

## $\alpha$ -Synuclein, and the Case of the Blocked ER-Golgi Pathway

**Michael White\***

Department of Biology  
Lake Forest College  
Lake Forest, IL 60045

### Summary

**Parkinson's disease has long been associated with Lewy Bodies composed of the protein  $\alpha$ -synuclein. A groundbreaking new study has demonstrated the pathological function of  $\alpha$ -synuclein may be impairment of ER-Golgi traffic.**

### Introduction

Parkinson's disease (PD) is a fatal neurodegenerative disorder of the brain. It affects 1 in 100 individuals over the age of 60 of which 5-10% of cases occur in individuals under 40, and another ~5-10% are familial (NPF, 2006). PD is the result of neuronal atrophy within the substantia nigra located in the brain stem. The substantia nigra is part of a complex circuit called the basal ganglia. It is responsible for the initiation of movement (Purves et al., 2004). The hallmark feature of PD is neurofibrillary inclusions, Lewy Bodies, composed primarily of the protein  $\alpha$ -synuclein ( $\alpha$ Syn; Spillantini et al., 1998). Familial forms of PD have been linked to the  $\alpha$ Syn mutations A30P (Krueger et al., 1998), A53T (Polymeropoulos et al., 1997), and recently E46K (Zarranz et al., 2004). However, the reason these cells are dying in PD patients remains unknown even after more than a decade of heavily funded research!

$\alpha$ Syn's pathological component has often been associated with its role in Lewy Bodies. One widely accepted hypothesis is that  $\alpha$ Syn is pathological when in a protofibrillar form that occurs between monomeric  $\alpha$ Syn disappearance and Lewy Body appearance (Lansbury et al., 2003). However, a remarkable new manuscript, " $\alpha$ -Synuclein Blocks ER-Golgi Traffic and Rab1 Rescues Neuron Loss in Parkinson's Models", by Lindquist et al. (2006) has demonstrated that the pathogenicity of  $\alpha$ Syn may be due to the impairment of ER-Golgi traffic, resulting in a halt of critical cellular secretory processes.

Prior to their research, little was known about  $\alpha$ -Syn's relationship with the ER-Golgi pathway. However,  $\alpha$ Syn expression led to the fragmentation of the Golgi apparatus (Fujita et al., 2006 and Gosavi et al., 2002). Notably, Gosavi et al. (2002) found Golgi fragmentation to occur before Lewy Body formation but after the disappearance of monomeric  $\alpha$ Syn. Contrary to the  $\alpha$ Syn-Golgi interaction, Lee et al. (2005) revealed  $\alpha$ Syn to be excreted from the cell via a vesicular, ER-Golgi independent, exocytotic pathway. Thus, debate exists over which pathway  $\alpha$ Syn is involved in.

### The Case of the Blocked ER-Golgi Pathway

In the recent Lindquist et al. (2006) study, they wanted to determine the effect of  $\alpha$ Syn on the ER-Golgi

pathway. In order to accomplish this task, they took two approaches; one genetic and the other cellular. Together these different pathways would converge to implicate  $\alpha$ Syn in the blocking of ER-Golgi traffic and cell death.

$\alpha$ Syn was expressed in yeast and regulated with a galactose inducible promoter. After  $\alpha$ Syn expression, ER stress was measured and found to be increased for cells expressing  $\alpha$ Syn-WT and further increased for the familial mutant  $\alpha$ Syn-A53T. Lindquist et al. (2006) hypothesized that  $\alpha$ Syn was causing ER stress by blocking the function of endoplasmic reticulum associated degradation (ERAD). As misfolded proteins accumulate in the ER, the ERAD process functions by retrotranslocating them back into the cytoplasm for proteasomal degradation (McCracken and Boodsy 2006). They found that out of two commonly misfolded proteins in the ER, CPY and Sec61-2p (both ERAD substrates), the rate of CPY degradation decreased even though proteasomal function was unaltered. Interestingly, Caldwell et al. (2001) demonstrated that ERAD degradation of CPY required transport through the Golgi.

Because the failure of the ERAD translocation through the ER to the Golgi during  $\alpha$ Syn expression may be an indicator of general pathway blockage, Lindquist et al. (2006) hypothesized that  $\alpha$ Syn may be blocking ER-Golgi traffic. To determine if this was the case, they followed two proteins, CPY and ALP, through the ER-Golgi circuit when  $\alpha$ Syn was expressed. Within three hours, ER-Golgi traffic was greatly reduced and at four hours nearly nonexistent. Simultaneously, cell growth inhibition also occurred. Thus,  $\alpha$ Syn blocks ER-Golgi traffic (2006).

