Parkinson's disease and alpha-synuclein:

on the hunt for novel therapeutics

Professor Stephan Witt is interested in the role that the protein, alpha-synuclein, plays in the development of Parkinson's disease. Together with his team at LSU Health Shreveport, USA, he has used yeast and worm models of Parkinson's disease to investigate the mechanisms and molecules that are involved in the development of disease. The hope is that with increased knowledge of how Parkinson's is caused, it will open the door to possible new therapies for patients.

Parkinson's disease affects 1-2% of the population over 65 years of age and is the most commonly occurring movement disorder. The disease is caused by selective degeneration of dopaminergic neurons in a region of the mid-brain called the substantia nigra. Although their numbers are few, these dopamine-producing neurons play an important role in the control of multiple brain functions, including voluntary movement, as well as a broad array of behavioural processes such as mood, reward, addiction, and stress. Loss of these neurons can lead to slow movement, rigidity and unstable posture. The affected neurons often show aggregates called Lewy bodies, whose main component is the protein alpha-synuclein. Although highly expressed in the brain, alpha-synuclein is also present in red blood cells, intestinal cells, liver cells and melanocytes, the cells that produce melanin.

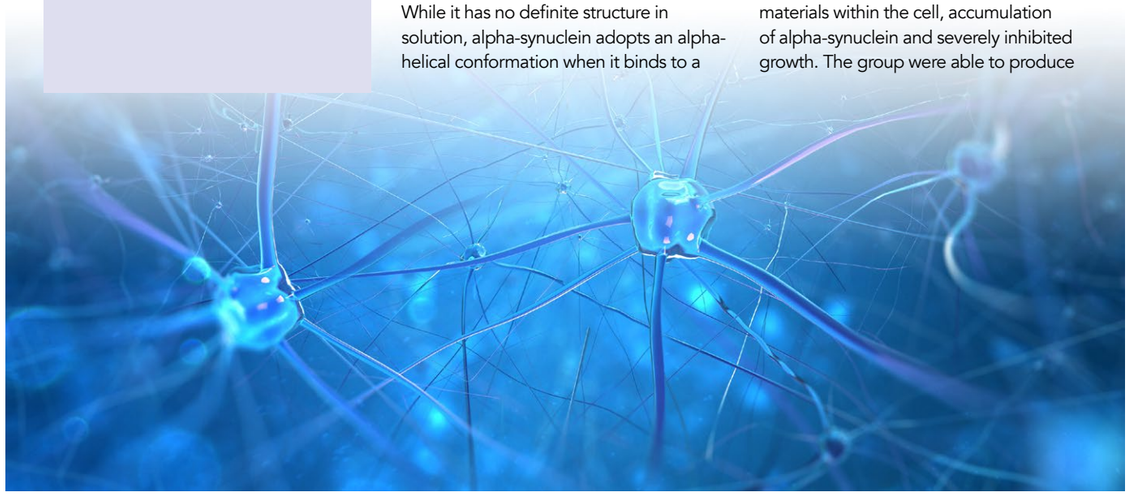
ALPHA-SYNUCLEIN AND PARKINSON'S DISEASE

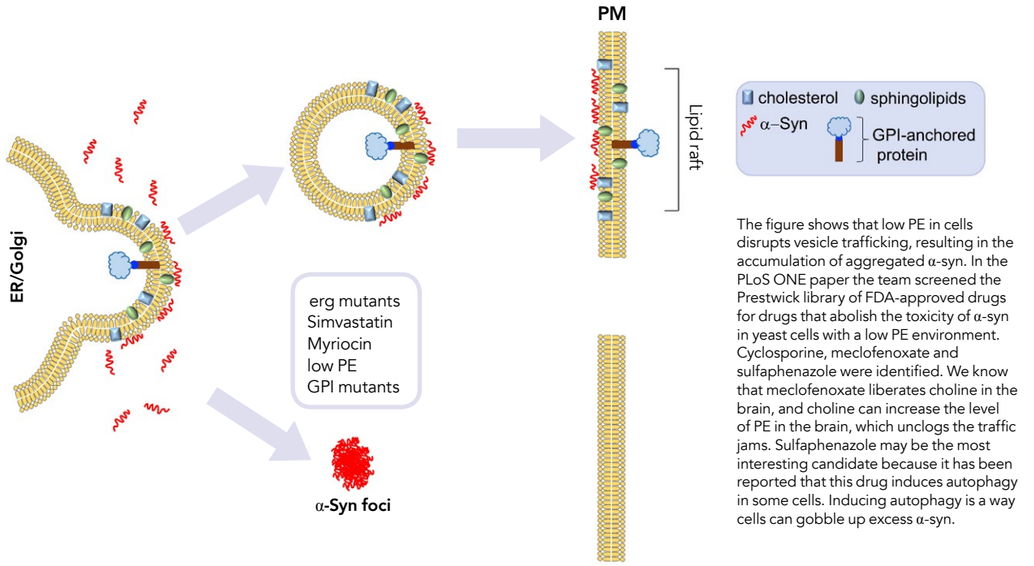
While it has no definite structure in solution, alpha-synuclein adopts an alpha-helical conformation when it binds to a membrane. If alpha-synuclein builds up in cells, then it may self-associate into a fibre-like structure. It is thought that, with age, alpha-synuclein slowly aggregates and forms inclusions in neurons.

