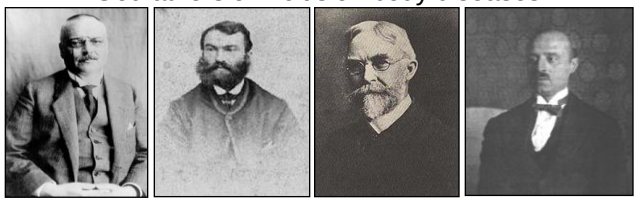
Glucan phosphatases link neurodegeneration with biofuel production.

Godfathers of inclusion body diseases



Alois Alzheimer James Parkinson George Huntington Gonzalo Lafora

Introduction

Multiple neurodegenerative diseases involve both malfunction of the ubiquitin proteasome pathway and aberrant intercellular accumulations, including Alzheimer's (AD), Parkinson's, (PD) Huntington's (HD), and Lafora's disease (LD). Emerging evidences suggest that some of these accumulations may be a protective mechanism and not the causative agent of the disease. Alternatively, the accumulations in other diseases may be the causative agent of the disease or a by-product of the disease. Lafora's disease falls into the latter category. While there are clearly aberrant accumulations in the cells of patients, their role in the disease is unknown.

LD was first described 100 years ago. It is a fatal, autosomal recessive disorder characterized by progressive neurological deterioration, myoclonus (i.e. severe muscle spasms), and epilepsy. The patients develop normally until age 15 when they experience an epileptic episode. These episodes increase in both severity and frequency until the patient dies around age 25. The diagnostic hallmark of LD is the accumulation of intracellular carbohydrate deposits found in most tissues, called Lafora bodies (LBs).

The combined efforts of multiple labs identified two genes that account for ≈90% of Lafora disease cases. *EPM2A* (epilepsy myoclonus type 2 gene A) encodes the bi-modular phosphatase laforin, and EPM2B (epilepsy myoclonus type 2 gene B) encodes the E3 ubiquitin ligase malin. Since LD is a recessive disease, mutations in both copies of either the gene encoding laforin or malin results in LD.

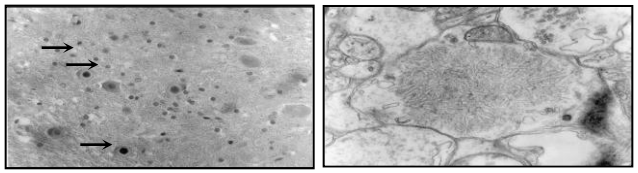


Fig. 1 Microscopic view of a Lafora body. A. PAS staining of LD patient tissue viewed via a light microscope. B. LB inside a neuron viewed by a transmission electron Microscope (TEM),

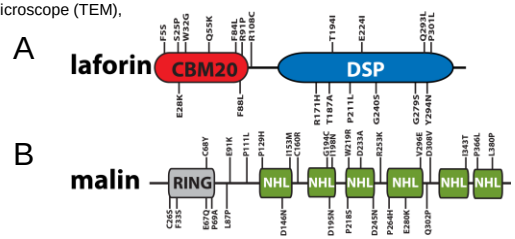


Fig. 2 Schematic of laforin and malin. **A.** Laforin contains an amino-terminal carbohydrate binding module (CBM) and carboxy-terminal dual specificity phosphatase domain (DSP). **B.** Malin contains an amino-terminal RING domain and 6 NHL domains at its carboxy-terminus. Disease causing missense mutations occur in each domain.

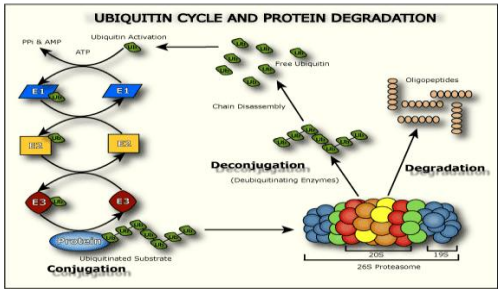


Figure 3 The ubiquitin proteasome pathway. In 2005, we discovered that malin is an E3 ubiquitin ligase and as such participates in a similar signaling cascade as shown here. Our current hypothesis is that malin monitors and manipulates the protein levels of multiple enzymes involved in carbohydrate metabolism via the ubiquitin proteasome pathway.