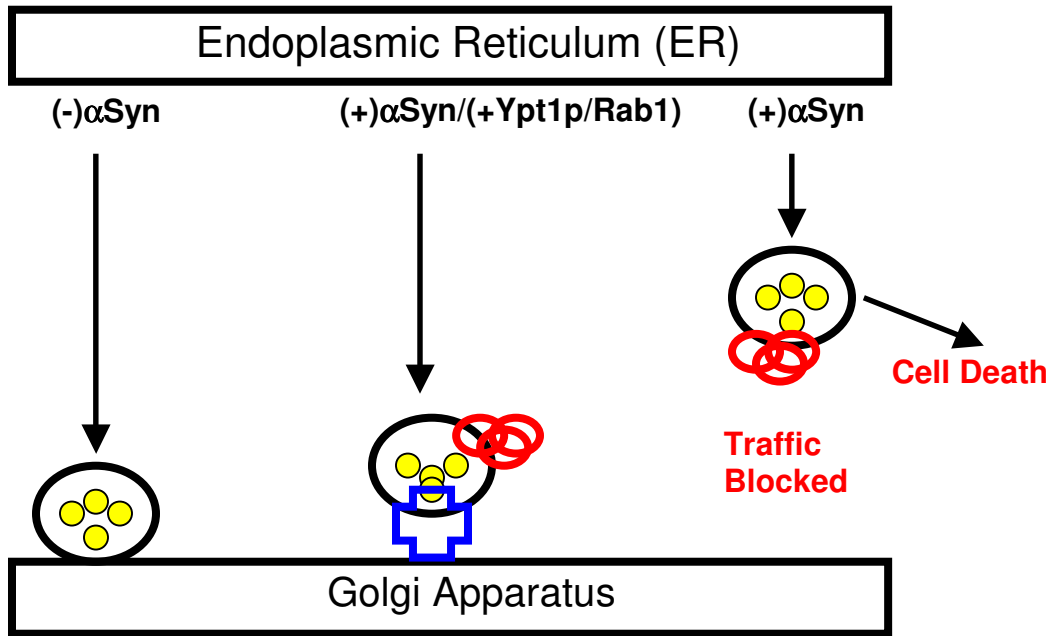
Following their cellular approach, Lindquist et al. (2006) initiated a genetics approach aimed at determining if genes that enhance ER-Golgi transport could reduce  $\alpha$ Syn's ability to block the pathway. They identified the yeast protein Ypt1p as a promoter of traffic, and Gyp8P as a suppresser of traffic. This finding led Lindquist et al. (2006) to hypothesize that over-expression of the Ypt1p (yeast) or Rab1 (mammalian) in a variety of models would rescue them from  $\alpha$ Syn toxicity.

This final study yielded profound results that provided the strongest evidence, yet, that  $\alpha$ Syn's impairment of ER-Golgi traffic was the source for toxicity. They overexpressed Rab1 along with  $\alpha$ Syn in *Drosophila melanogaster* (fruit fly), *C. elegans* (worm), and mammalian dopaminergic neurons to determine if Rab1 would prevent  $\alpha$ Syn toxicity by enhancing ER-Golgi traffic. In all three models, the cells were rescued from death when overexpressing Rab1.

As a result of Ypt1p/Rab1 re-establishing ER-Golgi traffic, it was hypothesized that  $\alpha$ Syn interacted at the ER-Golgi junction. This was based on two lines of evidence; 1) CSP requires transport into the Golgi to be degraded and 2) Ypt1p/Rab1 functions within the ER-Golgi vesicular binding pathway. Therefore, when Ypt1P/Rab1 is over-expressed, vesicular binding efficiency increases.

Returning to the Gosavi et al. (2002) and Lee et al. (2005) manuscripts, the Lindquist et al. (2006) data provides two established lines of evidence (i.e. detailed previously) supporting the Gosavi et al. (2002)

\*This paper was written for BIOL 493 Independent Study taught by Dr. Shubhik K. DebBurman.