FROM YEAST TO WORMS

Professor Stephan Witt and his team have been studying the roles of two such compounds, called phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in modulating the solubility of alpha-synuclein. One source of PE in cells is an enzyme called phosphatidylserine decarboxylase (PSD). Other sources of PE include enzymes in the cytoplasm, the fluid that fills a cell, and in the endoplasmic reticulum (ER), which acts as the manufacturing and packaging component of the cell.

The group at LSU Health Shreveport discovered a 60% reduction in PE in yeast cells when they deleted the gene for PSD. They also found that this resulted in ER stress, defects in trafficking of materials within the cell, accumulation of alpha-synuclein and severely inhibited growth. The group were able to produce





The figure shows that low PE in cells disrupts vesicle trafficking, resulting in the accumulation of aggregated α-syn. In the PLoS ONE paper the team screened the Prestwick library of FDA-approved drugs for drugs that abolish the toxicity of α-syn in yeast cells with a low PE environment. Cyclosporine, meclofenoxate and sulfaphenazole were identified. We know that meclofenoxate liberates choline in the brain, and choline can increase the level of PE in the brain, which unclogs the traffic jams. Sulfaphenazole may be the most interesting candidate because it has been reported that this drug induces autophagy in some cells. Inducing autophagy is a way cells can gobble up excess α-syn.

similar results using a worm model, *C. elegans*. These worms express human alpha-synuclein in their neurons, so they make a good model for Parkinson's disease in humans. Both experiments showed that moderate levels of human alpha-synuclein and low levels of PE were toxic. Interestingly, moderate levels of alpha-synuclein or low PE levels have little or no toxicity on their own; it is only the combination of the two which is extremely toxic.

Professor Witt also investigated whether it is possible to reduce this level of toxicity and found that administration of a single compound called ethanolamine could rescue the toxicity of low PE and alpha-synuclein. Ethanolamine does this by stimulating the synthesis of PE through a particular enzymatic pathway. Overall, they concluded that low PE in cells causes traffic jams of aggregated alpha-synuclein on the internal highways of the cell and that these traffic jams ultimately kill the cell.

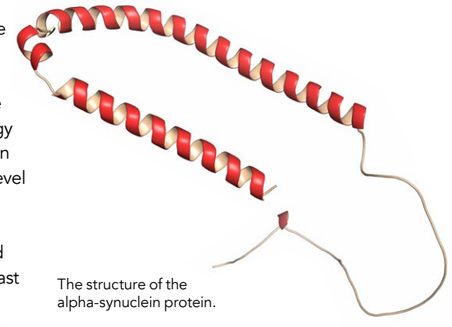
RE-DEFINING EXISTING DRUGS

Leading on from this, Professor Witt's team went on to screen the Prestwick library of Food and Drug Administration (FDA) approved drugs for drugs which would abolish the toxicity of alpha-synuclein in yeast cells with low PE levels. They chose drugs based on reports that they were able to rescue the slow growth phenotype of yeast cells without the gene for PSD, and which expressed human alpha-synuclein. Their work screened 1121 drugs and identified three possible candidates; cyclosporine (CsA), which is a powerful immunosuppressant, meclofenoxate (MFX) and sulfaphenazole (SUL), which is an antibiotic. Previous studies suggest that these drugs work in different ways. Cyclosporine is thought to prevent alpha-synuclein induced damage to mitochondria, the energy-producing powerhouse of the cell. Meclofenoxate hydrolyses into choline, which can increase the level of PE in cells and unclog the traffic jams. Sulfaphenazole has been reported to induce autophagy in cells, a process by which the cells can self-destruct and safely decrease the level of alpha-synuclein.

