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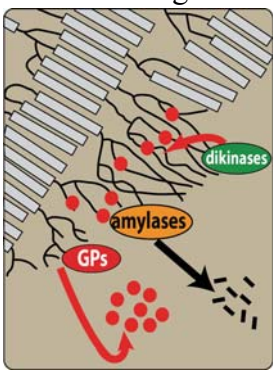
Grant Title: Glucan phosphatases link neurodegeneration and biofuels research

Biofuels research and research into the fatal neurodegenerative disease called Lafora disease (LD) are intimately linked by a newly described family of enzymes called glucan phosphatases. Glucans are the most abundant polymer in plants and algae, with cellulose (β-1-4 linkages) serving as the major structural component and starch (α-1-4 and α-1-6 linkages) as the major energy reserve. Due to its abundance and energy-rich status, starch is a common 1st generation biofuels feedstock. Instead of starch, humans utilize glycogen as their primary carbohydrate energy storage molecule. Both starch and glycogen metabolism is dependent on the action of glucan phosphatases.

Plants release the energy in starch via a recently identified three-step process: starch phosphorylation, degradation, and dephosphorylation (Figure). Plants phosphorylate the outer starch glucose units to make them water-soluble and enzyme accessible so that amylases can degrade the starch into glucose, maltose, and oligosaccharides. Following amylase activity, the phosphate must be removed by glucan phosphatases. This three-step cycle is repeated to allow efficient, processive starch degradation. In the absence of phosphorylation or dephosphorylation, the amylases are very inefficient and plants are unable to degrade the starch that it produces.



Starch degradation. Glucan dikinases phosphorylate starch to facilitate starch solubility. Amylases release glucose. Glucan phosphatases (GPs) dephosphorylate starch so the process can repeat.

In humans, glycogen synthase erroneously introduces a phosphate group ~1/10,000 glucose units. The human glucan phosphatase laforin removes phosphate from glycogen. In the absence of laforin activity, glycogen transforms into a hyper-phosphorylated, water-insoluble, starch-like Lafora body (LB). LBs are the suspected cause of neuronal apoptosis, neurodegeneration, and eventual death of LD patients.

The **objectives** for this proposal are to 1) determine the structure of glucan phosphatases and 2) define the molecular enzymology of laforin and laforin orthologs. The **methods** utilized for this proposal were purification of recombinant proteins, x-ray crystallography, mutagenesis, biochemical assays, and enzyme kinetics. Our **results** allowed us to successfully complete both objectives. We optimized protocols to produce recombinant glucan phosphatases, we established reproducible crystallization strategies to crystalize glucan phosphatases, and we have defined the biochemical properties of glucan phosphatases. These results were published in two articles: **MK Brewer *et al.*, BMC Biochemistry, 2014; M Raththagala *et al*., Molecular Cell, 2015.**

While much progress has been made concerning the biology of reversible starch phosphorylation little was known about the molecular mechanisms regulating glucan phosphatase function. The work in this proposal addressed critical information gaps of this essential pathway. This completed work defines this novel enzyme family and impact both biofuels research and the field of neurodegeneration.