POINT OF VIEW

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Rescuing Defective Vesicular Trafficking Protects against α-Synuclein Toxicity in Cellular and Animal Models of Parkinson's Disease

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ABSTRACT Studies in yeast are providing critical insights into the mechanisms of neurodegeneration in Parkinson's disease (PD). A recent study shows that disruption of vesicular trafficking between the endoplasmic reticulum (ER) and the Golgi, caused by the overexpression and/or aggregation of α-synuclein, is linked to degeneration of dopamine neurons. Overexpression of proteins that are known to enhance ER-to-Golgi transport rescue defective trafficking in yeast, worm, fly, and cellular models of PD.

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The failure of proteins to fold correctly or to remain folded is the primary cause of several systemic and neurodegenerative diseases that affect a significant portion of the world's population (1). To protect against aberrant folding, living organisms have evolved efficient protein synthesis and quality-control machinery. This system relies on close cooperation between the chaperone and the protein-degradation machinery to ensure control over proper folding, targeting, and degrading of proteins.

Protein folding occurs in the cytosol or within the lumen of the endoplasmic reticulum (ER). Proteins that are destined to go through the secretory pathway must pass through a series of quality-control checkpoints to ensure their correct processing and targeting to the extracellular space, the plasma membrane, or their final destination within the cell (Figure 1). The first checkpoint along this pathway resides in the ER, where soluble proteins are translated into the ER lumen and transmembrane proteins are translated and integrated into the ER membrane. Concomitant to translation, a specialized set of chaperones and the quality-control machinery of the ER ensure correct folding. In addition, post-translational modifications such as disulfide bonds or N-linked glycosylations are introduced. Once a protein is properly folded and modified and has passed the quality-control process, it is directed to a specialized ER exit site, where it is integrated into transport vesicles that bud from the ER membrane. These vesicles are anterogradely transported to the Golgi, where they dock and fuse with the membrane of the cis-Golgi (see ref 2 for a detailed description of trafficking at the ER and Golgi apparatus). The major function of the Golgi is to fine-tune the added sugar residues and to sort the different cargo proteins into particular vesicles to be transported along distinguished trafficking pathways. These include routes to the plasma membrane for secretion and insertion of surface proteins or to other intracellular compartments such as the endosomal/lysosomal system. Each transport step also engages in a retrograde transport activity. This is particularly important for transport between the ER and the Golgi in order to recapture components of the vesicle-trafficking machinery and ER resident proteins.

However, this process is not perfect, and many newly synthesized proteins misfold and rapidly degrade. Proteins that fail to fold properly are retrotranslocated at the level of the ER to the cytosol for degradation by the ubiquitin proteasome system (UPS). If the ER folding machinery and the UPS cannot keep up with protein misfolding, the accumulation and/or aggregation of misfolded proteins induces cellular stress by multiple mechanisms. The result is cellular dysfunc-
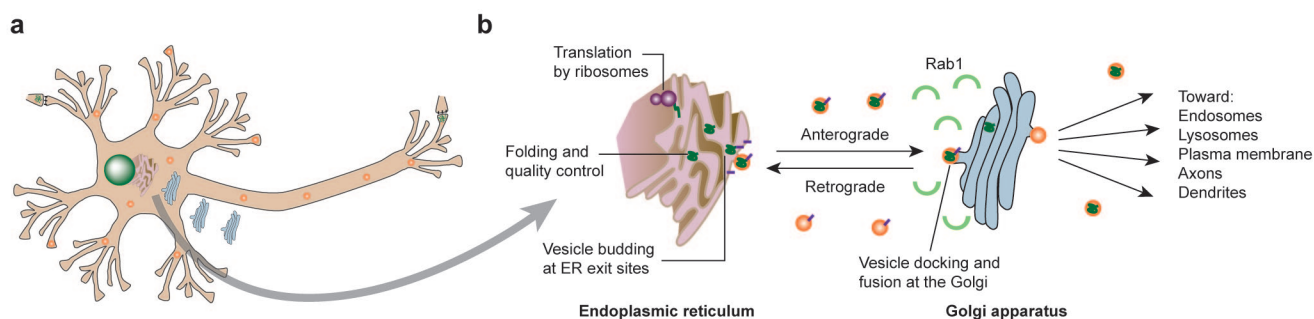
Figure 1. Vesicular trafficking in neurons. a) The extended morphology of neurons showing the cell body, dendrites, axons, and two synapses that are the contact sites with upstream or downstream cells in the network. **b)** Like all eukaryotic cells, neurons possess a set of organelles that form the secretory pathway. Protein synthesis takes place on ribosomes that associate with the ER membrane. Chaperones assist in proper folding of these newly translated polypeptide chains. A complex quality-control machinery that recognizes misfolded proteins allows only correctly folded proteins to reach ER exit sites, where transport vesicles containing the cargo proteins are formed. These vesicles are transported to the cis-side of the Golgi, where they dock and fuse with its membrane. This process is dependent on the small GTPase Rab1. After further protein modification, cargo proteins are packaged into specific vesicles that take on diverse transport routes in the cell.