Firstly, the drug candidates were tested for their ability to inhibit ER stress in yeast cells, a phenomenon which may also be associated with an increase in alpha-synuclein aggregation and increased cell death. The yeast cells didn't contain the gene for PSD, and therefore produced about 50% of the basal level of PE. Whilst SUL decreased ER stress to the greatest extent compared to the control, CsA decreased ER stress to a lesser extent and MFX slightly increased it.

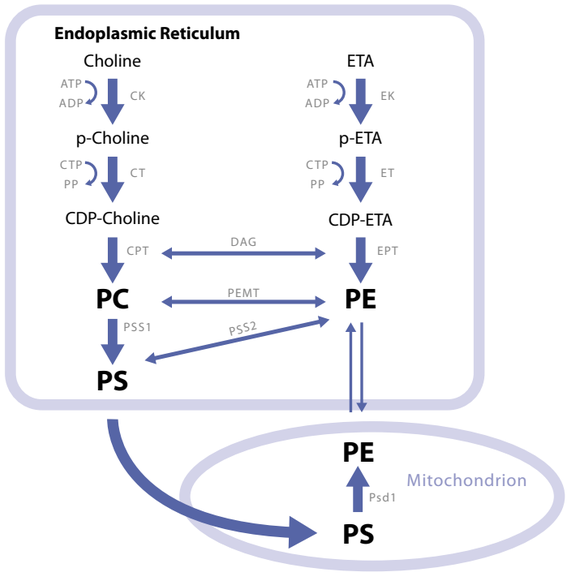
The drugs were then tested in a worm neurodegeneration model, which could be used to mimic Parkinson's disease

Parkinson's disease affects 1-2% of the population over 65 years of age and is the most commonly occurring movement disorder.



The structure of the alpha-synuclein protein.

Synthesis of PE via the two major pathways in cells, the Kennedy pathway (ER) and the PSD reaction (mitochondria). The two parallel branches of the Kennedy pathway are the CDP-ethanolamine pathway and the CDP-choline pathway. The four precursors needed for these reactions are choline, ethanolamine, cytosine triphosphate (CTP), and diacylglycerol (DAG). PS is synthesised in the ER via two base-exchange reactions (PSS1 and PSS2). The enzyme PEMT methylates PE to PC. PE is decarboxylated in the inner mitochondrial membrane by PSD (Psd1). CDP: cytidyl diphosphate; CTP: cytidyltriphosphate; DAG: diacylglycerol.



in humans. All three drugs were able to rescue dopaminergic neuron loss, and in fact protected the dopaminergic neurons that expressed alpha-synuclein, but not PSD, from degeneration. One way this neuroprotection is thought to occur is through a reduction in alpha-synuclein gene expression. However, Professor Witt determined that alpha-synuclein expression was unchanged following treatment with the drug candidates, but that the neuroprotection may occur via different pathways for each drug: MFX may scavenge free radicals, which cause damage to the body; CsA may inhibit pores associated with calcium transport, which may cause both neurodegeneration and cardiac damage; and SUL may inhibit cell death pathways.

CsA and MFX had previously been reported to protect against neurodegeneration, but this work presents the first report that SUL is able to protect against alpha-synuclein-induced dopaminergic neuron loss.

CsA and MFX had previously been reported to protect against neurodegeneration, but this work presents the first report that SUL is able to protect against alpha-synuclein-induced dopaminergic neuron loss.

Parkinson's disease affects 1-2% of the population over 65 years of age.

ANOTHER ROLE FOR ALPHA-SYNUCLEIN

Further work carried out by Professor Witt's group has investigated the effect of alpha-synuclein on the intracellular trafficking of iron transporters in yeast and worm models of Parkinson's disease. Alpha-synuclein interferes with the recycling of molecules which normally act as transporters for moving iron into and out of cells. Their findings suggest that dopaminergic neurons expressing alpha-synuclein become more susceptible to iron neurotoxicity with age, whereby excess iron enhances alpha-synuclein-induced neurodegeneration.