**Figure 1: Rab1 enhances ER-Golgi vesicular binding affinity.** This diagram portrays the ER-Golgi junction and the vesicular transport that occurs between the two organelles. (+) indicates the presence of the indicated protein and (-) indicates its absence. The black circles represent a vesicle full of cargo (ex. CPY), the red circles αSyn, and the blue cross Ypt1p/Rab1. αSyn blocks ER-Golgi traffic and leads to cell death (far right). Overexpression of Ypt1p/Rab1 rescues cells from atrophy by increasing the affinity of the vesicle for the Golgi (middle). Vesicular transport without αSyn is shown on the left.

conclusion that αSyn interacts with the ER-Golgi to yield toxicity. Though αSyn is continuously being secreted through a Golgi-ER independent pathway (Lee et al., 2005), it is plausible that a defect in this excretory system may function to exacerbate toxicity, but not produce it.

#### Future Research

The Lindquist et al. (2006) manuscript has provided the Parkinson's disease community with what appears to be an opened door, leading to a whole new frontier in PD research and understanding. As with the relentless pursuit of the protofibrillar discovery by Dr. Lansbury, all methods of research must be exhausted on finding the mechanism by which αSyn is able to turn off the ER-Golgi pathway. It is feasible that the same lentivirus used by Lindquist et al. (2006) to carry the Rab1 gene into mammalian neurons in their experiments could be re-configured to enter the cells of PD patients and re-establish traffic between the ER and Golgi. If this is, in fact, the reason these cells are dying, one of the most prevalent and debilitating neurodegenerative diseases could be cured.

*Note: Eukaryon is published by students at Lake Forest College, who are solely responsible for its content. The views expressed in Eukaryon do not necessarily reflect those of the College. Articles published within Eukaryon should not be cited in bibliographies. Material contained herein should be treated as personal communication and should be cited as such only with the consent of the author.*

#### References

- Caldwell, Sabrina R., Hill, Kathryn J., and Cooper, Antony A., Degradation of Endoplasmic Reticulum (ER) Quality Control Substrates Requires Transport between the ER and Golgi, *Journal of Biological Chemistry*, volume 276, no. 26, pages 23296-23303, 2001.
- Fujita, Tukiyo et al., Fragmentation of Golgi apparatus of nigral neurons with α-synuclein-positive inclusions in patients with Parkinson's disease, *Acta Neuropathol*, volume 112, pages 261-265, 2006.
- Gosavi, Nirmal et al., Golgi Fragmentation Occurs in the Cells with Prefibrillar α-Synuclein Aggregates and Precedes the Formation of Fibrillar Inclusion, *Journal of Biological Chemistry*, volume 277, no. 50, pages 48984-48992, 2002.
- Lansbury, Peter Jr., and Volles, Michael J., Zeroing in on the Pathogenic Form of α-Synuclein and Its Mechanism of Neurotoxicity in Parkinson's Disease, *Biochemistry*, volume 42, no. 26, pages 7871-7878, 2003.
- Lee, He-Jin et al., Intravesicular Localization and Exocytosis of α-Synuclein and its Aggregates, *Journal of Neuroscience*, volume 25, issue 25, pages 6016-6024, 2005.
- Lindquist, Susan et al., α-Synuclein Blocks ER-Golgi Traffic and Rab1 Rescues Neuron Loss in Parkinson's Models, *Science*, volume 313, issue 5785, pages 324-328, Epub 2006.
- McCracken, A., and Brodsky, J., Recognition and Delivery of ERAD Substrates to the Proteasome and Alternative Paths for Cell Survival, *CTMI*, volume 300, pages 17-40, 2006.
- PD Statistics Provided by the National Parkinson Foundation. Retrieved on 2 September 2006 from <http://www.parkinson.org/site/pp.asp?c=9dJFJLPwB&b=71354>.
- Purves, Dale et al., *Neuroscience* 3<sup>rd</sup> Edition, Sinauer Associates Inc., Maryland, 2004.

Smith, Wanli et al., Endoplasmic reticulum stress and mitochondrial cell death pathways mediate A53T mutant alpha-synuclein-induced toxicity, *Human Molecular Genetics*, volume 14, no. 24, pages 3801-3811, 2005.

Spillantini, Maria G. et al.,  $\alpha$ -Synuclein in filamentous inclusions of Lewy Bodies from Parkinson's disease and dementia with Lewy Bodies, *PNAS*, volume 95, pages 6469-6473, 1998