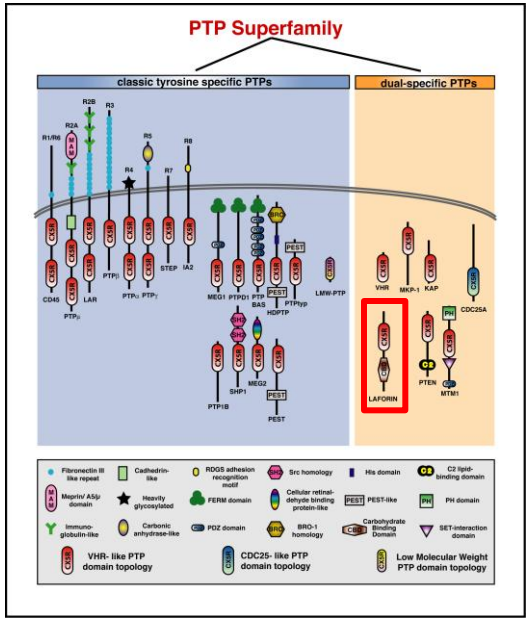


Fig. 4 Protein Tyrosine Phosphatase (PTP) Superfamily. Kinases and phosphatases are the backbone of cellular signaling. While kinases add phosphate groups to proteins, lipids, and/or carbohydrates to turn signaling events on or off, phosphatases remove phosphates. The PTP superfamily contains ≈100 human genes & is divided into classical PTPs and dual specificity phosphatases (DSPs) that all share the same CX_5R domain (CXXGXR). Most PTPs also have an additional domain that acts as a "zip-code" and targets the phosphatase to a specific sub-cellular location. Laforin contains a carbohydrate binding module (CBM) that targets it to the location of carbohydrate synthesis within cells. We recently demonstrated that laforin dephosphorylates carbohydrates, making it a unique class of PTP.

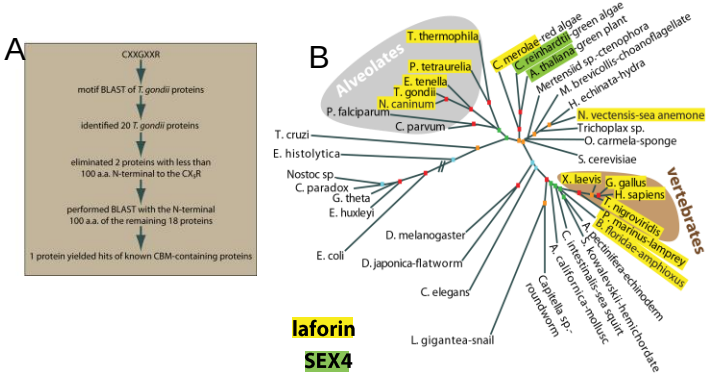
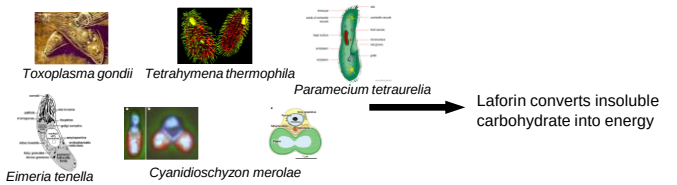


Figure 5 Our bioinformatic screen identified new laforin orthologs. Previously, laforin was thought to only be conserved in vertebrates. To gain insights into what biological processes laforin regulates, we devised a bioinformatic screen to identify laforin orthologs in new organisms. We discovered genes encoding a laforin ortholog in five different unicellular organisms (i.e. protists). Highlighted in yellow are protist's genomes that contain laforin. These analyses also uncovered a glucan phosphatase in all members of Kingdom Plantae called Starch Excess 4 (SEX4), these are highlighted in green.



