy, several explanations for α-synculein toxicity have been proposed. Many agree that unfolded synuclein monomers slowly polymerize to form β-sheet-like oligomeric fibrils. Eventually, these fibrils become large enough to be regarded as Lewy bodies, which are accompanied by a host of toxic phenotypes including vesicle transport blockage and mitochondrial dysfunction (Cookson, 2009). These toxic insults may be coupled with oxidative stress (Dawson and Dawson, 2003), dispersed pigmentation (Halliday et al., 2005), and cell membrane injury (Takeda et al., 2006), finally resulting in the apoptosis of dopamine-producing neurons (Cookson, 2009). Yeast models have also established a potential role for serine phosphorylation in α-synuclein aggregation (Fiske and DebBurman, 2010). Although this cascade of neuronal insults may not follow directly from the formation of Lewy bodies, it is believed that neuronal cell death in synucleinopathies may critically depend upon α-synculein aggregation (Cookson, 2009) and spreading (Desplats et al., 2009), providing evidence for prion-like behavior of α-synuclein. Like the infectious protein particles known as prions, altered α-synculein proteins may bind together to form aggregates that are pathogenic under certain intracellular conditions.

Despite the enormous attention α-synuclein aggregation has received, it remains unknown whether

aggregated fibrils constitute a direct cause of neuro-degeneration (Lansbury and Lashuel, 2006; Cookson, 2009). Instead of drawing a causal link between α-synuclein fibrils and neuronal cell death, it is possible that misfolding and aggregation prevent α-synuclein from performing some essential wild-type role. Chandra et al, (2005) suggested that wild-type α-synuclein compliments the function of cysteine-string protein-α (CSPα), a chaperone protein responsible for the proper folding of SNARE proteins, which are needed for functional release of dopamine. Accordingly, transgenic expression of α-synuclein had been shown to abolish a lethal phenotype produced by deletion of CSPα in mice (Chandra et al., 2005). Scott et al. (2010) reported an absence of processed presynaptic SNARE proteins in synapses overexpressing α-synuclein.

These absences, potentially caused by the loss of WT α-synuclein function due to oligomerization, could be responsible for the observed disruption of endocytosis and vesicle recycling in affected neurons. In contrast, the familial A53T α-synuclein mutant binds to ER-Golgi SNAREs and directly inhibits their functions in vesicle trafficking (Thayanidhi et al., 2010). It had also been suggested that under non-pathological conditions, wild-type α-synuclein negatively regulates mitochondrial complex I activity (Loeb et al., 2010).

In assuming these wild-type functions, it is possible that sequestration of α-synuclein prevents it from properly assisting SNARE protein folding, producing vesicle trafficking impairments, toxic buildup of dopamine in the cytosol, and mitochondrial dysfunction. Thus, it seems likely that loss of normal α-synuclein function and not the proteinaceous fibrils themselves may have a causal role in the precipitation of toxic phenotypes. However, since α-synuclein is not native to yeast, and since the yeast model has nonetheless recapitulated mammalian toxic phenotypes, it is equally possible that α-synuclein itself, and not loss of its normal function, is responsible for cytotoxicity. If loss of wild-type α-synuclein function were required for toxicity, then introducing the mammalian protein into yeast would not produce the expression-dependent cytotoxicity effect observed. This evidence favors an intrinsic role of α-synuclein in conferring cytotoxicity. Yet, what causes α-synuclein to be expressed at a toxic dosage in idiopathic PD remains unknown. It has been hypothesized that loss of methylation at the *SNCA* locus, as observed in the substantia nigra of PD patients, may produce excessive expression of α-synuclein that leads to neuropathology (Jowaed et al., 2010). If the mechanism of α-synuclein toxicity, in part or whole, is indeed the same in yeast and mammals, then comprehensive screens of the yeast ORFeome are expected to supply valuable information on genetic processes that underlie α-synuclein toxicity in both organisms.