tion, the initiation of ER-induced apoptosis, and ultimately disease manifestation.

Several misfolding diseases are caused by mutations that result in the loss of protein function due to improper folding, trafficking, and/or enhanced intracellular degradation by the UPS (*e.g.*, cystic fibrosis, sickle-cell anemia, α-1-antitrypsin deficiency, familial hypercholesterolemia, and some forms of cancer) (*1*). If the rate of protein misfolding is faster than that of degradation because of mutations and/or impaired quality-control machinery within the cell, then the misfolded proteins accumulate and self-associate to form highly ordered β-sheet-rich toxic aggregates. The presence of aggregates of misfolded protein in the form of intracellular inclusions or extracellular deposits in the vicinity of dying neurons is a defining hallmark of several neurodegenerative diseases (NDDs), including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis, and polyglutamine and prion diseases (*3*).

Increasing evidence from neuropathologic, genetic, animal modeling, biochemical, and biophysical sources points toward protein misfolding and aggregation as the primary cause of several NDDs. However, the exact mechanisms by which these pro-

cesses cause neurodegeneration and cell death remain a subject of intense investigation and debate. Studies on cellular and animal models of protein-aggregation diseases suggest that the pathogenesis is complicated, and it is likely that neurodegeneration occurs by more than one mechanism. Oxidative stress, membrane disruption, ER stress, altered chaperone activity, impairment of the UPS, mitochondrial deficit, transcriptional dysregulation, axonal transport abnormalities, and Golgi fragmentation are all possible consequences of protein aggregation and are thought to play key roles in the initiation and/or progression of neurodegeneration.

Yeast Sheds Light on Neurodegeneration in PD. The discovery of disease-associated mutations in the genes encoding the aggregating proteins inspired the development of genetic animal and cellular models as tools for understanding the relationship between protein aggregation and disease. The existing genetic models of protein aggregation diseases are all based on the massive overexpression of the gene coding for the wild-type protein or disease-associated mutants in mouse, rat, *Drosophila*, and *Caenorhabditis elegans*. These models recapitulate some features of the disease, but none has

been shown to reproduce the complete disease phenotype observed in humans.

Baker's yeast (*Saccharomyces cerevisiae*), a single-celled organism with <6000 genes, was once thought to be too simple for modeling complex pathologies of the nervous system, but it is now emerging as a powerful tool for modeling NDDs. Despite significant differences between yeast cells and neurons, many of the basic cellular processes, such as protein folding and the quality-control machinery, are conserved in both eukaryotic cells. In a recent study published in *Science* Express, Antony Cooper (University of Missouri, Kansas City), Susan Lindquist's team (Massachusetts Institute of Technology), and colleagues from several other research groups (*4*) took advantage of these similarities by using a yeast model to elucidate a new molecular mechanism underlying the pathogenesis of PD. This neurodegenerative movement disorder is characterized by the loss of dopamine (DA) neurons from the *substantia nigra* (SN) and the formation of intraneuronal proteinaceous inclusions, referred to as Lewy bodies (LBs).