WHAT COMES NEXT

The next question is whether the drug candidates identified by Professor Witt's work could potentially be used, singly or in combination, to protect against alpha-synuclein associated pathology in cells derived from patients with Parkinson's disease. If this is indeed the case, there is the possibility that by interfering with the pathways by which alpha-synuclein causes disease, we could develop novel therapeutic strategies to tackle a distressing degenerative disorder.

The work done by the lab investigating the link between iron levels and alpha-synuclein may also present potential therapeutic targets. Using approaches that can target the iron transporter system may make it possible to prevent neurodegeneration via this route. Leading on from work done in yeast and worm models, it will also be interesting to see the findings from Professor Witt's lab translated into mammalian models of Parkinson's disease.

Behind the Research

Professor Stephan Witt

E: switt1@lsuhsc.edu **T:** +1 318 675 5163 **W:** www.lsuhscbiochemistry.com/stephan-n-witt-phd

Research Objectives

Professor Witt's work focuses on investigating Parkinson's disease and the role played by alpha-synuclein.

Detail

Professor Stephan Witt
LSU Health in Shreveport
Department of Biochemistry & Molecular Biology
1501 Kings Highway
PO box 33932
Shreveport, LA 71130-3932
USA

Bio

Witt is a professor and chairman of the department of Biochemistry & Molecular Biology at LSU Health in Shreveport, Louisiana. His PhD and postdoc research were at Caltech and Stanford. His research on chaperones and Parkinson's disease has been funded by the NIH and other agencies for over 16 years. He is on the editorial board of Cell Stress and Chaperones, PLoS ONE, and the Journal of Biological Chemistry.

Funding

American Parkinson's Disease Association; National Institutes of Health /NINDS 1R01 NS057656-05; American Parkinson's Disease Association; National Institutes of Health / NINDS R21 NS053678

Collaborators

Guy A. Caldwell and Kim A. Caldwell were key collaborators on this work. Prof Witt's co-authors are as follows:

- Shaoxiao Wang, Louisiana State University Health Sciences Center
- Siyuan Zhang, The University of Alabama & Louisiana State University Health Sciences Center
- Chuan Xu, The University of Alabama & Louisiana State University Health Sciences Center
- Addie Barron, Louisiana State University Health Sciences Center
- Floyd Galiano, Louisiana State University Health Sciences Center
- Dhaval Patel, Louisiana State University Health Sciences Center
- Yong Joo Lee, Louisiana State University Health Sciences Center
- Guy A. Caldwell, The University of Alabama
- Kim A. Caldwell, The University of Alabama

References

Wang S, Zhang S, Xu C, Barron A, Galiano F, Patel D, et al. (2016) Chemical Compensation of Mitochondrial Phospholipid Depletion in Yeast and Animal Models of Parkinson's Disease. *PLoS ONE* 11(10): e0164465. https://doi.org/10.1371/journal.pone.0164465

Patel D, Xu D, Nagarajan S, Liu Z, Hemphill W, Shi R, Uversky V, Caldwell G, Caldwell K and Witt S (2018) Alpha-synuclein inhibits Snx3–retromer-mediated retrograde recycling of iron transporters in *S. cerevisiae* and *C. elegans* models of Parkinson's disease *Human Molecular Genetics*, Vol. 27, No. 9 1514–1532, doi: 10.1093/hmg/ddy059

Witt Lab, LSU Health Shreveport. Available at http://www.lsuhscshreveport.edu/departments/BasicScience/BiochemistryandMolecularBiology/labs/stephan-witt-Lab/index

Personal Response

Are there any other indirect effects of low/no PE which don't relate to Parkinson's disease, and would the lack of PE cause a problem, for example in a mouse model without the PSD gene?

" A study of a mouse knock out model of PSD was published in 2005. Disruption of both copies of Pisd -/- in mice caused lethality between days 8 and 10 of embryonic development. Cells exhibited misshapen and fragmented mitochondria. In contrast, disruption of only one copy of Pisd -/+ had no effect on development. However, to compensate for the lower level of PE in Pisd -/+ mice levels of the enzymes of the CDP-ethanolamine pathway were significantly upregulated. Collectively, the results were consistent with the CDP-ethanolamine pathway being incapable of compensating for the disruption of both copies of Pisd.

Steenbergen R, Nanowski TS, Beigneux A, Kulinski A, Young SG, Vance JE. Disruption of the phosphatidylserine decarboxylase gene in mice causes embryonic lethality and mitochondrial defects. *J Biol Chem*. 2005 Dec 2;280(48):40032-40. "

LSU Health SHREVEPORT