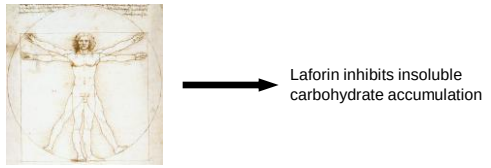


Figure 6 Our hypothesis of laforin's role in protists and vertebrates. Cumulatively, our work suggests that in protists laforin functions to convert insoluble carbohydrates into energy. Conversely, laforin inhibits insoluble carbohydrate accumulation in vertebrates.

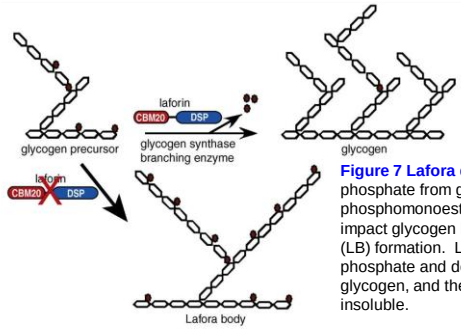


Figure 7 Lafora disease model. Laforin removes phosphate from glycogen. In the absence of laforin, phosphomonoesters accumulate and negatively impact glycogen branching and lead to Lafora body (LB) formation. LBs contain increased amounts of phosphate and decreased branching compared to glycogen, and these two characteristics make LBs insoluble.

Insights from our work on LD have allowed us to propose mechanisms that regulate starch metabolism in plants. Starch is integral to biofuels in organisms as diverse as corn and micro-algae.

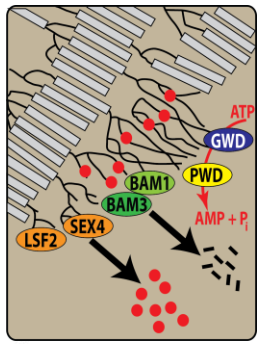


Figure 8 Starch degradation model. The dikinases GWD and PWD phosphorylate (red circles) the outer glucans to facilitate glucan solubility. β-amylases (BAM) release maltose and malto-oligosaccharides. SEX4 and LSF2 dephosphorylate surface glucans and phospho-oligosaccharides so the process can repeat.

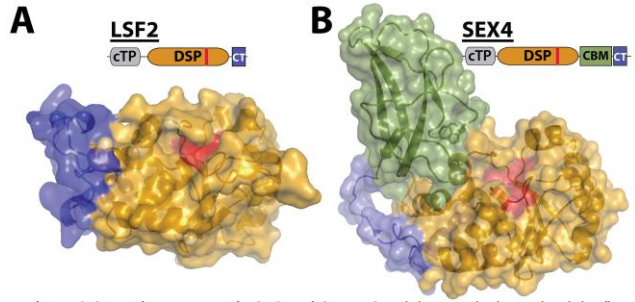


Figure 9 Crystal structure of LSF2 and SEX4. Our lab recently determined the first crystal structures of glucan phosphatases, LSF2 and SEX4. We are using these structures to engineer glucan phosphatase activity and modulate biofuels production.

Recent Select Lab Publications

Gentry MS, Roma-Mateo C, and PSanz. 2012. Laforin, a protein with many faces: glucan phosphatase, adapter protein, et alii. ***The FEBS Journal.***

Santelia D, Kötting O., Seung D, Schubert M, Thalmann M, Bischof S, Meekins DA, Lutz A, Patron N, Gentry MS, H.-T. Allain F, and SC Zeeman. 2011. Inhibition of phosphoglucan phosphatase LSF2 (Like Sex Four 2) in Arabidopsis leads to modified starch with elevated C3-bound phosphate content. ***The Plant Cell.***

Dukhande VV, Rogers DM, Romá-Mateo C, Donderis J, Taylor AO, Sanz P, and Gentry MS. 2011. Laforin, a dual specificity phosphatase involved in Lafora disease, is present mainly as monomeric form with full phosphatase activity. ***PLoS ONE.***

Romá-Mateo C, Solaz-Fuster C, Gimeno-Alcañiz J, Dukhande VV, Donderis J, Koller A, Cordoba S, Gentry MS and Sanz P. 2011. Laforin, a dual specificity phosphatase involved in Lafora disease, is phosphorylated at Ser25 by AMP-activated protein kinase. ***Biochemical Journal*.**