α-Synuclein Aggregation and Defective Trafficking. Lindquist and colleagues (*4, 5*) created a yeast model of PD based on

Colocalization of Ypt1p with α-synuclein suggests that α-synuclein toxicity may involve the sequestration of proteins that play a critical role in the ER-to-Golgi transport.

increased expression of the presynaptic protein α-synuclein, the primary constituent of LBs. Expression of mutant α-synuclein or the wild-type protein is sufficient to cause familial PD (6–9). α-Synuclein aggregation and fibrillogenesis are also implicated in the pathogenesis of several NDDs, including AD, multiple-system atrophy, dementia with LBs, Down syndrome, and neurodegeneration with brain iron accumulation, collectively referred to as synucleinopathies (10).

In the yeast system, the expression of α-synuclein (wild-type or disease-associated mutant A53T) can be tightly experimentally regulated and its effects monitored in real time; thus, detection of the early events involved in α-synuclein toxicity is possible (4). Within the first 4–8 h, the presence of α-synuclein aggregates and increased ER stress were observed to coincide with growth arrest and loss of cell viability. ER and proteasome-specific substrates were used to show that the expression of α-synuclein does not affect the general proteasome activity. However, it significantly impairs the turnover of substrates for which degradation requires trafficking from the ER to the Golgi as well as the transport of proteins that traffic through this pathway. Detailed dissection of the early events occurring during the first 4 h demonstrated that the first detectable defects in cell growth coincide with the impairment of vesicular transport from the ER to the Golgi and occur before the induction of ER stress.

Rescuing Defective Vesicular Trafficking. Next, Lindquist and colleagues performed a complementation screen for modifiers of α-synuclein toxicity, which identified 34 genes that suppressed α-synuclein toxicity and 20 genes that increased it. Many suppressor genes that were specific for α-synuclein toxicity encode proteins that are also involved in ER-to-Golgi transport. If toxicity from increased α-synuclein levels and/or aggregation occurs through disruption of the ER-to-Golgi transport machinery, then promoting the forward transport from the ER to
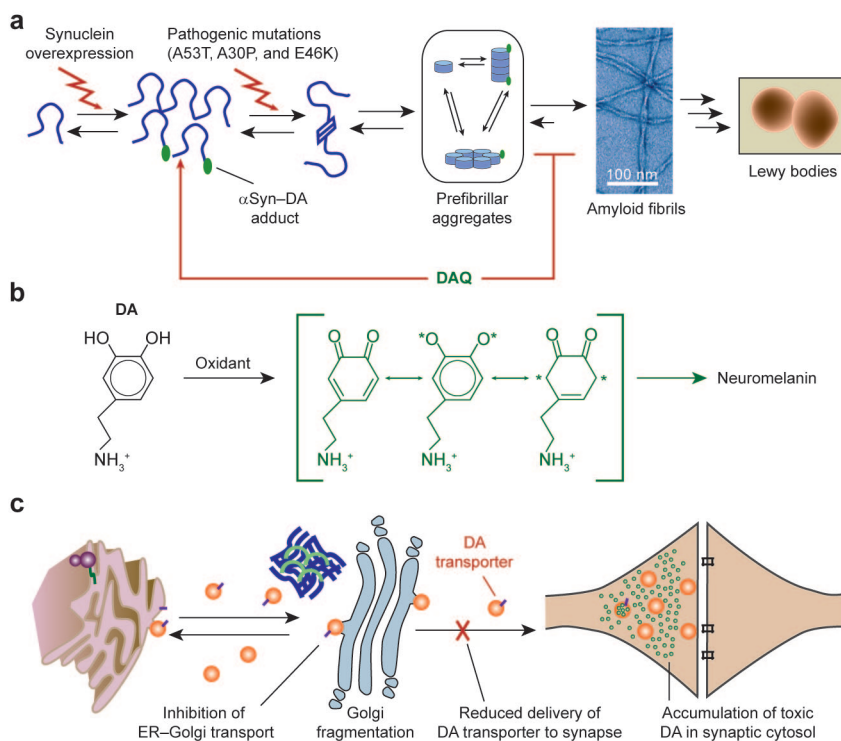
Figure 2. Potential toxic mechanisms linking protein aggregation, defective trafficking, and selective degeneration of DA neurons in PD. a) Schematic depiction of the current understanding of the aggregation pathway of α-synuclein based on *in vitro* biophysical studies. **b)** DA oxidation results in the production of reactive oxygen species and quinone and semiquinone intermediates, all of which are highly cytotoxic. To protect against the toxic properties of DA metabolites, the majority of DA is stored in vesicles before its release. **c)** The study by Cooper *et al.* shows that α-synuclein aggregates interfere with a Rab1-dependent step of ER-to-Golgi transport. This could lead to fragmentation of the Golgi due to an imbalance of incoming and outgoing vesicles, reduced targeting of DA transporters to synaptic vesicles in the presynaptic terminal, and, as a consequence, reduced uptake of DA into vesicles and an accumulation of DA in the cytosol. Formation of toxic DAQ intermediates has been shown to covalently modify α-synuclein and enhance α-synuclein toxicity through the kinetic stabilization of toxic prefibrillar aggregates.