Yeast is thus a rather fortuitous model organism with which to study synucleinopathy, according to those engaged in the early toxicity screens. Not only does α-synuclein exert toxic phenotypes in yeast that are similar to those produced in mammals—the yeast genome is well-characterized and readily-manipulated, and many yeast gene interactions have already been documented (Cooper, 2006; Gitler, 2008; Yeger-Lotem et al., 2009). As investigators continue to screen for genes that modify α-synuclein toxicity, a more complete picture of neurodegenerative pathways is emerging. Yet, several challenges remain.

SCREENING CHALLENGES AHEAD

To improve screen methodology and analysis, yeast screening protocols must be optimized with suitable definitions for toxicity enhancement and suppression. Next, to gauge interactions between genes, a new type of screen is needed. This new screen will most likely take advantage of α-synuclein's dose dependence (Cooper et al., 2006). Overexpression of one suppressor gene alone is not enough to rescue a yeast cell from a highly toxic dose of α-synuclein; instead, two suppressor genes that rely on one another must be co-overexpressed in order to overcome high toxicity. Such combinatorial screens with two library genes may reveal protein-protein or protein-gene interactions with net suppressor effects. Conversely, toxicity enhancers can be identified by over-expressing libraries of yeast ORFs along with a low dose of α-synuclein, as a true enhancer is needed for the low dose to confer any toxicity. The Lindquist Laboratory has developed a methodology to screen the yeast ORFeome for genetic enhancers of α-synuclein cytotoxicity. This methodology relies on high-throughput matings between yeast containing integrated human α-synuclein and yeast containing a yeast ORF. Enhancer candidates identified by the high-throughput screen are then validated by further screens that rely on transformation instead of mating. Importantly, orthologs of all validated enhancers (originally yeast ORFs) must be confirmed in higher organisms with neurons.

The subsequent challenge will be piecing together the massive amounts information that these high-throughput screens will uncover. To help ease this data bottleneck, bioinformatics initiatives at the Whitehead Institute have developed a computational algorithm to elucidate genetic pathways that may be involved in the α-synuclein toxicity mechanism. The algorithm, ResponseNet, integrates screen data with data obtained from mRNA profiling to diagram the most likely molecular pathways given sets of screen results (Yeger-Lotem et al., 2009). ResponseNet output may reveal whether α-synuclein truly is a "nodal point" that integrates genetic and environmental information, as suggested by Yeger-Lotem et al. (2009).

As high-throughput screens are slowly unraveling the

fibers in our understanding of the α-synuclein toxicity mechanism, our broader perception of neurodegenerative disease is beginning to shift. In the clinic, PD is often seen as an idiopathic disorder that manifests itself spontaneously with age. As a result of the knowledge garnered from preliminary screens, incidence of PD and related synucleinopathies may be deemed less sporadic than previously thought. Synucleinopathies may result from the interplay of a variety of genetic and environmental nodes built into a network that affects the toxicity of α-synuclein or its aggregates. Luckily, evolution has conserved much of this network from yeast to human, rendering some findings from rapid genetic screens in yeast applicable to human disease. Although these screens may not capture the full range of genetic interactions present in human neural tissue, they may uncover useful clues for understanding, treating, and preventing a large class of neurodegenerative diseases.

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REFERENCES

Chandra S, Gallardo G, Fernández-Chacón R, Schüter OM, Südhof TC (2005). α-Synuclein cooperates with CSPα in preventing neurodegeneration. Cell., 123: 383-396.

Cookson MR (2009). α-Synuclein and neuronal cell death. Mol. Neurodegener. 4.

Cooper AA, Gitler AD, Cashikar A, Haynes CM, Hill KJ, Bhullar B, Liu K, Xu, K, Strathearn KE, Liu F, Cao S, Caldwell, KA, Caldwell GA, Marsischky, G, Kolodner RD, LaBaer J, Rochet JC, Bonini NM, Lindquist S (2006). α-Synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. Science, 313: 324-328.

Dawson TM, Dawson VL (2003). Molecular Pathways of Neurodegeneration in Parkinson's Disease. Sci., 302: 819-822.

Desplats P, Lee, HJ, Bae EJ, Patrick, C, Rockenstein E, Crews, L, Spencer B, Masliah E, Lee SJ (2009). Inclusion formation and neuronal cell death through neuron-to-neuron transmission of α-synuclein. Proc. Natl. Acad. Sci. USA., 106: 13010-13015.