Romá-Mateo C, Moreno D, Vernia S, Rubio T, Bridges TM, Gentry MS and Sanz P. 2011. Lafora disease E3-ubiquitin ligase malin is related to TRIM32 at both the phylogenetic and functional level. ***BMC Evolutionary Biology***

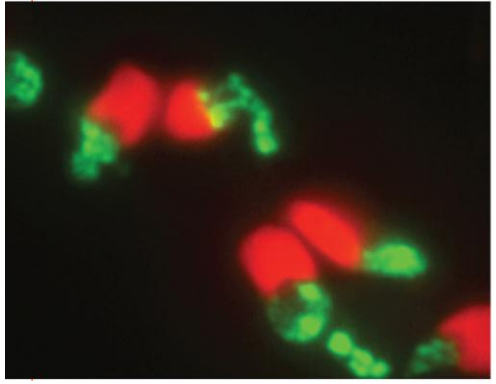
Vander Kooi CW, Taylor AO, Pace RM, Meekins DA, Guo H-F, Kim Y, Gentry MS. 2010. Structural basis for the glucan phosphatase activity of Starch Excess4. ***Proceedings of the National Academy of Sciences*.**

Hsu S, Kim Y, Li S, Durrant ES, Pace RM, Wood VW, Gentry MS. 2009. Structural insights into glucan phosphatase dynamics using amide hydrogen-deuterium exchange mass spectrometry. ***Biochemistry***.

Gentry MS, Dixon JE, Worby CA. 2009. Lafora disease: Insights into neurodegeneration from plant metabolism. ***Trends in Biochemical Science***.

Gentry MS and Pace RM. 2009 Conservation of the glucan phosphatase laforin is linked to rates of molecular evolution and the glycogen metabolism of the organism. ***BMC Evolutionary Biology.***

Gentry MS, Dowen RH, Worby CA, Mattoo S, Ecker J and JE Dixon. 2007. The phosphatase laforin crosses evolutionary boundaries and links carbohydrate metabolism to neuronal disease. *The Journal of Cell Biology*



Laforin (green) dephosphorylates starch granules in this red alga.

Research Highlight in *The Journal of Cell Biology*

Solubilizing stubborn carbs

Plants and protists may not have a nervous system, but they can help elucidate the mechanism of a fatal neurodegenerative disease, as Gentry et al. reveal on page 477. The organisms helped researchers pin down the function of a protein suspected of sparking destructive carbohydrate accumulations.

Lafora disease is a fatal form of epilepsy whose symptoms usually begin between the ages of 10 and 20. In neurodegenerative diseases such as Parkinson's and Alzheimer's, globs of insoluble proteins amass in neurons. But in Lafora disease, neurons harbor clumps of insoluble carbohydrates similar to amylopectin, a component of starch. Several mutations can trigger the illness, including glitches in the gene for laforin. This protein carries two key modules: one that grabs carbohydrates, and a second that slices off phosphate groups. Although mouse models develop the symptoms of Lafora disease, researchers still don't understand how their faulty laforin protein elicits neurological damage.

Gentry et al. thus went hunting for other laforin-making organisms. They trolled protist genomes and pinpointed genes for laforin in organisms as different as the pathogen behind toxoplasmosis and a type of red alga. Previously, researchers had thought that only vertebrates manufacture the protein. Like human laforin, the protist version grips carbohydrates and lops off a phosphate. Where laforin hangs out has been a mystery, but the researchers determined that, in one type of protist, it congregates around starch granules.

The team also found that plants carry an unrelated protein with the same function, known as SEX4. Arabidopsis plants with a defective form of SEX4 built up excess starch. The presence of proteins with the same job in such a range of organisms suggests that phosphate removal is crucial for breaking down insoluble carbohydrates. Why persistent phosphates lead to carbohydrate buildup remains unclear. The scientists now want to investigate whether the carb clusters from patients with Lafora disease carry excess phosphates. JCB

UK

The Gentry Lab

http://gentrylab.com/wordpress/

matthew.gentry@uky.edu

Elucidating the role of glucan phosphatases in neurodegenerative disease and biofuels production.

Molecular and Cellular Biochemistry
Center for Structural Biology
University of Kentucky