the Golgi should reverse α-synuclein toxicity. Indeed, this was the case. This was particularly striking with Ypt1p and Rab1 (the human homologue of Ypt1p). In yeast, *Drosophila*, *C. elegans*, and rat DA neurons, the overexpression of Ypt1p/Rab1 resulted in significant reduction of α-synuclein-induced neurodegeneration.

The small GTPases of the Rab family play essential roles in vesicle docking and fusion (Figure 2, panel c) (11). Rab1 has been

shown to be specifically involved in ER-to-Golgi trafficking and the docking of ER-derived transport vesicles at the Golgi membrane. This suggests that the transport step affected by α-synuclein toxicity is vesicle docking/fusion at the Golgi membrane. The colocalization of Ypt1p with α-synuclein in cytosolic inclusion suggests that α-synuclein toxicity may involve the sequestration of proteins that play a critical role in the ER-to-Golgi transport and, eventually, the

disruption of the secretory pathway. Further biochemical and biophysical characterization of the α-synuclein aggregates could provide important insights into the mechanism of α-synuclein toxicity in yeast. Whether α-synuclein has a physiological role in ER-to-Golgi trafficking or transport of synaptic vesicles remains to be determined.

α-Synuclein-Induced Disruption of ER-to-Golgi Transport. Given the essential role of the Golgi apparatus in the processing and targeting of proteins through the secretory pathway, any disruption at this level is likely to have detrimental consequences for the function of the cell. Furthermore, a delicate balance between anterograde and retrograde membrane traffic through the Golgi is critical to avoid its fragmentation. A link between α-synuclein aggregation and Golgi fragmentation is supported by previous findings demonstrating that formation of α-synuclein aggregates, particularly prefibrillar aggregates that precede LB formation (Figure 2, panel a), causes fragmentation of the Golgi in cellular models of synucleinopathies (12) as well as in nigral neurons of PD patients (13). Further studies are required to elucidate the exact mechanisms by which α-synuclein aggregation disrupts ER-to-Golgi transport and cause Golgi fragmentation.