Fiske M, DebBurman S (2010). Contributions of familial mutation E46K, phosphorylation, protein domains, and alanine-76 to α-synuclein toxicity in yeasts. FASEB J., 708.

Gitler AD (2008). Beer and bread to brains and beyond: Can yeast cells teach us about neurodegenerative disease? Neurosignals, 16: 52-62.

Gitler AD, Chesi A, Geddie ML, Strathearn KE, Hamamichi S, Hill KJ, Caldwell KA, Caldwell GA, Cooper AA, Rochet JC, Lindquist S (2009). α-Synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. Nat. Genet., 41: 308-315.

Goedert M (2001). Alpha-synuclein and neurodegenerative diseases. Nat. Rev. Neurosci., 2: 492-501.

Halliday GM, Ophof A, Broe M, Jensen PH, Kettle E, Fedorow H, Cartwright MI, Griffiths FM, Shepherd CE, Double KL (2005). α-Synuclein redistributes to neuromelanin lipid in the substantial nigra early in Parkinson's disease. Brain, 128: 2654-2664.

Jankovic J (2005). Searching for a relationship between manganese and welding in Parkinson's disease. Neurology, 64: 2021-2028.

Jowaed A (2010). Methylation Regulates Alpha-Synuclein Expression and Is Decreased in Parkinson's Disease Patients' Brains. J. Neeurosci., 30: 6355-6359.

Lansbury PT, Lashuel HA (2006). A century-old debate on protein aggregation and neurodegeneration enters the clinic. Nature, 443: 774-779.

Mosharov EV, Staal RGW, Bové J, Prou D, Hananiya A, Markov D, Poulsen N, Larsen KE, Moore CMH, Troyer MD, Edwards RH, Przedborski S, Sulzer D (2006). α-Synuclein Overexpression Increases Cytosolic Catecholamine Concentration. J. Neurosci., 26: 9304-9311.

Outeiro TF, Lindquist S (2003). Yeast cells provide insight into alpha-synuclein biology and pathobiology. Science, 302: 1772-1775.

Ross CA, Poirier MA (2004). Protein aggregation and neurodegenerative disease. Nat. Med., 10: S10-S17.

Scott DA, Tabarean I, Tang Y, Cartier A, Masliah E, Roy S (2010). A Pathologic Cascade Leading to Synaptic Dysfunction in -Synuclein-Induced Neurodegeneration. J. Neurosci., 30: 8083-8095.

Takeda A, Hasegawa T, Matsuzaki-Kobayashi M, Sugeno N, Kikuchi A, Itoyama Y, Furukawa K (2006). Mechanisms of neuronal death in synucleinopathy. J. Biomed. Biotechnol., pp. 1-4.

Thayanidhi N, Helm JR, Nycz DC, Bentley M, Liang Y, Hay JC (2010). α-Synuclein Delays Endoplasmic Reticulum ER-to-Golgi Transport in mammalian Cells by Antagonizing ER/Golgi SNAREs. Mol. Biol. Cell., 21: 1850-1863.

Thiruchelvam M, Richfield EK, Baggs RB, Tank AW, Cory-Slechta DA (2000). The Nigrostriatal Dopaminergic System as a Preferential Target of Repeated Exposures to Combined Paraquat and Maneb: Implications for Parkinson's Disease. J. Neurosci., 20: 9207-9214.

Watabe M, Nakaki T (2007). Mitochondrial Complex I Inhibitor Rotenone-Elicited Dopamine Redistribution from Vesicles to Cytosol in Human Dopaminergic SH-SY5Y Cells. J. Pharmacol. Exp. Ther., 323: 499-507.

Yeger-Lotem E, Riva L, Su LJ, Gitler AD, Cashikar AG, King OD, Auluck PK, Geddie ML, Valastyan JS, Karger DR, Lindquist S, Fraenkel E (2009). Bridging high-throughput genetic and transcriptional data reveals cellular responses to alpha-synuclein toxicity. Nat. Genet., 41: 316-323.