**Identification of phosphorylation sites required for *PEX1* function in peroxisome biogenesis**

Zellweger Syndrome Spectrum (ZSS) diseases are a collection of autosomal recessive genetic diseases for which there is no cure.[[1]](#endnote-1) These diseases affect the production of peroxisomes, organelles important for breaking down cell wastes, such as very long chain fatty acids (VLCFAs) and making fatty acids, such as plasmalogens. Patients experience a variety of organ failures including, liver, hear, and kidney dysfunction and many patients do not make it to adulthood.[[2]](#endnote-2) The most common genetic mutation affects the *peroxisomal biogenesis factor 1* (*PEX1*).[[3]](#endnote-3) *However, it is not entirely clear what is required for proper PEX1 function during peroxisome biogenesis*.

My ***primary goal***is to understand the crucial role PEX1 plays in protein import into the peroxisome. PEX1 interacts with PEX6 via two AAA ATPase domains to recycle PEX5, responsible for the transport of peroxisomal proteins that contain peroxisomal targeting sequences (PTS). PEX1 cannot properly interact with PEX6 if this portion is misfolded, because peroxisomal protein import becomes dysfunctional.[[4]](#endnote-4), [[5]](#endnote-5) Fly and mouse models of ZSS with mutations in the *PEX1* gene recapitulate the phenotypes observed in human patients due to this dysfunction.[[6]](#endnote-6),[[7]](#endnote-7),[[8]](#endnote-8) Drugs that act as generic chemical chaperones have been shown to restore PEX1 function in cell culture, suggesting that an increase in folding stability is sufficient to restore peroxisome biogenesis.[[9]](#endnote-9) The *overall objective* of this study is to further investigate the role phosphorylation in the proper folding conformation and activity of PEX1, specifically its AAA ATPase domains. This will be studied using phosphoproteomics and by applying findings to *in vivo* models. The *rationale* for this is that protein phosphorylation is known to affect protein function in a diverse number of ways, which in this case could include the conformation of the protein and its AAA ATPase domains.

Understanding the nature of PEX1 protein folding will uncover the mechanisms of peroxisome biogenesis. I am proposing to test the ***central hypothesis*** that phosphorylation of PEX1 affects its tertiary structure, and therefore, its function with the following specific aims:

***Aim 1:*** To identify predicted and conserved phosphorylation sites in the PEX1 protein

**Hypothesis*:*** The use of NetPhos 2.0 phosphorylation prediction algorithm will allow for the identification of phosphorylation sites on PEX1, PEX1 will have multiple phosphorylation sites because it contains multiple residues that can be phosphorylated, and those essential for its function will be evolutionarily conserved.

**Approach*:***

1. Use NetPhos 2.0 to predict phosphorylation sites on the human PEX1 protein
2. Use Clustal Omega to align protein sequences and determine which phosphorylation sites are evolutionarily conserved, and therefore, most likely to play a role in PEX1 function

***Aim 2:*** To determine which of these sites are actually phosphorylated in human fibroblast cell culture.

**Hypothesis:** The predicted phosphorylation sites that are highly conserved and that were predicted with high confidence will be phosphorylated in cell culture.

**Approach:**

1. Generate mutant cell lines corresponding to single amino acid substitutions that prevent phosphorylation at predicted sites
2. Use tandem mass spectrometry to compare the phosphorylation states of PEX1 in wild type and mutant lines

***Aim 3:*** To determine which phosphorylation sites are necessary for proper PEX1 function.

**Hypothesis:** Mutated phosphorylation sites necessary for PEX1 function will yield phenotypes similar to those established in fly and mouse models of ZSS.

**Approach:**

1. Use FLP-FRT recombinase system to generate knock-in fly models with specific phosphorylation sites blocked
2. Use Cre-Lox recombination system to generate knock-in mouse models with specific phosphorylation sites blocked
3. Analyze mutant phenotypes using growth and survival assays, RT-PCR, and tandem mass spectrometry to study levels of peroxisome products and metabolites

The identification of the phosphorylation sites of PEX1 will contribute to the understanding of amino acids necessary for proper PEX1 conformation and function. This will further contribute to the understanding of PEX1 structure and function in the context of peroxisome biogenesis and protein import.

1. "Zellweger spectrum." *Genetics Home Reference*. U.S. National Library of Medicine, 4 Mar. 2014. Web. 10 Mar. 2014. <http://ghr.nlm.nih.gov/condition/zellweger-spectrum>. [↑](#endnote-ref-1)
2. "NINDS Zellweger Syndrome Information Page." *National Institute of Neurological Disorders and Stroke*. National Institutes of Health. U.S. Department of Health and Human Services, 22 Oct. 2012. Web. 24 Jan. 2014. <<http://www.ninds.nih.gov/disorders/zellweger/zellweger.htm>>. [↑](#endnote-ref-2)
3. Hans R. Waterham, Merel S. Ebberink, Genetics and molecular basis of human peroxisome biogenesis disorders, Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, Volume 1822, Issue 9, September 2012, Pages 1430-1441. PMID: [22871920](http://www.ncbi.nlm.nih.gov/pubmed/22871920). [↑](#endnote-ref-3)
4. Grou C, Carvalho A, Pinto M, Alencastre I, Rodrigues T, Freitas M, Francisco T, Sa-Miranda C, Azevedo J, The peroxisomal protein import machinery—a case report of transient ubiquitination with a new flavor, Cell Mol. Life Sci. 66 (2009) 254–262. PMID: 18810320. [↑](#endnote-ref-4)
5. Tamura S, Matsumoto N, Imamura A, Shimozawa N, Suzuki Y, Kondo N, Fujiki Y, Phenotype-genotype relationships in peroxisome biogenesis disorders of PEX1-defective complementation group 1 are defined by Pex1p-Pex6p interaction, Biochem J. 357 (2001) 417-426. PMID: 11439891. [↑](#endnote-ref-5)
6. Hiebler S, Masuda T, Hacia J, Moser A, Faust P, Liu A, Chowdhury N, Huang N, Lauer A, Bennett J, Watkins P, Zack D, Braverman N, Raymond G, Steinberg S, The Pex1-G844D mouse: A model for mild human Zellweger spectrum disorder, Mol Genet Metab. 14 (2014) epub. PMID: 24503136. [↑](#endnote-ref-6)
7. Chen H, Liu Z, Huang X, Drosophilamodels of peroxisomal biogenesis disorder: peroxins are required for spermatogenesis and very-long-chain fatty acid metabolism, Human Mol Genet. 19 (2010) 494-505. PMID: 19933170. [↑](#endnote-ref-7)
8. Mast F, Li J, Virk M, Hughes S, Simmonds A, Rachubinski, A Drosophilamodel for the Zellweger spectrum of peroxisome biogenesis disorders, Dis Model Mech. 4 (2011) 659-672. PMID: 21669930. [↑](#endnote-ref-8)
9. Zhang R, Chen L, Jiralerspong S, Snowden A, Steinberg S, Braverman N, Recovery of PEX1-Gly843Asp perxosiome dysfunction by small-molecule compounds, PNAS 107 (2010) 5569-5574. PMID: 20212125. [↑](#endnote-ref-9)