Defective Vesicular Trafficking and DA Neurons. Although α-synuclein is an abundant protein in different parts of the brain (up to 1% of total proteins), α-synuclein aggregation in PD occurs primarily in DA neurons of the SN. The wide distribution of α-synuclein in the brain suggests that α-synuclein on its own cannot explain the selective degeneration of DA neurons in PD.

Overexpression of human α-synuclein in transgenic flies (14) or specifically in the SN of rats (15) and primates (16) results in selective DA neuronal death and in the formation of α-synuclein-containing inclusions. Overexpression of PD-linked α-synuclein mutations in human mesencephalic cell lines leads to an impaired storage and secretion of DA, causing an increase in cytosolic DA and

enhanced oxidative stress (17, 18). Xu et al. (19) reported that blocking DA synthesis in cultured DA neurons prevents α-synuclein toxicity, consistent with toxicity being mediated by interactions between the two molecules. These observations are consistent with the known hypersensitivity of DA neurons that express high levels of cytoplasmic DA (e.g., the SN rather than the ventral tegmental area) to cell death in PD.

The selective vulnerability of DA neurons of the SN to α-synuclein toxicity in PD may be related to the toxicity and increased concentration of cytoplasmic DA in these cells; this suggests that improper packaging, secretion, and/or oxidation of DA might explain the selective degeneration of DA neurons (17, 18). DA oxidation and formation of DA orthoquinone (DAQ) *in vitro* covalently modifies α-synuclein and results in kinetic stabilization of toxic α-synuclein aggregates (20). Therefore, the simultaneous contribution of several factors, including α-synuclein oligomerization, DA metabolism, and oxidative stress, might be required for selective degeneration of DA in the SN of PD brains. Disruption of vesicular trafficking is likely to hasten cell death by simultaneously increasing the levels of cytosolic DA and enhancing α-synuclein aggregation.

The findings by Cooper and colleagues offer new insight into the mechanisms by which α-synuclein overexpression and/or aggregation interferes with normal function and viability of neurons and reveal new targets for therapeutic intervention in PD and related synucleinopathies. In addition to being a good model with which to study genetic diseases, the yeast system is also demonstrated by these researchers to offer an excellent platform on which to screen for druglike molecules as modifiers of α-synuclein function(s), aggregation, and toxicity. The identification of specific pharmacological agents that suppress α-synuclein-induced ER-to-Golgi trafficking defects and prevent or reverse neurodegeneration in mouse models of PD as well as in clinical studies is the ulti-

mate proof of the therapeutic potential of these findings.

REFERENCES

1. Cohen, F. E., and Kelly, J. W. (2003) Therapeutic approaches to protein-misfolding diseases, *Nature 426,* 905–909.
2. Lee, M. C., Miller, E. A., Goldberg, J., Orci, L., and Schekman, R. (2004) Bi-directional protein transport between the ER and Golgi, *Annu. Rev. Cell Dev. Biol. 20,* 87–123.
3. Ross, C. A., and Poirier, M. A. (2005) Opinion: What is the role of protein aggregation in neurodegeneration? *Nat. Rev. Mol. Cell Biol. 6,* 891–898.
4. Cooper, A. A., Gitler, A. D., Cashikar, A., Haynes, C. M., Hill, K. J., Bhullar, B., Liu, K., Xu, K., Strathearn, K. E., Liu, F., Cao, S., Caldwell, K. A., Caldwell, G. A., Marsischky, G., Kolodner, R. D., Labaer, J., Rochet, J. C., Bonini, N. M., and Lindquist, S. (2006) Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models, *Science 313,* 324–328.
5. Outeiro, T. F., and Lindquist, S. (2003) Yeast cells provide insight into alpha-synuclein biology and pathobiology, *Science 302,* 1772–1775.
6. Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E. S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., Di Iorio, G., Golbe, L. I., and Nussbaum, R. L. (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease, *Science 276,* 2045–2047.
7. Kruger, R., Kuhn, W., Muller, T., Woitalla, D., Graeber, M., Kosel, S., Przuntek, H., Epplen, J. T., Schols, L., and Riess, O. (1998) Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease, *Nat. Genet. 18,* 106–108.
8. Singleton, A. B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R., Lincoln, S., Crawley, A., Hanson, M., Maraganore, D., Adler, C., Cookson, M. R., Muenter, M., Baptista, M., Miller, D., Blancato, J., Hardy, J., and Gwinn-Hardy, K. (2003) Alpha-synuclein locus triplication causes Parkinson's disease, *Science 302,* 841
9. Zarranz, J. J., Alegre, J., Gomez-Esteban, J. C., Lezcano, E., Ros, R., Ampuero, I., Vidal, L., Hoenicka, J., Rodriguez, O., Atares, B., Llorens, V., Tortosa, E. G., Del Ser, T., Munoz, D. G., and De Yebenes, J. G. (2004) The new mutation, E46K, of alpha-synuclein causes parkinson and Lewy body dementia, *Ann. Neurol. 55,* 164–173.
10. Trojanowski, J. Q., and Lee, V. M. (2003) Parkinson's disease and related alpha-synucleinopathies are brain amyloidoses, *Ann. N.Y. Acad. Sci. 991,* 107–110.
11. Zerial, M., and McBride, H. (2001) Rab proteins as membrane organizers, *Nat. Rev. Mol. Cell Biol. 2,* 107–117.

12. Gosavi, N., Lee, H. J., Lee, J. S., Patel, S., and Lee, S. J. (2002) Golgi fragmentation occurs in the cells with prefibrillar alpha-synuclein aggregates and precedes the formation of fibrillar inclusion, *J. Biol. Chem.* *277,* 48984–48992.

13. Fujita, Y., Ohama, E., Takatama, M., Al-Sarraj, S., and Okamoto, K. (2006) Fragmentation of Golgi apparatus of nigral neurons with alpha-synuclein-positive inclusions in patients with Parkinson's disease, *Acta Neuropathol.* DOI: 10.1007/s00401-006-0114-4.

14. Feany, M. B., and Bender, W. W. (2000) A Drosophila model of Parkinson's disease. *Nature 404,* 394–398.

15. Lo Bianco, C., Ridet, J. L., Schneider, B. L., Deglon, N., and Aebischer, P. (2002) Alpha-synucleinopathy and selective dopaminergic neuron loss in a rat lentiviral-based model of Parkinson's disease, *Proc. Natl. Acad. Sci. U.S.A. 99,* 10813–10818.

16. Kirik, D., Annett, L. E., Burger, C., Muzyczka, N., Mandel, R. J., and Bjorklund, A. (2003) Nigrostriatal alpha-synucleinopathy induced by viral vector-mediated overexpression of human alpha-synuclein: a new primate model of Parkinson's disease, *Proc. Natl. Acad. Sci. U.S.A. 100,* 2884–2889.

17. Lotharius, J., and Brundin, P. (2002) Pathogenesis of Parkinson's disease: dopamine, vesicles and alpha-synuclein, *Nat. Rev. Neurosci. 3,* 932–942.

18. Lotharius, J., and Brundin, P. (2002) Impaired dopamine storage resulting from alpha-synuclein mutations may contribute to the pathogenesis of Parkinson's disease, *Hum. Mol. Genet. 11,* 2395–2407.

19. Xu, J., Kao, S. Y., Lee, F. J., Song, W., Jin, L. W., and Yankner, B. A. (2002) Dopamine-dependent neurotoxicity of alpha-synuclein: a mechanism for selective neurodegeneration in Parkinson disease, *Nat. Med. 8,* 600–606.

20. Conway, K. A., Rochet, J. C., Bieganski, R. M., and Lansbury, P. T., Jr. (2001) Kinetic stabilization of the alpha-synuclein protofibril by a dopamine-alpha-synuclein adduct, *Science 294,* 1346–1349.